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# Advances in Animal Biosciences

## The Challenge of Change

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# Proceedings

of the  
British Society of Animal Science

## 2020

### Advances in Animal Biosciences

This book is part of a series which is a companion to the journal ANIMAL



The Proceedings of the British Society of Animal Science constitute summaries of papers originally scheduled to be presented at the Society's Annual Conference, BSAS 76th Annual Conference 2020 held at the East Midlands Conference Centre (EMCC) on 30 March - 1 April 2020.

The summaries have been edited. Views expressed in all contributions are those of the authors and not those of the BSAS.

This publication contains all the summaries that were available at the time of going to press.

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# Proceedings

## of the British Society of Animal Science Annual Conference 2020

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Feed Efficiency and Global Food Security	004 - 008
Small Ruminants	009 - 017
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# The role of ruminant livestock - sustainability metrics associated with product quality and land use.

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Agricultural land and human food security are coming under great pressure from climate change, rapidly increasing human population, urbanisation, demand for biofuels, and demand for animal protein. This situation led us to re-assess the role of ruminant livestock in meeting our requirements for key nutrients in the broader context of global warming potential and land use. To date, several studies have examined environmental consequences of different food consumption patterns at the diet level; however, few have addressed nutritional variations of a single commodity attributable to on-farm strategies, leaving limited insight into how agricultural production can be improved to better balance environment and human nutrition. Using seven livestock production systems encompassing cattle, sheep, pigs and poultry as examples, we propose a novel approach to incorporate nutritional value of meat products from two different sustainability driven metrics:

- i) Global warming potential (GWP) - mass of nutrient provision per kg CO<sub>2</sub>eq; and
- ii) Arable land use (ALU) - mass of nutrient provision per m<sup>2</sup> of arable land.

To assess overall value of the product associated with human nutrition we used a nutrient index based on Saarinen et al., (2017), a scalar value to combine information on multiple nutrients, both beneficial and detrimental to human health. The beneficial nutrients considered included protein content, essential fatty acids as well as key minerals and vitamins and the negative included saturated fatty acids and sodium. The nutrient index rankings of the livestock products dramatically altered between chosen sustainability driven metrics, for GWP overall: poultry < pigs < cattle < sheep; whereas the inverse relationship was calculated for ALU.

These findings suggest that, for livestock systems to contribute to sustainable agriculture, a shift towards quality-oriented market structure is required with consideration across multiple sustainability driven metrics included.

# The effect of protein quality on interpreting agricultural greenhouse gas emissions

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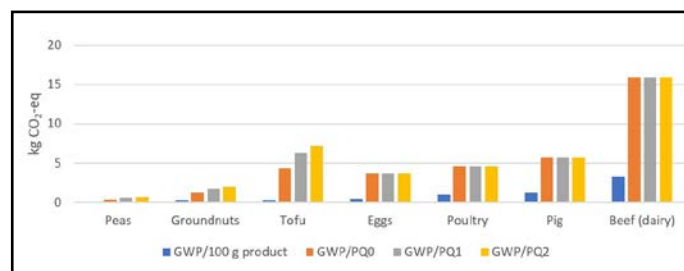
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**Application** Total protein content is a common functional unit in sustainability assessments. By accounting for the *quality* of protein, these assessments can be further integrated into health sciences for One Health solutions.

**Introduction** Nutritional composition of food products is becoming a focal point of discussion in the field of life cycle assessment (LCA) (McAuliffe et al., 2019). One of the most common methods to integrate this concept into the framework is to quantify environmental burdens per protein content of a given product. However, this approach implicitly assumes protein to be a homogenous nutrient and consequently fails to address complexities including amino acid content, ileal digestibility and bioavailability. This paper investigates the quantitative importance of considering protein quality when evaluating global warming potentials (GWP) of multiple food products.

**Materials and methods:** Emissions intensities (reported as kg CO<sub>2</sub>-eq/100 g) of products commonly consumed as protein sources were acquired from Poore and Nemecek (2018). For each product, effective protein content was obtained under three methods: (1) mass of total protein (PQ0); (2) PQ0 corrected by digestibility corrected amino acid scores (PQ1); and (3) PQ0 corrected by digestible indispensable amino acid score (PQ2). PQ1 and PQ2 were calculated using coefficients reported in Ertl et al. (2016) for plant-based products, whereas for animal-based products the values were truncated at 100% to disallow compensation by complementary commodities (Ertl et al., 2018). GWP values from Poore and Nemecek (2018) were subsequently recalculated under the functional units of kg CO<sub>2</sub>-eq/100 g protein, whereby the denominator was derived as per the quality correction specification detailed above.

**Results** Under the mass-based assessment, animal-based products showed a higher GWP in all instances. When total protein (g protein/100 g product) was used as the functional unit, however, the carbon footprint of tofu became comparable to eggs. Results under PQ1 and PQ2 were largely comparable to each other: under both methods, tofu showed higher carbon footprints than all monogastric products (eggs, chicken and pork), by up to 94% against eggs under PQ2. Contrary to an earlier study based on nutrient density scores that encompass all major nutrients including minerals and vitamins (McAuliffe et al., 2018), dairy beef systems had the highest GWP regardless of the functional unit in this analysis.



**Figure 1.** Global warming potentials for selected food products commonly consumed as protein sources

**Conclusion** Although LCA practitioners are increasingly accounting for human nutrition in environmental assessments of agri-food systems, many studies which do so opt for simplistic denominators. As demonstrated by this study, assumptions on nutrient quality can have a significant impact on relative carbon footprints of different products, particularly in studies which compare animal- and plant-based products. Looking ahead, future LCA research should aim to improve the understanding of nutritional quality of food by also accounting for bioavailability of individual amino acids as well as micronutrients to truly elucidate the benefits of individual food items to human nutrition.

**Acknowledgements** This work was funded by BBSRC (BBS/E/C/00010320, BBS/E/C/000J0100).

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# Feed efficiency and global food security

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**Application** Feed efficiency is important for global food security. Better use of land and feed resources, with lower environmental impacts, comes from study of feed efficiency at the whole farm, human edible and environmental levels.

**Introduction** Livestock production is an essential component of global food security. Global food demand will increase over the next 30 years to meet the needs of the increasing human population. Demand for animal products (meat, milk and eggs) is likely to increase faster than demand for cereals as developing countries achieve higher incomes. Some say that a sustainable food strategy should rely on crop production, and that grain, protein crops and fish products fed to animals should be for direct human consumption. This strategy ignores the essential role of animals in global food security, whereby animals generate nutritious products from non-arable land and agrifood by-products. Only one quarter of agricultural land can grow crops; the remainder is used for livestock.

**Feed conversion efficiency (FCE)** Animal Scientists typically define FCE as performance (e.g. live-weight gain, milk yield, egg production) of an animal or bird per unit feed intake (e.g. fresh weight, dry matter, nitrogen intakes). The inverse of FCE is feed conversion ratio (FCR). FCE and FCR have been the focus of genetic selection for decades, leading to significant increases in efficiency. Geneticists also use residual feed intake (observed minus expected feed intake); animals that eat less than expected are more efficient. The main driver of FCE is animal performance: higher yields dilute maintenance requirements; faster growth rates reduce days to slaughter.

**Whole-farm feed efficiency (WFFE)** extends FCE to look at all animals and feed on a farm. Performance is still a main driver, but other factors come into play. High replacement rate in a dairy herd, for example, reduces WFFE due to large numbers of youngstock eating feed but not producing milk. WFFE uses total feed available, rather than only feed eaten. Feed and land utilisation are important, and dairy systems based on mixed rations with high proportions of concentrates show greater WFFE than systems based predominantly on grazing (Garnsworthy et al., 2019).

**Human edible feed efficiency (heFCE)** Livestock consume  $\approx 6$  billion tonnes of feed dry matter annually, including one third of global cereal production. The majority (87%) of livestock feed, however, comprises materials that are not eaten by humans. Ruminant diets contain  $>57\%$  grass and forages, whereas non-ruminants consume 72% of grain fed to livestock (Mottet et al., 2017). Thus, although ruminant livestock have a greater environmental impact than non-ruminants, they have greater potential to utilise land that cannot grow crops for direct human consumption. In terms of human-edible feed conversion efficiency, beef and lamb (21-43% heFCE) are comparable with pigs and poultry (26-43% heFCE), although milk production (237% heFCE) is by far the most efficient animal production system (Wilkinson, 2011).

**Environmental feed efficiency** Several metrics can relate FCE to environmental impact. Feed carbon footprint, nitrogen use efficiency, and methane emissions per kg of product were used to explore formulation of dairy diets with least environmental cost rather than simply least cost (Wilkinson and Garnsworthy, 2016). Generally, by-product feeds showed lower environmental impacts than primary product feeds. Biodiversity is greater in high-yield livestock systems with land sparing than in extensive systems with land sharing; high-yield systems have greater FCE and lower environmental impact (Balmford et al., 2018). In many parts of the world, silvopastoral systems combine fodder plants with shrubs and trees for sustainable and resilient livestock production that has a high efficiency of land use.

**Conclusion** To ensure global food security within a finite amount of land, efficiency of livestock production must continue to increase whilst environmental impacts decrease. Feed efficiency provides useful indications of resource use at the animal, farm or global level, and aids selection of best systems for different scenarios and objectives.

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# Healthy livestock produce sustainable food - how do we quantify the impacts of cattle health on industry sustainability?

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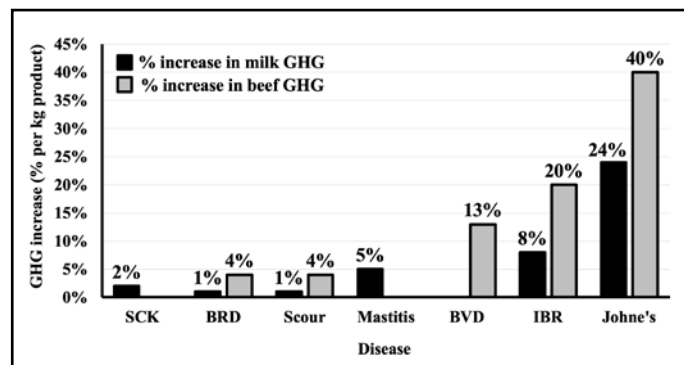
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**Application** Cattle diseases have negative impacts on productivity, economic cost and greenhouse gas emissions per unit of milk or meat produced, yet better data is required to accurately quantify sustainability impacts.

**Introduction** Animal health is key to sustainable food production, with over 20% of global animal protein losses attributed to disease (OIE, 2008). Healthy cattle produce a greater quantity of milk or meat in a lesser time, using fewer resources (feed, land, water, fuel, etc) and with lower greenhouse gas (GHG) emissions per unit of product (Capper and Bauman, 2013); thereby potentially reducing economic costs and environmental impacts. To date, however, there has been little attempt made to quantify the sustainability impacts of cattle disease.

**Material and Methods** The economic and environmental impacts of cattle disease were assessed by examining the effects of disease on key performance indicators (KPI: milk or meat yields, fertility, growth rate and mortality), economic costs of production and GHG emissions (as a proxy for environmental impact); weighted for the incidence of the disease in the population. A comprehensive literature search using PubMed (National Center for Biotechnology Information, Bethesda MD, USA), ScienceDirect (Elsevier, Amsterdam, NL) and Google Scholar (Google, Mountain View CA, USA) was executed to gather data relating to the impact of cattle diseases on KPI and, where available, on resulting GHG emissions and economic



**Table 1** Impacts of cattle disease on key performance indicators and industry economic costs

cost. Where sufficient data were not available, economic costs and GHG emissions were calculated based on the best available data.

**Results** Considerable literature gaps existed – although the impacts of disease on KPIs were well-defined, these were rarely translated into sustainability metrics. Economic metrics tended to be dated or to describe an extremely wide range of economic costs (Table 1). The impacts of cattle disease on GHG emissions varied from 1-40% with little apparent association between GHG emissions (Figure 1) and industry-wide economic cost.

**Conclusion** Improving cattle health reduces GHG emissions and economic costs. Accurate quantification of the sustainability impacts of cattle diseases can only be achieved if knowledge gaps relating to KPI, economic costs and GHG emissions are assessed using a common baseline with up-to-date data inputs.

**Acknowledgments** Funding from MSD Animal Health is gratefully acknowledged.

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**Figure 1.** Impacts of cattle disease on greenhouse gas emissions per unit of beef or milk. Diseases are defined as follows: SCK = sub-clinical ketosis; BRD = Bovine respiratory disease; BVD = Bovine viral diarrhoea; IBR = Infectious bovine rhinotracheitis

	Milk yield	Fertility	Growth rate	Mortality	Annual industry cost (£)
Sub-clinical ketosis	Reduced	Reduced	-	-	48-212 million
Bovine respiratory disease	-	-	Reduced	Increased	60 million
Scour	-	-	Reduced	Increased	11 million
Mastitis	Reduced	Reduced	-	Increased	125-384 million
Bovine viral diarrhoea	Reduced	Reduced	Reduced	Increased	162 million
Infectious bovine rhinotracheitis	Reduced	Reduced	Reduced	Increased	45 million
Johne's disease	Reduced	Reduced	Reduced	Increased	2.8 million

# Feed wastage in milking parlours on GB dairy farms: money down the drain

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**Application** Minimising feed wastage in the dairy parlour can save farmers thousands of pounds per year.

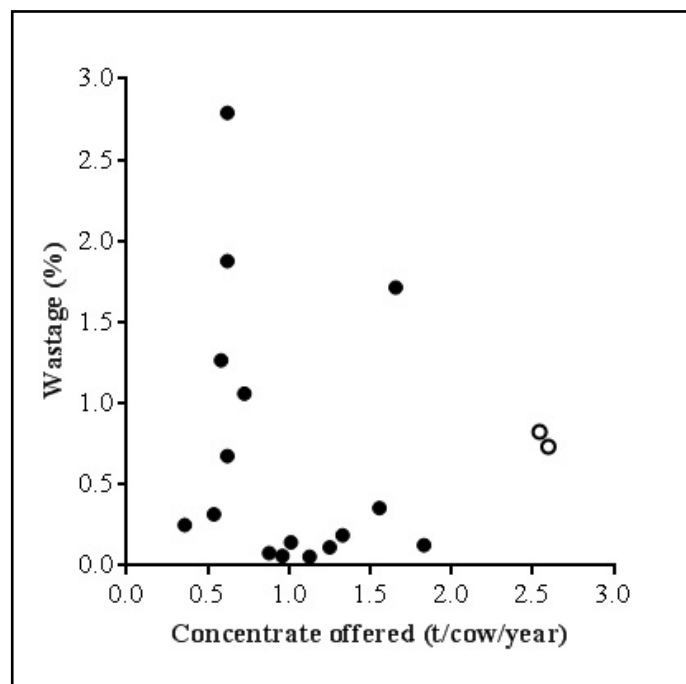
**Introduction** Concentrate is the most expensive feed utilised within the milking herd so farmers should be aiming to minimise wastage. When asked about feed wastage within their parlour or milking robot, most farmers assumed that this was negligible. An on-farm study showed that calibrating in-parlour feeders correctly can eliminate over-feeding of concentrates (Farmers Weekly case study, 2012), but concentrate waste due to spillage has not, to our knowledge, been studied previously. The aim of this research was to determine the direct economic cost of concentrate waste during milking.

**Material and methods** Concentrate wastage during milking was measured three times on each of 18 GB dairy farms throughout 2017. In conventional parlours (n=16), the floor was cleared of all debris prior to milking. After the first row of cows on each side exited the parlour (two rows if the parlour was less than 10 cows in length), milking was paused and concentrate on the floor was collected using shovels, dustpans and brushes. This wasted concentrate was weighed and then dried to determine dry matter (DM) content. Samples of concentrate offered were also taken for DM analysis. Concentrate wastage was calculated as weight of DM on the floor as a percentage of DM offered. For farms using robotic milking (n=2), the same method was used, but milking was stopped after ten cows went through the robot to allow collection of waste. Total concentrate supplementation during milking, concentrate cost and average herd size were recorded throughout 2017 for each farm.

**Results** Concentrate wastage varied from 0.06% to 2.79% of DM offered during milking. This equated to a cost of between £0.12 and £5.56 per cow per year (Table 1). Concentrate wastage was not correlated with the amount of concentrate offered (Figure 1).

	Mean	Min.	Max.
Herd size, n	257 (124)	68	550
Conc. fed during milkGreen Tinting, t/cow/year	1.16 (0.66)	0.36	2.60
Wastage during milking, %	0.70 (0.77)	0.06	2.79
Wastage, kg/cow/year	7.45 (8.48)	0.57	28.39
Wastage cost, £/cow/year	1.48 (1.66)	0.12	5.56

**Figure 1.** Concentrate wastage during milking in 16 conventional (solid circles) and two robotic (open circles) milking parlours during 2017



**Conclusion** Concentrate wastage during milking was a small proportion of total concentrate offered, but was costing some farmers more than £1,000 per year. In addition to the direct cost of concentrate loss, there is a hidden cost in terms of loss of potential milk production and fertility problems caused by reduced nutrient intake. On some farms the cause of wastage was obvious, such as excessive metalwork causing animals to stretch for feed, holes in feed troughs or damaged pipework. One farmer enrolled on this study noticed the amount of waste collected during the first visit and reduced their waste from 1.74% to 0.44% by repairing holes in feed troughs, representing a saving of £1,139/year. Simple, cost-effective interventions can prevent money from going down the drain.

**Acknowledgements** Research was funded by AHDB Dairy - Nottingham Partnership on Health, Welfare and Nutrition. We thank the participating farmers.

## Reference

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**Table 1.** Concentrate wastage during milking at 18 GB dairy farms during 2017

# Calibration of in-parlour concentrate feeding systems is critical for precision feeding of dairy cows

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**Application** Poorly calibrated in-parlour feeding systems can cause significant in-accuracies in concentrate feeding for dairy cows.

**Introduction** The concentrate component of dairy cow diets represents between 60 – 70% of the variable costs of milk production on many dairy farms. Given the significant cost of concentrates, it is important that concentrates are accurately allocated to cows. Indeed, many modern concentrate feeding systems, both in-parlour and out-of-parlour systems, allow concentrates to be allocated to individual cows based on their current milk yields. However, the accuracy of these feeding systems can be seriously impacted if concentrates are not dispensed accurately. This study was conducted to examine the accuracy with which in-parlour concentrate feeding systems on commercial farms dispensed concentrates.

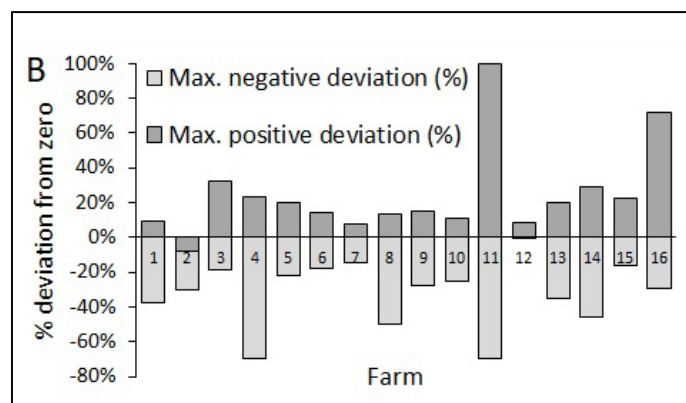
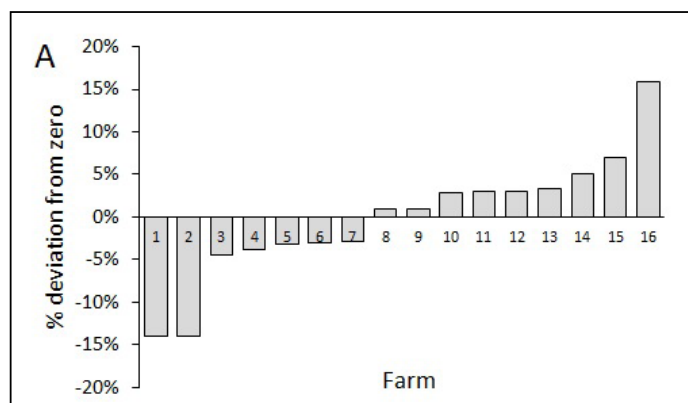
**Material and methods** The study was conducted on 16 Northern Ireland dairy farms, and measurements were conducted over a six week period during November and December 2018. The 16 farms were all participants in a study involving an evaluation of 'feed-to-yield' concentrate feeding systems, and had a mean herd size of 174 (s.d., 66.6) cows, and a mean annual milk yield of approximately 8,618 (s.d., 1235.2) kg. Each farm was visited by an AFBI staff member who tested all in-parlour feeders for accuracy. A total of 490 feeders were tested, ranging from 16 - 48 feeders per farm. This test involved allowing a pre-programmed quantity of concentrates (normally between

500 – 2000 g, depending on the feeder calibration setting) to be dispensed from each feed hopper into a plastic bucket, and weighing this on a tared weigh-scale. This information was then used to determine the percentage deviation of the dropped weight of concentrate from the target weight. The information on the actual weight of concentrate dropped was then used to recalibrate the weigh cell in each feed hopper using the inbuilt computer software.

**Results** Across all feeders on each farm, the average deviation from target (zero) was 0.2% (s.d., 7.4), while at the extremes, Farm 1 had a mean average deviation of -14% across all feeders, while Farm 16 had a mean average deviation of +16% (Figure 1A). However, these mean values hide individual feeder variations on many farms. For example, when averaged across the 16 farms the maximum positive deviation (most extreme feeder on each farm) from target was +24% (s.d., 26.3%), while the mean maximum negative deviation from target was -32% (Figure 1B). Of the 16 farms examined, the maximum positive deviation from zero was observed on Farm 11 where one feeder was overfeeding by 100%, while the maximum negative deviation from zero was observed on Farm 4 where one feeder was underfeeding by 70%. The implications of the average deviation of all feeders on Farms 1 and 16 (Figure A) is examined for a 100 cow herd offering an average of 6.0 kg concentrate/cow/day through in-parlour feeders over a 180 day winter period. The target concentrate usage in this situation is 108 t concentrate over the winter. However based on the mean deviations observed, Farm 1 would actually have fed only 3 t concentrate, while Farm 16 would have actually fed 125 t concentrate, representing underfeeding and overfeeding of 15 t and 17 t respectively.

**Conclusion** Poorly calibrated feeders are common on many Northern Ireland dairy farms, and can result in substantial underfeeding or overfeeding of concentrates. On farms where the average feeder performance is close to target, individual feeders can still be inaccurate resulting in significantly overfeeding or underfeeding of individual cows on a daily basis.

**Acknowledgements** This work was co-funded by DAERA and AgriSearch through the Research Challenge Fund.



**Figure 1** Average percentage deviation from target (zero) of all in-parlour feeders on each of the 16 farms (A) and percentage deviation from target (zero) of the in-parlour feeder on each farm with the largest positive and negative deviation from zero (B)

# Comparison of nitrogen excretion rate of dairy cattle at different physiological states (calf vs growing vs lactating vs dry period)

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**Application** Dairy cattle utilise feed nitrogen with different efficiencies at different rearing stages (calve to adult cows), which provides useful information to quantify N input and excretion in dairy production systems.

**Introduction** The nitrogen excretion rate (NER) of dairy cattle can be influenced by many animal and dietary factors. Perhaps, the most important factor is the physiological state of dairy cattle. However, there is no direct comparison of NER of dairy cattle at different rearing stages. The objective of the present study was to evaluate if there was a significant difference in NER between calf, growing cattle, lactating cow and dry cow.

**Material and methods** Dairy cattle data (n = 1916) used were collated from 63 studies undertaken at the Agri-Food and Biosciences Institute between 1984 and 2017. The dataset comprises data from 54 calves, 454 growing cattle, 954 lactating cows and 454 dry cows of pure Holstein-Friesian and crossbreed, Norwegian (lactating cows only n = 40) and Swedish Red (lactating cows only n = 18). All calf data were obtained before weaning and offered milk replacer with free access to water, starter concentrates and forage. Dry cows were offered fresh grass-only diets at maintenance levels. Growing cattle and lactating cows were offered ad libitum forage-only diets or mixed diets of forage and concentrates. Before commencing digestibility trials, all animals were housed in loose cubicle accommodation and offered experimental diets for at least 20 d, and then transferred to metabolism crates/units and housed there for 8 d with total feed intake and faeces and urine outputs recorded daily. The data were used to compare the NER between the 4 groups of cattle (calf vs growing vs lactating vs dry) using the ANOVA. The NER between the 4 groups was further compared using the REML procedure in linear relationships between manure N (MN) output and N intake (NI) with a fixed intercept for each group which is a sum of endogenous faecal and urine N calculated using the recommendation of NRC (2001).

**Results** Calf data had a mean N intake 69 (s.d. 25.0) g/d, faecal N output 18 (s.d. 7.8) g/d, urine N output 16 (s.d. 6.7) g/d and total manure N output 34 (13.7) g/d. The corresponding data for growing cattle are respectively 169 (48.8), 53 (15.2),

76 (27.6) and 129 (39.5) g/d; lactating cows 494 (121.0), 149 (35.8), 197 (68.0) and 345 (90.1) g/d; dry cows 138 (39.8), 34 (6.3), 87 (34.5) and 121 (35.5) g/d. The ANOVA comparison of NER of dairy cattle at different stages is showed in Table 1. Calves had the lowest ratios of faecal N (FN), urine N (UN) and MN outputs over NI, while the highest FN/NI was produced by growing cattle, and the highest UN/NI and MN/NI were with dry cows. The UN accounted for less than 50% of MN with calves, but with growing cattle and adult cows this value was over 50%. A similar result was also observed when using the linear regression to compare NER, when intercepts were fixed as calculated endogenous faecal and urine N outputs (Table 2). Dry cows excreted more (60.8%) consumed N in manure than calves, growing cattle and lactating cows, and calves had lowest N losses. The slope with lactating cows is lower than growing cattle although the difference does not reach significance.

**Table 1:** Nitrogen excretion of dairy cattle at different physiological states

	Calf	Growing cattle	Lactating cow	Dry cow	s.e.d.	P
FN/NI	0.249 <sup>a</sup>	0.318 <sup>d</sup>	0.306 <sup>c</sup>	0.267 <sup>b</sup>	0.0062	< 0.001
UN/NI	0.244 <sup>a</sup>	0.449 <sup>c</sup>	0.396 <sup>b</sup>	0.634 <sup>d</sup>	0.0121	< 0.001
MN/NI	0.493 <sup>a</sup>	0.767 <sup>c</sup>	0.702 <sup>b</sup>	0.901 <sup>d</sup>	0.0123	< 0.001
UN/MN	0.490 <sup>a</sup>	0.580 <sup>b</sup>	0.560 <sup>c</sup>	0.697 <sup>d</sup>	0.0086	< 0.001

<sup>a,b,c,d</sup> Means within a row with different superscript letter differ significantly (P < 0.05).

**Table 2:** Comparison of manure N output in dairy cattle at different physiological states

	Equation	R <sup>2</sup>	RMSE
Calf	MN = 0.336 <sup>a</sup> (0.0600) * NI + 12.4	0.92	7.0
Growing cattle	MN = 0.562 <sup>b</sup> (0.0087) * NI + 37.1	17.8	
Lactating cow	MN = 0.550 <sup>b</sup> (0.0021) * NI + 77.8	40.6	
Dry cow	MN = 0.608 <sup>c</sup> (0.0106) * NI + 37.7	24.9	

<sup>a,b,c</sup> Means of slope with different superscript letter differ significantly (P < 0.05).

**Conclusion** Dairy calves can utilise feed N more efficiently than growing cattle and adult cows, and lactating cows have a lower proportion of N excretion in faeces and urine than growing cattle. The present result can help dairy producers budget N input and output for achievement of sustainable and environment-friendly dairy production.

**Acknowledgements** This project was funded by the Department of Agriculture, Environment and Rural Affairs of Northern Ireland (17-S-267).

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# A decision support system for sustainable meat sheep production

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**Application** The iSAGE decision support system (DSS) helps meat sheep farmers to make annual management planning decisions by testing future scenarios. It promotes profitability and production optimisation for sustainable farming.

**Introduction** Meat sheep production in Europe is faced with great challenges. The sector is characterised by low incomes and increased production costs (European Parliament, 2018). The majority of farmers do not follow an established management plan, thus operating inefficiently and depending heavily on subsidies to remain economically sustainable (Dýrmundsson et al., 2006; Bernués et al., 2011). Moreover, there is a shortage of suitable and user-friendly tools to facilitate decision making by farmers. Hence, the objective was to create a model-driven DSS for sustainable meat sheep production. Material and methods Data were obtained for the United Kingdom (UK) from the Agriculture and Horticulture Development Board and the National Sheep Association and from Oviaragón - Pastores Grupo Cooperativo for Spain. The data were used to set default values and acceptable ranges for all the important parameters that characterise a meat sheep farm in UK and Spain, respectively. Such parameters included flock size, meat production, grazing, feeding, costs and product prices. An algorithm was developed based on energy and protein requirements of different categories of sheep (lambs, ewes and rams), according to their production stage. The idea was to assess nutritional management and its impact on production and economic performance. The equations for calculating nutrient requirements of sheep were obtained from existing literature using the Agriculture and Food Research Council system (AFRC, 1993). A cloud-based DSS was developed allowing users to input data to designated web forms. Data input is checked for correctness and then compared with theoretical minimum and maximum limits per category. The data of each farm are centrally collected and stored securely on a cloud server. These data are processed with a model algorithm, which provides results that serve as a guide to management decisions.

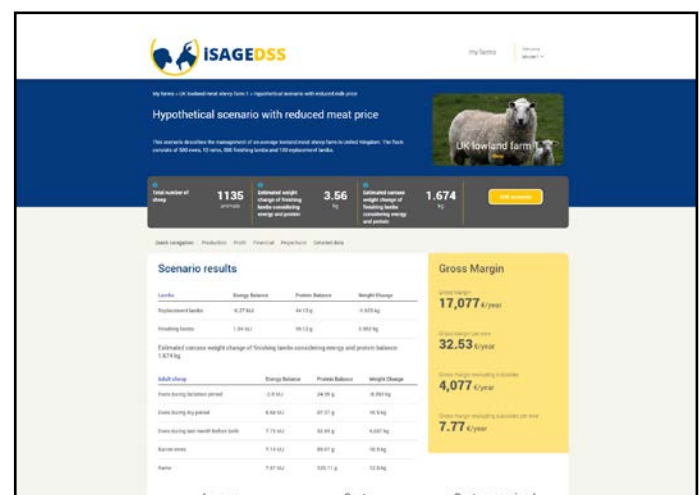
**Results** The developed DSS provides action plans for farm management through simulations of future scenarios. The generated reports (Figure 1) are focused on profitability and productivity making the impact of management decisions explicit. Farm income, variable costs and gross margin are estimated taking into account production estimates such as live weight and carcass weight of finishing lambs based on their nutritional management. Moreover, the DSS provides comprehensible charts of income and cost analyses and generates projections of important farm parameters, such as flock size, in relation to gross margin. Finally, farmers are able to create and compare multiple scenarios and they are provided with human readable advice which helps towards testing more viable solutions.

**Conclusion** The iSAGE DSS focuses on efficient management of meat sheep farms. It allows users to test future management decisions by estimating both financial and production related figures. In this regard, it is expected to help farmers respond better to the challenges faced by the sector and remain sustainable.

**Acknowledgements** This work was undertaken as part of the iSAGE project (<https://www.isage.eu/>) with financial support from the European Union's Horizon 2020 research and innovation programme (Grant Agreement No 679302).

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**Figure 1** Section of the iSAGE DSS report page with estimated gross margin and production estimates based on energy and protein balance.

# Redefining Key Performance Indicators for sheep production systems

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**Application.** Quantification of information values associated with sheep KPIs can help define what information should be recorded, and need not be recorded, to improve system-wide productivity and profitability.

**Introduction.** Growing global population and wealth are driving an increasing demand for livestock products (Hayes et al., 2013). Given that competition for alternative land-use limits the expansion potential for livestock production (Wilkinson and Lee, 2018), these demands can only be met through improvements in livestock production efficiency. As with many European countries, sheep farming in the UK has long been associated with low profit margins with low efficiency (Lima et al., 2018). To overcome this issue, various metrics to assist on-farm decisions, generally referred to as Key Performance Indicators (KPIs), have been proposed (AHDB, 2019). However, as the list of KPIs has grown, so has confusion amongst commercial producers regarding which measurements should be prioritised given their time and budgetary constraints. Using 5-year (2014–2018) high resolution data from the North Wyke Farm Platform BBSRC National Capability in Devon, UK, this study constructed a framework for ranking existing standard KPIs based on their information value for UK sheep producers.

**Material and methods.** Based on an extensive literature search, a complete list of animal-level KPIs in common usage was compiled and separated into performance predictors and performance outcomes. The information value of each performance predictor was then defined as the difference in realised performance outcome between the top third (high) and bottom third (low) groups, when ordered by predictor variable, with differences between groups assessed using independent samples T-test. This difference represents the economic benefit of an animal's improvement from low to

high group under each predictor. Both lambs' pre-weaning weights and the ewe's pre-weaning body scores (BCS) were considered as potential performance predictors. Calculations were repeated twice, initially with the total carcass value and secondly with carcass value divided by finishing age, the latter to account for variable (non-capital) production cost. Finally, these values were compared against the cost of measuring each predictor, composed of physical cost (financial cost of equipment necessary to collect the data) and labour cost (time taken to collect and evaluate the data), to rank all predictors according to their cost effectiveness. Physical cost and labour time were sourced from the NWFP's farm management records, with the latter converted to monetary value using the governments recommended agricultural workers wage.

**Results.** Lambs' pre-weaning weights and ewes' pre-weaning BCS were both shown to be positively (and largely significantly) correlated with realised carcass values (Figure 1). Ewe-level performance indicators showed the highest information value when the benefit was defined as gross carcass value, with body condition score (BCS) at lambing valued at £3.17. When the cost of production was considered, however, lamb-level growth metrics presented a higher information value, with weaning weight valued at £0.23 per day. This indicates that, in situations where pasture utilisation is fully optimised, information obtained from lambs' early life may be more valuable than that obtained from their mothers.

**Conclusion.** This study demonstrated that measuring carefully selected performance predictors can provide an efficient way to improve the profitability of sheep systems, provided that on-farm options are available to manipulate these predictors earlier in the animal's lifecycle. To the best of our knowledge, this is the first attempt to define the relative values of KPIs based on quantitative evidence.

## Acknowledgements

This work was funded by AHDB (61100030) and BBSRC (BBS/E/C/000J0100 and BBS/E/C/000I0320).

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	Outcomes		Benefit	
	Slaughter age (days)	Carcass score (%)	Carcass value (£)	Carcass value/day (£)
Birth weight	-37.58 ***	-0.35	1.90 ***	0.13 ***
Weaning weight	-77.42 ***	5.41 **	0.41	0.23 ***
DLWG birth to wean	-74.66 ***	8.39 ***	0.25	0.22 ***
Ewe BCS at lamb	-8.70 **	0.40	3.17 ***	0.05 ***
Ewe BCS at wean	-10.34 ***	-2.45	2.39 ***	0.06 ***
Ewe BCS at tupping	13.28 ***	-4.77 .	1.96 ***	-0.02
Ewe BCS change tup to lamb	-17.03 ***	3.57	1.52 **	0.06 ***
Ewe BCS change lamb to wean	1.92	-4.58 *	0.08	0.01
Ewe BCS change tup to wean	-17.03 ***	-0.24	-0.23	0.05 ***

Signif. codes: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; .  $P < 0.1$

**Figure 1.** Correlations between various predictors and outcomes (left), and economic benefits of measuring these predictors (right).

# What role do preventative footbath regimes have on the prevalence of antimicrobial resistant bacteria on the ovine interdigital skin?

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**Application** Understanding the role that non-antibiotic biocidal footbaths play in co-selection for antibiotic resistance allows informed decision making on how to best prevent lameness in sheep.

**Introduction** Lameness is a significant problem for the UK sheep industry, with approximately 2/3 of the industries total antibiotic use being prescribed for infectious lameness diseases. The overuse of antibiotics in both human and animal medicine has intensified selective pressure on antibiotic resistance in bacteria, impacting future food security and human health. Farmers and veterinarians are under increasing pressure to reduce their antibiotic use, meaning more emphasis will be placed on preventative measures. Preventative antimicrobial footbaths are commonly used to reduce the spread of bacteria, however their effect on the prevalence of antibiotic resistance in the bacterial population of the ovine hoof is unknown. The three most common antibiotic classes used in the sheep industry are tetracycline, penicillin & aminoglycoside, in this context the aim of the present study was to investigate the proportion of antibiotic resistance bacteria to these commonly used antibiotics on the ovine interdigital skin, before and after the application of different footbath regimes.

**Materials and Methods:** Bacterial swabs of the interdigital skin were taken at multiple time points before and after the application of: (1) single footbath (2% glutaraldehyde) using six randomly selected sheep from an outdoor flock and (2) once weekly footbaths for six weeks, using six sheep from an indoor flock and another six sheep as non-footbath control from the same flock. Swab samples were plated on MacConkey agar in the presence or absence of tetracycline, ampicillin and spectinomycin (latter for indoor study only) and followed by bacterial counts.

**Results** A transient decrease ( $2 \times 10^5$ ) in the total number of bacteria on the ovine interdigital skin was observed immediately after a single footbath. This was accompanied by an approximate 10-fold (0.01-0.2%) increase in the proportion of tetracycline resistant bacteria, recovering to pre-footbath levels after 10 days. The proportion of ampicillin resistance was not affected by the single footbath.

The proportion of tetracycline resistant bacteria only marginally increased by 2 & 3% over the 6 weeks in both, footbath and non-footbath control feet respectively. The proportion of spectinomycin resistant bacteria increased by 18% over 6 weeks in both, footbath and non-footbath control feet. No ampicillin resistant bacteria were found in the indoor flock, either before or after footbathing.

**Summary:** Although a single footbath transiently increased the proportion of tetracycline resistant bacteria, the weekly footbath did not lead to a significant overall increase in the prevalence of tetracycline resistant bacteria. The steady increase in the prevalence of spectinomycin resistant bacteria could have been due to the footbath selecting for the resistant bacteria with cross-contamination between the footbath and control groups as the sheep were housed together. Alternatively, this increase could have been due to the indoor housing environment allowing spectinomycin resistant bacteria to thrive, or a combination of the two. Resistance to spectinomycin is of concern as this antibiotic is commonly used to treat neonatal lambs with colibacillosis (watery mouth). Further studies are required to better understand the effects of preventative footbathing.



# Genetic control of CT-measured body dimensions in Texel rams that could be associated with lambing ease

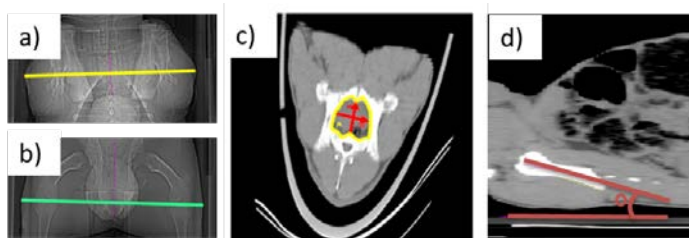
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**Application** Novel traits associated with shoulder, hip and pelvic regions of ram lambs were all found to be heritable. Further investigation to assess their association with lambing ease would be beneficial to reduce levels of dystocia at lambing time. Introduction Lamb losses at lambing time due to dystocia can have a significant impact on animal welfare and flock productivity. New information available from computer tomography (CT) scans has allowed a further set of novel traits to be investigated for the first time, relating to shoulder, hip and pelvic measurements of Texel sheep. The potential relationships between these traits (measured in male lambs from routine CT scanning) and dystocia (of lambs being born or their female relatives giving birth) is of particular interest based on farmer feedback and previous research conducted at both SRUC and elsewhere. The aim of this study was to estimate preliminary genetic parameters for these new CT traits prior to assessing their relationship with lambing difficulty records.

**Material and methods** Data was available for 437 commercial Texel ram lambs, CT scanned by SRUC between 2000 and 2015. The traits investigated included: Hip width (HW), Shoulder width (ShW), Pelvic opening width (PW), height (PH), area (PArea) and Pelvic angle (PAng) – the angle of the pelvis relative to the table, as the lambs lay on their back (Figure 1).



**Figure 1** Computer tomography (CT) images of Shoulder width (a); Hip width (b); Pelvic area, height and width (c) and Pelvic angle (d) measurements.

Genetic analyses were performed using ASReml (Gilmour et al., 2009), to estimate heritabilities for these traits. The animal model fitted was: CT Trait = CT live weight + litter size + dam age + birth year + flock. Where, live weight at CT scanning was fitted as a covariate. Litter size that the lamb was reared in (2 levels; 1 and >2) and age of the lamb's dam at lambing (4 levels; 2 to >5), birth year (14 levels) and flock (58 levels) were fitted

as fixed effects. Each fixed effect was significant for the majority of traits and to remain consistent, the same models were fitted across the different traits. The full Texel pedigree was pruned using the RelaX2 pedigree analysis programme (Stranden and Vuori, 2006) to retain only information required for estimating variance components. The pedigree file used included sire and dam information for 32,168 animals.

**Results** Heritabilities estimated were low to moderate in magnitude, ranging from 0.16-0.65, with standard errors ranging from 0.15-0.17. The highest estimates were associated with the pelvic traits, particularly PArea, PAng and PW (all >0.50) (Table 1).

**Table 1** Genetic parameters (genetic variance, VA; phenotypic variance, VP; heritability, h<sup>2</sup>) for the new computer tomography (CT) traits (s.e. in parenthesis).

Trait	VA	VP	h <sup>2</sup>
Hip Width (HW; mm)	10.87	47.12 (3.93)	0.23 (0.17)
Shoulder Width (ShW; mm)	19.73	127.11 (10.32)	0.16 (0.15)
Pelvic Width (PW; mm)	12.82	19.63 (1.92)	0.65 (0.16)
Pelvic Height (PH; mm)	8.11	20.83 (1.93)	0.39 (0.17)
Pelvic Area (PArea; mm <sup>2</sup> )	108493	199250 (18688)	0.54 (0.15)
Pelvic Angle (PAng; o)	7.34	13.16 (1.27)	0.56 (0.17)

**Conclusion** These preliminary results indicate that the traits investigated, from the CT scans of ram lambs, are under genetic control. The highest heritability estimates were associated with the pelvic measurements, suggesting substantial scope for future genetic selection. The next stage of the study will be to increase the number of animals with these traits measured and assess the relationships between these and dystocia (at the lamb or ewe level), as assessed by lambing difficulty records.

**Acknowledgements** The authors are grateful for the financial support from the Scottish Government's Rural and Environmental Science and Analytical Services Division. Many thanks also to the Texel Sheep Society, AHDB's Signet Sheep Breeding Services and staff at SRUC's CT Scanning service.

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- Figure/image 1

# The effect of breed type and silage type offered during late gestation on ewe and lamb performance

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**Application** Ewes receiving whole crop wheat (WCW) silage had similar metabolisable energy (ME) intake to the ewes receiving grass (G) silage but apparent increased energy utilisation. Mule ewes had lower ME intake compared to both the Belclare X and Lley X but had increased apparent efficiency of ME utilisation.

**Introduction** Number of lambs born is a key factor influencing the profitability of seasonal sheep meat production systems (Bohan et al., 2018). A second is the ewe's ability to utilise intake of home grown forage into energy for both her maintenance and the growing foetus. The foetus undergoes ninety percent of all growth in the final third of gestation (Redmer et al., 2004), so high energy and protein levels are essential during this period and grass silage alone cannot meet these needs as foetal growth restricts the space available for the rumen (Kaske & Growth, 1997). A novel feed source, WCW silage which is becoming more readily available in Ireland is now a possible winter feed for sheep in the final weeks of gestation. The objective of this study was to examine WCW silage versus traditional G silage for prolific ewes during late gestation on ewe intake and performance.

**Material and methods** A 2 × 3 factorial design experiment involving 90 twin-bearing ewes was conducted during the final eight weeks of pregnancy with ewes offered 100% of predicted ME requirements (AFRC, 1993; Robinson et al. 2002). One (n=45) of two silage types, G or WCW, were offered to three (n=30) prolific breed types, (Mule [Bluefaced Leicester X Blackface Mountain], Belclare X and Lley X). Concentrates were offered as required to achieve 100% of ME requirements to individually penned ewes. Predicted ME was calculated using the ewe's mature live weight, day of gestation and predicted combined litter weight (CLW) (11kg). Feed intake was recorded daily with water intake recorded on three x 48-hour periods. Body condition score (BCS) was recorded on day 91 of gestation and at parturition. CLW was recorded at parturition. Ewe apparent efficiency of energy utilisation was estimated as per the equation: (Ewe predicted required daily ME intake for actual CLW / Ewe actual daily ME intake) \*100.

**Statistical analysis** Data was analysed using repeated measures (Feed and water intake as well as weekly apparent ewe efficiency of energy of utilisation) and "proc glimmix" (BCS percentage changes, CLW and lamb average daily gain) in SAS (Version 9.4) and it was checked for normality using "proc univariate". Model selection via linear regression was set at P<0.25.

**Results** Ewes offered WCW silage had significantly lower water intake from within feed (P<0.001) and significantly higher voluntarily water intake (P<0.001) in comparison to ewes offered G silage. Ewes offered WCW silage had greater apparent efficiency of energy utilisation compared to ewes offered G silage (P<0.01). Mule ewes consumed significantly less ME per day than other breed types (P<0.05). However, Mule ewe's apparent efficiency of energy utilisation was greater than other breed types (P<0.01). WCW silage ewes produced lower CLW (P<0.05). Diet by breed interactions showed Mule ewes offered WCW silage produced a lower CLW than Mule ewes offered G silage (P<0.01). Within silage type, Mule ewes offered G silage had

a higher CLW than Belclare X ewes offered G silage (P<0.05) while within WCW silage Belclare X ewes had a higher CLW than Lley X ewes (P<0.05).

**Conclusion** Ewes had greater apparent efficiency of energy utilisation when offered WCW silage but subsequently increased voluntary water intake. Mule ewes consumed lower ME levels but had higher apparent efficiency of energy utilisation in this experiment.

**Acknowledgements** The authors gratefully acknowledge funding from the Irish Research Council and Germinal Ireland LTD for this experiment.

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**Table 1** The effect of silage type and breed on intake and animal performance (LS Means ± SEM)

	Silage type			Breed			
	G	WCW	SEM	Belclare	Lley	Mule	SEM
<b>Water intake (litres/day)</b>							
<b>From fed</b>							
Day 112-118	3.55c,x	1.82d,x	0.098	2.81x	2.81x	2.45x	0.120
Day 126-132	3.51c,x	1.57d,y	0.116	2.71a,x	2.77a,x	2.13b,y	0.141
Day 140-147	2.73c,y	1.29d,y	0.153	2.01y	2.02y	1.99y	0.187
SEM	0.081	0.082		0.100	0.970	0.100	
Mean	3.34c	1.72d	0.078	2.63a	2.63a	2.34b	0.096
<b>Voluntary</b>							
Day 112-118	0.62c,x	2.82d,x	0.305	1.73x	2.08x	1.35x	0.374
Day 126-132	1.99c,y	4.59d,y	0.389	3.23y	3.81y	2.82y	0.476
Day 140-147	2.84c,z	3.98d,z	0.382	3.26y	4.07y	2.88y	0.468
SEM	0.210	0.212		0.261	0.251	0.262	
Mean	1.81c	3.8d	0.316	2.74ab	3.32b	2.35a	0.387
<b>Total intake (feed + voluntary)</b>							
Day 112-118	4.16x	4.64x	0.380	4.53x	4.88x	3.80x	0.465
Day 126-132	5.46y	6.16y	0.381	5.93ab,y	6.58a,y	4.92b,y	0.466
Day 140-147	5.56y	5.26x	0.380	5.27xy	6.10y	4.86y	0.465
SEM	0.227	0.229		0.282	0.272	0.284	
Mean	5.06	5.35	0.331	5.24ab	5.85b	4.53a	0.405
<b>Feed</b>							
Mean daily DM intake (kg)	1.22	1.24	0.033	1.30a	1.26a	1.13b	0.041
Mean daily ME intake (MJ/ME/day)	13.7	13.5	0.33	14.2a	13.8a	12.8b	0.40
Mean ME from forage (%)	81.3c	75.8d	1.44	79.6ab	80.6a	75.5b	1.76
Mean apparent efficiency of energy utilisation (%)	106.4c	122.6d	5.01	106.9a	107.2a	129.6b	6.14
<b>Water intake (per kg DM)</b>							
Day 112-118	3.7	4.2	0.29	3.7	4.3	3.7	0.36
Day 126-132	3.6	4.3	0.23	3.8	4.3	3.8	0.28
Day 140-147	4.0	4.1	0.31	3.6	4.5	4.2	0.38
SEM	0.18	0.18		0.22	0.21	0.22	
Mean	3.8	4.2	0.23	3.7	4.4	3.9	0.29
<b>Animal performance</b>							
Ewe BCS change Day 91 of gestation to parturition (%)	-9.4	-8.2	3.48	-9.7	-5.6	-11.0	4.26
Combined litter weight (kg)	10.47c	9.83d	0.279	10.22	9.86	10.36	0.342
Lamb average daily gain Birth to 6 weeks old (g/day)	261	252	8.4	264	245	260	10.3
Lamb average daily gain Birth to weaning (g/day)	242	237	5.5	240	236	243	6.9

a,b: between breed, means with different superscripts are significantly different P<0.05  
c,d: between silage type, means with different superscripts are significantly different P<0.05  
x,y,z: within column, means with different superscripts are significantly different P<0.05

# The effect of breed type and silage type fed during late pregnancy on ewe colostrum yield and composition

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**Application** Ewes offered whole crop wheat (WCW) silage produced colostrum with an elevated concentration of total solids compared to ewes offered grass (G) silage. Mule ewes produced a lower yield of colostrum with elevated total solids content compared to Belclare and Lleyen ewes.

**Introduction** Timely production of adequate volumes of high-quality colostrum is essential for lamb survival and subsequent lamb growth rate (Dwyer et al., 2016) which in turn influences the profitability of sheep meat production systems. Late pregnancy nutrition and the associated nutrient intake are key drivers of colostrum production (McGovern et al., 2015) with breed also having a major influence on colostrum production and quality (Campion et al., 2019). Grass silage is the predominant winter feed offered in Irish production systems (Keady & Hanrahan, 2008) but alternative feedstuffs such as WCW are increasingly available. Nutrient requirements to support mammogenesis and lactogenesis increase in the final eight weeks of ovine gestation and in housed systems this necessitates supplementation with concentrate feeds. The objective of this study was to determine the impact of feeding WCW vs G during late pregnancy and of ewe breed type on colostrum yield and composition.

**Material and methods** A 2 × 3 factorial design experiment involving 90 twin-bearing ewes was conducted during the final eight weeks of pregnancy with ewes offered 100% of predicted metabolisable energy (ME) requirements. One (n=45) of two (GS or WCW) silage types were offered to three (n=30) prolific breed types, (Mule [Bluefaced Leicester X Blackface Mountain], Belclare X and Lleyen X). This resulted in six treatments (n=15). When silage intake was insufficient to meet 100% of predicted ME requirements on an individual animal basis, concentrates were offered to reach 100% of predicted ME allocation per ewe per day. At the point of parturition an udder cover was placed on each ewe to prevent lambs suckling for the first twenty-four hours post-partum. Colostrum yield was measured at one, ten and eighteen hours post-partum, with samples collected at each timepoint for compositional analysis. Lambs were fed colostrum via stomach tube at a rate of 20-50ml/kg birth weight depending on colostrum yield of the dam. Colostrum compositional analysis was completed using wet chemistry techniques for total solids (fat, protein, lactose & ash), crude protein and ash.

**Statistical analysis** Data was analysed using repeated measures and "proc glimmix" in SAS (Version 9.4) and it was checked for normality using "proc univariate". Total solids, crude protein, ash and yield per timepoint of the colostrum were analysed using repeated measures. The total colostrum yield over the eighteen-hour period was analysed using "proc glimmix". Model selection via linear regression was set at P<0.25.

**Results** Ewes offered WCW silage produced similar volumes of colostrum to ewes offered G silage (P>0.05). There were elevated average total solids (P<0.05) and crude protein (P<0.05) concentrations in the colostrum of the ewes offered WCW silage over the first eighteen-hours. Breed did not impact on colostrum volume at individual timepoints. Belclare X ewes produced a significantly larger volume of colostrum over the combined eighteen-hour period than Mule ewes (P<0.05) but the colostrum from Mule ewes had elevated concentrations of total solids and crude protein compared to colostrum from either the Belclare X (P<0.05) and Lleyen X (P<0.01) ewes, respectively. No interactions between breed type and silage type were detected.

**Table 1** The effect of silage type and breed on colostrum yield and composition (LS Means ± SEM)

		Silage type			Breed			
		G	WCW	SEM	Belclare	Lleyen	Mule	SEM
<b>Colostrum yield (ml)</b>								
	1 hr	541 <sup>x</sup>	543	73.6	635	602	389 <sup>x</sup>	90.0
	10 hr	692 <sup>y</sup>	628	54.5	708	623	650 <sup>y</sup>	66.6
	18 hr	683 <sup>y</sup>	618	51.2	672	610	669 <sup>y</sup>	62.7
	SEM	44.3	45.7		71.2	54.2	56.2	
	Total	1953	1763	137.9	2011 <sup>a</sup>	1895 <sup>ab</sup>	1668 <sup>b</sup>	168.9
<b>Colostrum composition (%)</b>								
Total solids	1 hr	38.2 <sup>a</sup>	41.1 <sup>x</sup>	1.70	37.4 <sup>x</sup>	37.6 <sup>x</sup>	44.0 <sup>x</sup>	2.09
	10 hr	29.7 <sup>xy</sup>	34.0 <sup>dy</sup>	1.44	31.0 <sup>aby</sup>	29.2 <sup>xy</sup>	35.3 <sup>by</sup>	1.76
	18 hr	23.3 <sup>z</sup>	25.8 <sup>c</sup>	1.09	23.5 <sup>z</sup>	23.0 <sup>z</sup>	27.2 <sup>z</sup>	1.34
	SEM	0.88	0.92		1.09	1.07	1.16	
	Mean	30.4 <sup>c</sup>	33.6 <sup>d</sup>	1.23	30.6 <sup>a</sup>	30.0 <sup>a</sup>	34.5 <sup>b</sup>	1.51
<b>Crude protein</b>								
	1 hr	19.7 <sup>a</sup>	21.2 <sup>x</sup>	0.74	20.0 <sup>x</sup>	19.2 <sup>x</sup>	22.1 <sup>x</sup>	0.91
	10 hr	12.7 <sup>y</sup>	14.6 <sup>y</sup>	0.73	13.0 <sup>aby</sup>	12.3 <sup>xy</sup>	15.6 <sup>by</sup>	0.89
	18 hr	7.8 <sup>z</sup>	8.9 <sup>z</sup>	0.73	8.0 <sup>z</sup>	7.7 <sup>z</sup>	9.3 <sup>z</sup>	0.90
	SEM	0.49	0.51		0.60	0.59	0.64	
	Mean	13.4 <sup>c</sup>	14.9 <sup>d</sup>	0.61	13.7 <sup>a</sup>	13.1 <sup>a</sup>	15.6 <sup>b</sup>	0.75

No silage x breed interactions occurred at any of the three timepoints.

a,b: denotes significant difference between breed P<0.05

c,d: denotes significant difference between silage type P<0.05

x,y,z: denotes significant difference between timepoints P<0.05

**Conclusion** Offering WCW silage increased total solid concentrations in colostrum for the first eighteen hours post-partum. Mule ewes produced lower volumes of colostrum, but their colostrum had elevated solid concentration across the first eighteen hours post-partum.

**Acknowledgments** The authors gratefully acknowledge funding from the Irish Research Council and Germinal Ireland Ltd. for this experiment.

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# Effects of age at first joining and ewe genotype, of ewes aged from 2 to 6 years, on lamb performance

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**Application** Prolific ewe genotypes (Belclare, Belclare×Suffolk) produce lambs that are drafted for slaughter at a younger age than lambs from ewes with >75% Suffolk ancestry. Lambs from crossbred ewes grow faster and are drafted at a younger age reflecting significant heterosis.

**Introduction** Replacements are a major cost in prime lamb production (Keady 2014). Litter size is a key determinant of efficiency of prime lamb production (Keady and Hanrahan 2006) and Keady and Hanrahan (2019) concluded that prolific ewe genotypes, when joined to lamb at 1 year of age, yield an extra lifetime output of at least 2 lambs reared per ewe joined. Keady et al. (2018) concluded that, over 12 years, high levels of lamb performance are consistently achievable from grass-based systems of prime lamb production and that all lambs were slaughtered (mean carcass weight 19.9 kg) prior to the end of the grazing season (early December) in the absence of concentrate supplementation (except for triplets pre-weaning). The aim of the current study was to evaluate the effects of age at first joining (~7 or 19 months) and ewe genotype, over the ages 2 to 6 years at lambing, on the performance of their lambs from grass-based system of lamb production.

**Material and methods** A total of 424 ewes [157 Belclare (B), 114 Belclare×Suffolk (BS), 153 Suffolk type (>75%S); 2 cohorts] were joined, annually, with Charollais rams, starting at 19 months of age. A random 50% of each genetic group had been joined at ~7 months, of which 85% produced lambs at 1 year. Ewes

left the flock when culled for natural reasons (e.g., udder and mouth issues) or when they died. Ewes lambed (late Feb – early Mar) indoors and a total of 2,544 lambs were born. Male lambs were reared entire. Ewes were put to pasture within 2 days of lambing. Ewes rearing singles or twins received no concentrate supplementation while those rearing triplets received concentrate (0.5 kg/d) for 5 weeks post lambing, and their lambs had access to concentrate (up to 300 g/head daily) until weaning. From weaning (at 14 weeks of age) until slaughter all lambs were managed as one flock and offered grazed grass as the sole diet. All lambs were slaughtered prior to the end of the grazing season. The data were analysed using the Proc MIXED of SAS.

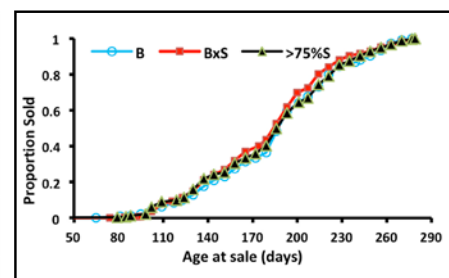
**Results** The effects of age at first joining and ewe genotype on lamb performance are presented in Table 1. There were no age-at-first-joining by ewe-genotype interactions ( $P > 0.05$ ). Ewes joined at 7 months produced heavier lambs at birth ( $P < 0.01$ ). Differences among ewe genotypes were significant ( $P < 0.01$ ) for all lamb growth traits, except weight at slaughter, and the differences were due in all cases to the superiority of the B×S dams (heterosis test; Table 1). Differences between B and >75%S were not significant ( $P > 0.05$ ). The effects of age at first joining and ewe genotype on lamb drafting pattern are in Figure 1. Progeny of B×S ewes were drafted at a younger age than lambs from the other 2 genotypes.

**Conclusion** Ewe age at first joining (7 or 19 months) had no adverse effect on the performance of their lambs from lambings at 2 to 6 years of age. Lambs born to B×S ewes had higher daily gain and were drafted for slaughter at a younger age than lambs from the other 2 genotypes, which did not differ.

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		First joining (FJ)			Ewe genotype (G)			Sig.		
		7 month	19 month	s.e.	B	B×S	>75%S	s.e.	FJ	Heterosis
Weight (kg)	birth	4.8	4.6	0.17	4.6	4.8	4.7	0.17	**	**
	weaning	33.0	32.7	0.28	32.5	33.7	32.2	0.32	NS	**
	slaughter	48.5	48.5	0.33	48.5	48.2	48.7	0.34	NS	NS
ADG (g/d)	0 – 5 weeks	302	302	5.5	300	311	295	5.9	NS	**
	5 – 14 weeks	286	286	5.9	276	285	271	6.3	NS	**
Carcass weight (kg)	21.1	20.9	0.39	21.1	20.9	21.0	0.39	NS	NS	
Age at slaughter (days)	176	177	4.2	177	171	181	4.3	NS	**	



**Figure 1** Lamb drafting pattern by ewe genotype

**Table 1** Effects of age at first joining and ewe genotype on lamb performance



# The effect of rotational paddock grazing on lamb performance

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**Application** Grazing lambs in a 4 paddock system results in higher lifetime average daily gain and lower days to slaughter than an 8-paddock system.

**Introduction** Grass utilisation is one of the key factors influencing profitability of grass-based livestock systems and currently there are inefficiencies in the level of herbage utilized ha<sup>-1</sup> within sheep production systems (Creighton, 2015; Earle et al., 2017). Several authors indicate that rotational grazing delivers higher levels of animal and grassland performance than set stocking (Sharrow, 1983); the question still remains on what is the optimal number of paddocks within a rotational system for animal performance and grass utilisation. This study set out to address this knowledge gap through examining the effect of 4 versus 8 paddock rotational grazing systems on animal and grassland performance.

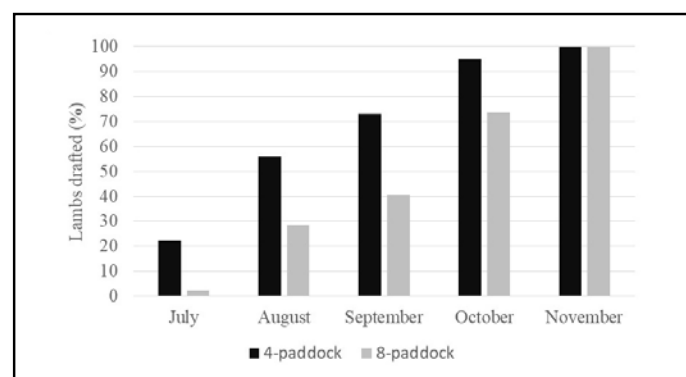
**Material and methods** The present study was conducted at AFBI, Hillsborough from April to November 2018, using a predominantly *Lolium perenne* sward. The study comprised of two grazing treatments (4 vs. 8 paddock rotational grazing system) which were balanced for ewe live weight, body condition score and lamb sire breed. Each system consisted of 1.6 ha which were rotationally grazed at a stocking rate of 14 ewes ha<sup>-1</sup> which equated to 22 twin-rearing ewes per treatment. The target pre-grazing cover was 2600 kg DM ha<sup>-1</sup> (7-8cm) for the duration of the experiment and the target post-grazing cover was 1600 kg DM ha<sup>-1</sup> (4cm) on both treatments. On average, the 8 paddock system rotated every 3-4 days, whereas, the 4 paddock system rotated every 7-8 days. Pre- and post-grazing compressed sward heights were taken on each paddock before and after grazing by taking 30 measurements across the diagonal of the paddock with a rising plate meter (Jenquip, New Zealand). Herbage mass was also recorded before and after each grazing by taking four quadrat (0.5 x 0.5m) cuts to ground level with Gardena hand shears. The harvested herbage was weighed and retained for dry matter determination. Herbage compositional quality was analysed at each grazing using near infrared reflectance spectroscopy. Lambs were weaned on average at 14 weeks of age, with a leader follower grazing system operated post-weaning. Lambs were weighed fortnightly using portable electronic scales (Shearwell, UK) and were drafted for slaughter on average at 45kg of liveweight. Data was analysed using linear mixed models in GenStat with ewe as a random effect and lamb breed, gender and treatment as fixed effects. Differences were considered significant at P<0.05.

**Results** Lambs grazing the 4-paddock rotational system had higher ADG from birth to weaning (P<0.001) compared to those grazing the 8-paddock system and this was mostly driven by higher ADG from 10 to 14 weeks of age. This resulted in higher weaning weights (P<0.01) for lambs on the 4-paddock rotational system and a higher lifetime average daily gain (P<0.001) compared to lambs on the 8-paddock system (Table 1). Subsequently, the 8-paddock lambs required longer to reach slaughter (P<0.001) but no differences in lamb drafting weight and carcass traits were observed. In the 4-paddock system, 95% of lambs were drafted for slaughter by October, in comparison the 8-paddock system only had 75% of lambs drafted (Figure 1). Grazing system had no significant effect on grass quality in terms of CP, ME, DM, ADF and WSC.

**Table 1** Effect of treatment on lamb performance, days to slaughter and carcass traits.

Variables	Paddock System		SED	Sig.
	4	8		
Pre-grazing height (cm)	9.0	8.7	0.08	NS
Post-grazing height (cm)	4.4	4.4	0.15	NS
Weaning weight (kg)	30.2	27.5	9.73	**
Lifetime ADG (g/day)	223	190	8.40	***
Days to slaughter	178	211	7.87	***
Drafting weight (kg)	45.2	46.1	0.54	NS
Carcass weight (kg)	20.1	20.3	0.36	NS

**Figure 1** Cumulative lamb drafting pattern



**Conclusion** In the context of this study, lamb performance was higher on the 4-paddock system compared to the 8-paddock rotational grazing system, however, there was no effect on carcass traits between the systems.

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# Comparative study of the fatty acid profile of retail goat and cow milk in the UK

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**Application** Consumption of goat milk, as opposed to cow milk, can reduce the intake of undesirable saturated fats in the UK diets although this would also reduce the intake of the beneficial omega-3 fatty acids.

**Introduction** Milk and dairy products are a major source of saturated fatty acids (SFA) in the UK diets; although some SFA may show a positive effect on human health (C8:0, C10:0) (Haug et al., 2007), SFA intakes of the population are considerably higher than recommended (Bates et al. 2014). Milk also contains monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) which are beneficial to human health, including the MUFA oleic acid (OA) and vaccenic acid (VA), the conjugated PUFA ruminic acid (RA), and the omega-3 PUFA (n-3)  $\alpha$ -linolenic acid (ALNA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Haug et al. 2007; Stergiadis et al. 2012). Dairy goat farming has gained interest in the UK over the last 25 years and there are 40,000–45,000 goats, producing 34 million litres of milk annually (Milking Goat Association, 2019), but the fatty acid (FA) profile of retail goat milk in the UK is unknown. The aim of this study was to perform a comparative assessment of milk FA profile of goat and cow retail milk throughout the year.

**Material and methods** Pasteurised retail milk bottles from 4 cow milk and 3 goat milk brands were purchased monthly, over 12 months, from retail outlets at Reading, UK (n=84). Milk FA profiles were analysed by gas chromatography in a Varian CP-SIL 88 fused silica column (100 m x 0.25 mm internal diameter x 0.2  $\mu$ m film thickness) and 81 FA were identified using external

standards and published chromatograms. Analysis of variance, by linear mixed effects models in GenStat 17th edition, used species (cow, goat), month (March 2016 to February 2017), and their interaction as fixed factors; and milk brand as the random factor.

**Results** Goat milk contained more SFA than cow milk, mainly due to the higher contents of C8:0 and C10:0, while it contained less C14:0 and C16:0. Concentrations of VA were higher in cow milk than in goat milk although MUFA and trans FA concentrations were similar. Total n-3 contents, including EPA, were lower, while DHA contents were higher in goat milk. The latter also contained more LA and omega-6 PUFA (n-6). A genetic/physiological effect could be the main driver for the higher concentrations of C8:0, C10:0, C12:0 and the lower concentrations of C14:0 and C16:0, but a potential effect of husbandry practices may explain differences in n-3, n-6 and VA concentrations. UK dairy goat systems are typically intensive with housed animals all year round, fed low forage:concentrate ratios; while cow conventional herds (which contribute most of the cow milk at retail) would typically allow access to pasture for 4–6 months throughout the year. These practices in goat farming may reduce n-3, VA and EPA and increase n-6 contents in milk.

**Conclusion** When compared with cow milk, goat milk showed some nutritionally desirable characteristics (e.g. more C8:0, C10:0 and DHA and less C14:0 and C16:0) and some undesirable characteristics (e.g. higher content of C12:0, and lower content of VA and EPA). These differences may be explained by husbandry practices in goat dairy systems, and in particular the lower pasture intake and forage:concentrate ratio; while intrinsic metabolic differences between the species may also play a role. The implications of these differences on human health were not assessed in the present study.

**Acknowledgements** The authors acknowledge funding from the University of Reading and the help of Ms Carolina Berlitz, Mr Ben Hunt and Sneha Garg with milk collection and analysis.

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Table 1

Fatty acid group	Cow	Goat	P-value	Fatty acid	Cow	Goat	P-value	Fatty acid	Cow	Goat	P-value
SFA	68.8	70.3	*	C8:0	1.00	2.07	***	LA	1.71	2.61	*
MUFA	27.3	25.3	ns	C10:0	2.51	8.00	***	RA	0.59	0.47	ns
PUFA	3.97	4.38	ns	C12:0	3.33	4.16	*	ALNA	0.44	0.34	ns
n	0.79	0.54	*	C14:0	11.1	10.3	**	EPA	0.05	0.04	*
n	2.09	2.94	*	C16:0	33.1	30.3	*	DPA	0.08	0.08	ns
trans	1.91	1.74	ns	VA	1.22	0.72	**	DHA	0.01	0.02	**

Significant differences were declared at \*\*\*, P<0.001; \*\*, P<0.01; \*, P<0.05; ns, P>0.05.

# Identification of the origin of host DNA content in the gastrointestinal tract of the broiler chicken

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**Application** Assessment of CYTB DNA present in the digesta, which apparently originates from intestinal epithelial cell sloughing, potentially indicates gut health, which has an impact on animal performance.

**Introduction** We previously hypothesised that cytochrome b (CYTB) DNA present in faeces originates from sloughed epithelial cells from the gastrointestinal tract and demonstrated that a yeast inclusion diet led to significant reductions in the amount of pig CYTB DNA present in faecal samples (Slinger et al., 2019), suggesting that the yeast reduced gut cell sloughing. However, it is unclear where in the gastrointestinal (GI) tract the host DNA originates from. Insoluble fibre has been shown to increase levels of RNA in digesta of broilers (Jin et al., 1994). Therefore, the aim of this study was to determine the origin of the DNA within the GI tract and examine the effect of a 20% wheat bran diet on levels of chicken CYTB and bacterial 16S DNA present in the digesta.

**Material and Methods** Newly hatched broilers (n=36) were acclimatised on a control diet for 4 days and on the 4th day were either maintained on the control diet (n=18, 2 birds in 9 replicate pens) or assigned to a diet containing 20% wheat bran (n=18, 2 birds in 9 replicate pens). Birds and remaining feed in troughs were weighed at the end of each week and feed intake was calculated for the pen. Birds were culled over 2 days at either 33 or 34 days of age. Digesta contents were collected from the duodenum, jejunum, ileum and colon and immediately placed on dry ice and then stored at -70 until processed. DNA was extracted from digesta from 9 birds per dietary group (1 bird per pen). 200mg of digesta was homogenised in MagNA Lyser Green Bead Tubes (Roche) in the presence of Buffer ASL (Qiagen) and then centrifuged. Supernatant was processed using the QIAamp® DNA Stool Mini Kit (Qiagen) resulting in DNA which was assessed by the NanoDrop™ 2000 (Thermo Scientific). SYBR Green (Roche) quantitative PCR (qPCR) was carried out on a LightCycler® 480 (Roche) instrument to assess the DNA composition of the digesta. DNA diluted to 5ng/μl were used to create DNA pools which were used to produce a 1:4 dilution series and this was used for standard curves. Chicken genomic

DNA was detected using chicken CYTB primers. Bacterial DNA was detected using published 16S primers (Mieszkin et al., 2009). qPCR analyses of digesta DNA (chicken CYTB DNA and bacterial 16S DNA) were analysed by two-way (diet x gut section) ANOVA in Genstat 19th edition, blocking for plate, followed by Bonferroni post hoc tests. Significance = P<0.05. Results There was only a significant (P<0.001) effect of gut section on the chicken CYTB DNA in the digesta. The duodenum contained significantly (P<0.001) more chicken CYTB DNA compared to the other gut sections (Table 1). Similarly, there was only a significant effect of gut section (P<0.001) on the bacterial 16S DNA contents in the digesta. The digesta from the colon contained significantly more bacterial DNA (P<0.001) compared to the earlier gut sections (Table 1).

**Table 1** The effect of gut section on digesta DNA composition in the broiler GI tract.

Digesta DNA component	Gut section				SED	P value
	Duodenum	Jejunum	Ileum	Colon		
Chicken CYTB	11.355 <sup>a</sup>	0.418 <sup>a</sup>	0.111 <sup>a</sup>	1.274 <sup>a</sup>	1.053	<0.001
Bacterial 16S	0.00007 <sup>a</sup>	0.00402 <sup>a</sup>	0.00736 <sup>a</sup>	0.06502 <sup>a</sup>	0.01532	<0.001

**Conclusion** A diet high in insoluble fibre had no apparent effect on gut cell sloughing as determined by assessing chicken CYTB DNA concentrations in the digesta. The duodenum was the region of the GI tract where there was the apparent greatest levels of gut cell sloughing. The subsequent decrease in CYTB DNA could be due to decreased sloughing or to nuclease activity, but this needs to be verified. However, any nuclease activity does not completely remove CYTB DNA. The bacterial 16S DNA was highest in the colon, which was expected, since it follows the caeca where the majority of bacterial fermentation takes place.

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# Effect of olive tree pruning by-products on intake, digestibility, growth performance and blood parameters of Shami goats

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**Application** Olive twigs treated with 4% urea and 10% molasses could replace up to 48.5% of conventional diets of growing Shami goat kids reduce fattening costs by 80% for farmers in olive-livestock mixed systems.

**Introduction** Chronic feed shortages is a major constraint to livestock production, resulting in subsequent increase in feeding costs a decrease in profitability of livestock production systems. Thus, development of co-products into palatable, high nutritional value feed materials represents a key route to increase sustainability of animal production (Bampidis and Robinson, 2006). Olive leaves and twigs are an important feed resource in the olive-livestock mixed system, however, this by-product is still underutilised. Using this by-product as ruminant feed would contribute significantly decreasing feed deficit and decrease feeding cost. Accordingly, the objective of this study is to analyse the possibility of replacing traditional fattening rations by olive twigs and leaves treated with urea and molasses on growth performance, nutrient utilization and health of growing Shami kids.

**Material and methods** The effect of replacing wheat straw and a portion of commercial concentrate with olive tree pruning by-products (twigs and leaves) treated with urea and molasses on nutrient digestibility, growth performance and blood parameters was determined using a 117 day combined growth and digestibility trial. Forty-two intact Shami goat kids (20±2.47 kg live weight and 133±5.44 days of age) were randomly allotted to three dietary treatment groups (n=14 for each group). Three diets were formulated; Control was fed a conventional ration (75% commercial concentrate mixture and 25% wheat straw), OTU (62.3% concentrate mixture +37.3% olive twigs and leaves treated with 4% urea for 14 days), OTUM (51.5 % concentrate mixture +48.5% olive twigs and leaves treated with 4% urea for 14 days then sprayed by 10% molasses). Goat were individually fed. The study consisted of a growth trial of 100 days (10 days of adaptation and 90 days of data collection) followed by a digestibility trial of 17 days (10 days of adaptation and 7 days of a total faeces and urine collection). Blood samples were collected from the jugular vein for each kid during the growth trial for haematology analyses. R package was used to analyse

growth performance and metabolism data using one-way ANOVA while blood data was analysed using repeated measures design.

**Results** There was no significant difference ( $P>0.05$ ) between OTU and OTUM compared to the control on daily dry matter intake, digestibility of crude protein, ether extract and nutrient detergent fibre. Nitrogen intake, nitrogen excretion in urine and faeces and nitrogen balance was not significantly affected ( $P>0.05$ ) by dietary treatment. Nor did dietary treatment have any significant effect on average daily gain, feed conversion ratio or blood metabolites ( $P>0.05$ ).

**Table 1** Effect of inclusion of olive twigs treated by urea and molasses on digestibility, growth performance and blood haematology Shami goat kids

	Control	OTU	OTUM	SEM
Daily dry matter intake (g/Live weight <sup>0.75</sup> )	78	77.6	79	0.76
Dry matter digestibility	0.691	0.659	0.674	0.029
Nitrogen balance (g/day)	5.33	4.98	4.88	0.126
Average daily gain (g)	149	144	142	7.56
Feed conversion ratio	6.17	6.34	6.45	0.292
Albumin (g/l)	36.3	39.5	39.2	2.46
Total protein (g/l)	68	71.3	71.4	3.49
Alanine transferase (IU/l)	15.5	14.4	15.8	2.5
Aspartate transferase (IU/l)	64.5	75.1	75.9	4.79
Urea (mg/dl)	53.6	56.8	56.6	7.59
Glucose (mg/dl)	72.5	75.5	75.9	4.7
Cholesterol (mg/dl)	50.2	41.6	42.6	2.82
Creatinine (mg/dl)	1.42	1.8	1.74	0.35
Packed cell volume (%)	29	30.1	28.5	3.55
Haemoglobin (g/dl)	11.3	12.1	11.5	1.37

**Conclusion** Olive twigs treated with 4% urea and 10% molasses could replace up to 48.5% of conventional diets of growing Shami goat kids without negative effects on growth performance nor health and potentially reduce fattening costs for farmers in olive-livestock mixed systems by 80%. This presents a substantial reduction in fattening cost for farmers in olive livestock mixed systems.

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# GeoNutrition: spatial aspects of diet quality

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**Application** The talk provides an overview of ongoing GeoNutrition projects and related activities. These activities are part of a longer-term vision to develop an Agriculture-to-Nutrition framework to help to reduce micronutrient deficiencies (MNDs) in sub-Saharan Africa (SSA), thereby improving human health and well-being (mapping to Sustainable Development Goal 2). The relevance of GeoNutrition activities for livestock nutrition will be described.

**Introduction** Micronutrients, including vitamins and minerals are required in small quantities in the diet for a range of functions in the body. Micronutrient deficiencies (MNDs) pose a serious risk to human health, for example, zinc deficiency in children increases risks of infection and stunting (low height for age), while adequate dietary selenium is essential for a healthy immune system and thyroid function. In SSA, the prevalence of MNDs including zinc and selenium is high, especially among poor and rural populations. There are multiple causes for MNDs, including nutrient-poor soils, a lack of access to diverse diets including animal source foods, low nutrient bioavailability in staple crops such as maize (corn), and increased nutrient losses following infection (e.g. diarrhoea). Other MNDs, including iodine, iron and vitamin A, are widespread.

**Material and methods** The research has several major components that span multiple academic disciplines, including: (1) spatial mapping of soil, crop, human and livestock micronutrient status; (2) determining the effectiveness of agricultural interventions on micronutrient dynamics in food systems (e.g. Joy et al., 2019); (3) exploration of socio-economic and ethical aspects of agricultural interventions; (4) research capacity strengthening. There are strong conceptual links between GeoNutrition activities in SSA and research on micronutrients in temperate livestock systems (e.g. Lark et al., 2014; 2019; Kumssa et al., 2019a,b), and for food systems research in South Asia (e.g. Lowe et al., 2018; Ohly et al., 2019).

**Results** A selection of case studies will be described, including outputs of mapping work in Ethiopia, Malawi, and Zimbabwe. These studies are providing the first nationally representative evidence of long-range variation in human micronutrient status in SSA. They also provide evidence of how this variation is associated with covariates including soil chemistry, food composition, and wider landscape and socioeconomic contexts (e.g. Phiri et al., 2019). Updates on the effectiveness of agricultural interventions and bioethical implications will also be reported, with a specific focus on Malawi. Implications for the livestock sector will be described.

**Conclusion** This overview provides an update on an active research programme whose primary focus is on improving human nutrition-related outcomes through a greater understanding of spatial drivers of MNDs and agricultural interventions focusing on arable crops used for direct human consumption. However, the work is highly relevant to the livestock sector and will be placed in this context, with references to (1) SSA food systems, and (2) to UK-focused research, which is exploring spatial drivers of forage crop quality, and the agronomic management of crop quality, for the ruminant livestock sector.

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# Assessing the attitudes of feed and land advisers towards reducing phosphorus losses from UK dairy farms

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**Application** Findings could guide strategies to better utilize feed and land advisers' influence over phosphorus management practices on UK dairy farms to minimise phosphorus loss and reduce costs on UK dairy farms.

**Introduction** Dairy cows in the UK are often fed P above NRC (2001) recommendations (Sinclair and Atkins, 2015). This practice is costly and generates P-rich manure, which when applied to land contributes more P than crops can utilise, leading to runoff into waterbodies, resulting in eutrophication. Feed advisers are key to reducing P overfeeding on dairy farms (Dou et al., 2003). In a survey of GB dairy farms, feed advisers were present on 70% of farms and their advice motivated farmers to lower their dietary P concentration. The current study aimed to better understand the attitudes of feed and land advisers towards P management on UK dairy farms, and to identify factors that could encourage or discourage them to recommend precision P management practices.

**Materials and Methods** Two questionnaires, targeting GB feed or land advisers, were published online. Questionnaires were accessed via anonymous links distributed through media and newsletters of stakeholders. Paper copies of the questionnaire were also distributed at relevant UK events. Participants were asked to answer questions based on one typical farm they advise on. The feed and land adviser questionnaires only differed when questioning the practices they advised, with questions being pertinent to adviser type. Postal responses were uploaded to the online questionnaire. Responses were grouped by herd size (< 100, 101 to 150, 151 to 200 and > 200 cows), region (England, Scotland and Wales) and one of 5 dairy classifications (Garnsworthy et al., 2019). Data were analysed in Minitab (2018) using descriptive statistics.

**Results** The 33 respondents (27 feed and 6 land advisers) had clients with an above UK average herd size (351; range 120-1300 cows) and with a feeding system more based on concentrate rather than grazing. Responses were across GB regions (England 78%, Scotland 13% and Wales 9%). Most

advisers encouraged forage P analysis (89%). Despite half of feed advisers reporting dietary P concentrations above NRC (2001) recommendations, only 37% of advisers recommended manure P analysis. Both feed and land advisers were motivated to improve P use efficiency primarily to achieve environmental (41%) and cost (34%) benefits. Land advisers stated P-rich manure generated from overfeeding prevented improvements in P use efficiency (43%). Major reasons for feed advisers not to reduce dietary P concentration were lack of information on P content in feed ingredients (28%), uncertainty of P availability (21%) and concerns over reduced productivity (21%). Most advisers (71%) felt that there was not enough P management training available to them.

**Conclusion** Advisers could be better utilized to improve P management on UK dairy farms by increasing availability of P management training to advisers. This training should emphasise the environmental and cost benefits of more efficient P use and promote manure P analysis. More research is needed to determine P availability in feed ingredients and to develop a simple tool to quantify P in feeding ingredients.

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# Survey of current phosphorus feeding practices on Great British dairy farms

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**Application** Findings could guide strategies to improve precision phosphorus (P) feeding on UK dairy farms to minimise P loss and reduce farmer feeding costs.

**Introduction** Dairy cows in the UK are often fed P above NRC (2001) recommendations (Sinclair and Atkins, 2015). Feeding closer to requirement can reduce feed costs (Kebreab et al., 2008) and faecal P excretion without compromising health, productivity or fertility (Ferris et al., 2009). Land application of manure rich in P over-applies P above crops' requirement, leading to accumulation in soil and runoff to waterbodies resulting in eutrophication. This study aimed to assess current P feeding practices on GB dairy farms and to identify the barriers to and motivators for achieving precision P feeding.

**Materials and Methods** A 29 question survey was posted to 2000 GB dairy farmers from a list provided by AHDB after a stratified random sampling according to herd size (< 100, 101 to 150, 151 to 200 and > 200 cows), and region (England, Scotland and Wales). The questionnaire was also accessible online via an anonymous link distributed through social media and various stakeholders. Postal responses were uploaded to the online questionnaire. Respondents were classified into one of 5 production systems (Garnsworthy et al., 2019). Data were analysed in Minitab (2018) using Chi Square Test and ANOVA.

**Results** Responding farms (139) were representative of the average UK milk yield (7,956±181 (SE) kg/cow), region in regard to producer distribution (58% England, 14% Wales and 28% Scotland) and dairy farm type classification, but were above UK average herd size (265±30 (SE) cows/herd). Most respondents (72%) did not know the P concentration in their lactating cow' diet; a third (33%) who stated they did know, fed above the recommended (NRC, 2001). Precision P feeding practices were rarely adopted, and manure P was tested by only 32% of respondents, and the proportion of farms testing manure increased with herd size group (P = 0.001). The level of farmer awareness of the consequences of overfeeding P increased with herd size group (P = 0.001), and this awareness affected feeding practices. Farmers were willing (96%) to reduce P concentration in dairy cow diets, mostly motivated by the prospect of reduced

feed costs (27%) and reduced environmental damage (26%) and if their nutritionist advised it (25%). The uncertainty of P availability in feed ingredients (30%) and the concerns over reduced fertility (22%) were main barriers to this. Presence of a feed professional on a farm had minimal impact on P feeding practices and tended to increase inorganic P supplement use (P = 0.081). Participants (91%) felt there was insufficient P management training available to dairy farmers.

**Conclusion** Future strategies should improve the availability of P management education for UK dairy farmers. This education should improve the awareness of the consequences of P overfeeding, counteract concerns over fertility associated with reducing dietary P concentration, and emphasise the environmental and feed cost benefit of precision P feeding. Nutritionists' influence over P feeding practice should also be more effectively utilized to reduce P overfeeding. Future recommendations should include research to address the uncertainty of P availability in feed ingredients.

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# Iron status of suckling pigs offered access to an iron supplemented anise-flavoured creep following maternal exposure to anise flavour

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Iron status of suckling pigs offered access to an iron supplemented anise-flavoured creep following maternal exposure to anise flavour

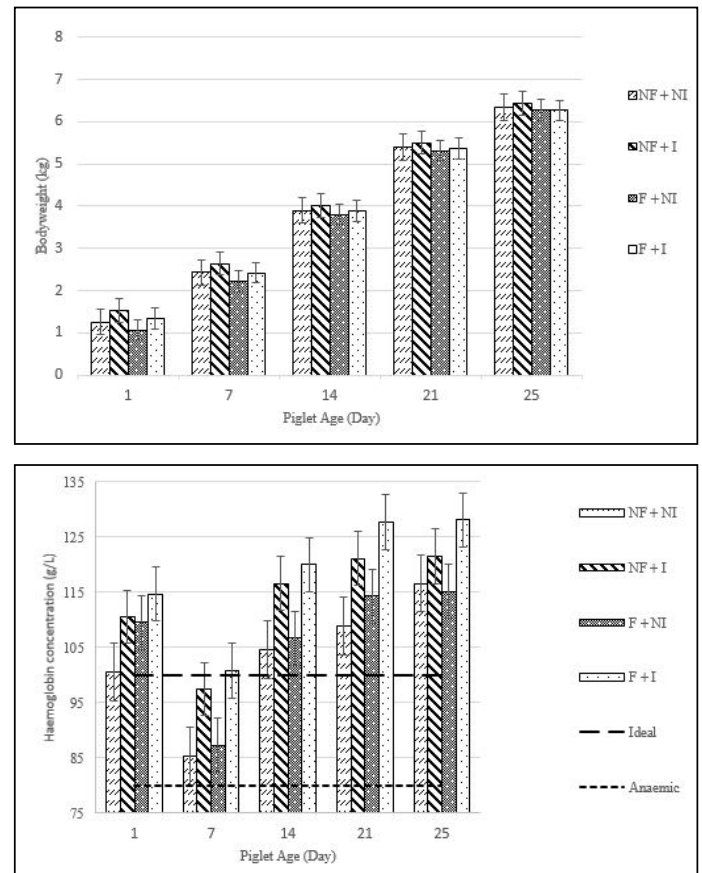
**Application** This preliminary study shows comparable iron status between injected and non-injected pigs following exposure to an iron-supplemented creep feed. The use of a maternal diet flavour to entrain creep intake was not persuasive.

**Introduction** Iron deficiency anaemia arises in indoor pigs due to an undersupply of iron in sow milk. Consequently it is a standard practice to provide supplementary iron intramuscularly to each piglet. This procedure is effective in protecting against iron deficiency but has disadvantages including increased labour, risk of infection and development of abscesses. In contrast, the augmentation of supplementary creep feed with iron is complicated by variable consumption of creep by piglets, poor absorption of dietary iron and / or increased risk of microbial overgrowth in the gastrointestinal tract. This study evaluated the role of maternal exposure to a flavour on the subsequent utilisation of a flavoured creep feed containing supplementary iron on piglet growth and haemoglobin status during the suckling period.

**Material and methods** Ethical approval was obtained from the University of Sydney animal ethics committee (2017/1256). The experiment treatments were arranged in a factorial design incorporating maternal exposure to aniseed and iron injection of the litter; sow-feed flavour added (F) versus no added flavour (NF) and piglet iron injection (I) or no injection (NI). Twenty-three multiparous sows were assigned to 1 of the 4 treatments 1 week prior to farrowing (n=5/6 per treatment group). Maternal exposure to aniseed (1ml/kg diet) commenced 1 week prior to farrowing until weaning. At birth, piglets were weighed, sexed and tagged, and litter size were then balanced within treatment groups. Entire litters were then assigned to iron injection or not (d 2). All piglets had access to an aniseed-flavoured, iron supplemented creep from day 2 until weaning. A combination of inorganic and organic iron was top dressed and mixed into the creep at a supplementary iron concentration of 8.75%. The utilisation of creep feed was determined daily while bodyweight

(all piglets) and blood haemoglobin levels (2 piglets per litter) were measured on d 1, 7, 14, 21 & 25. Data were analysed using Genstat (v.18.2) and were analysed by REML modelling. The experimental unit was the sow and her litter. Models included the main effects of treatment groups, day and their interaction to investigate effects during each week after parturition.

**Results** Neither the addition of the flavour to sow diets or iron injection or not of the piglets influenced daily or total creep intake (data not presented). The response of bodyweight and blood haemoglobin status to dietary treatments are presented in Figure 1. There was no significant difference between treatments for bodyweight or haemoglobin status throughout the experimental period.



**Figure 1** Response of bodyweight (a) and haemoglobin status (b) of piglets consuming iron-supplemented, anise flavoured creep following maternal exposure to aniseed. (NF; no maternal flavour, F; maternal flavour; I; piglet iron injection, NI; no piglet iron injection).

**Conclusion** Piglets receiving access to an iron-supplemented, aniseed flavoured creep had comparable final bodyweight and haemoglobin status regardless of receiving an iron injection. Maternal aniseed exposure did not influence anise-flavoured creep intake.

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# Effect of dietary copper supply during the rearing phase on holstein-friesian heifer performance, fertility, and hepatic copper concentration.

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**Application** Feeding copper (Cu) to replacement heifers above requirements may marginally improve heifer growth rate but will have a large and negative effect on conception rates. Copper should therefore be fed at the recommended concentration.

**Introduction** Copper is an essential trace element that can influence dairy cow performance, health and fertility (Suttle, 2010). There is recent evidence of over-supplementation of Cu in the UK during the winter-feeding period, with a mean concentration in herds surveyed in Northern and Central England of 28 mg Cu/kg DM (Sinclair and Atkins, 2015), 2.5 times the guideline of 11 mg Cu/kg DM for this class of stock (NRC, 2001). This over-supply of Cu is reflected in approximately 38% of Holstein-Friesian cow livers entering the food chain with toxic Cu concentrations (Kendall et al., 2015). Most studies that have been conducted with replacement heifers have been short-term, and the effects on health and fertility have not been reported (Suttle, 2010). The aim of the present study was to determine the long-term consequences of feeding dietary Cu in excess of requirements on replacement heifer performance, fertility, and hepatic Cu status.

**Material and methods** Eighty Holstein-Friesian heifer calves weighing 137 kg (SE ± 2.4) at 4.1 months of age (SE ± 0.1) were paired based on their live weight, condition score, and predicted transmitting ability for milk, fat, and protein yield, and randomly allocated to one of two dietary Cu concentrations. The control (C) basal diet contained 15 mg Cu/kg DM, which was predicted to meet the animal's requirements (NRC, 2001). Heifers on the high (H) concentration received an additional 15 mg Cu/kg DM supplied via two intra-ruminal Cu oxide boluses (Agrimin Ltd, Kirmington, UK), to provide a total dietary concentration of approximately 30 mg Cu/kg DM, which was similar to the mean concentration reported on UK dairy farms by Sinclair and Atkins (2015). The two dietary treatments were therefore: C [15 mg Cu/kg DM -basal ration], and H [30 mg Cu/kg DM- basal ration + copper oxide]. Heifers were weighed and condition scored on a fortnightly basis between 4 and 22 months of age, and served by artificial insemination from 13.5 months of age. Liver biopsy samples were collected at 7, 13 and 22 months of age, immediately snap frozen in liquid nitrogen and stored at -80 °C prior to being digested at 60°C in concentrated nitric acid, and

analysed for Cu concentration by ICP-MS (Perkin Elmer, UK). Mean growth rate was determined by linear regression of the fortnightly live weight measurements. Performance parameters and hepatic Cu concentration were analysed by ANOVA, and binomial data by linear model regression using Genstat (v18).

**Results** Over the duration of the study heifers on treatment H had a 0.04 kg/d (P < 0.05) higher growth rate and 0.17 greater gain in condition score compared to those on C (Table 1). There was no difference (P > 0.05) in pregnancy rate to 1st service. In contrast, the combined conception rate to 1st and 2nd service was lower at 75% for heifers on H compared to 97% for those on C (P < 0.01). Heifers on treatment H had hepatic Cu concentrations that were 155, 155, and 165 mg/kg DM higher (P < 0.01) than those on C at 7, 13 months of age, and 22 months of age respectively.

**Table 1.** The effects dietary Cu concentration on Holstein-Friesian heifer performance and fertility.

	Cu supplementation level		sed	CI (95%)	P-value
	C	H			
Live weight change	0.76	0.79	0.014		0.033
Change in body condition score	0.09	0.26	0.072		0.025
Pregnancy to 1	60	47		0.44, 1.77	0.486
Pregnancy to 1	97	75		1.35, 6.68	0.008
Hepatic Cu (mg/kg DM) at 7 months of age	643	798	47.7		0.002
Hepatic Cu (mg/kg DM) at 13 months of age	195	350	22.4		<0.001
Hepatic Cu (mg/kg DM) at 22 months of age	128	293	27.0		<0.001

<sup>1</sup>over the 18-month study period

**Conclusions** Feeding Cu in excess of requirements to replacement dairy heifers increased liver Cu concentration and resulted in a small but significant increase in growth rate and body condition, although there was a large reduction in conception rate. The biological mechanisms behind these effects are unclear and require further investigation.

**Acknowledgements** The authors gratefully acknowledge funding from the AHDB.

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# Effect of breed type and starting live weight on live-weight gain, grade and time required to finish differing breeds and sizes of dairy bull calves

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**Application** Growth performance of Holstein and Jersey crossbred dairy bull calves were similar and rearing of a variety of commonly available dairy bred calves of all sizes was viable for beef and veal production, which would reduce on-farm culling and enhance farm income from bull calves.

**Introduction** The agricultural industry aspires to utilize more dairy bull calves for the production of beef and veal (CHAWG, 2018). The value of Holstein-Friesian bull calves of a higher  $\geq 50$  kg live weight (LW) for beef and veal production is well established (Calf Forum, 2013). However, not all dairy bull calves are  $\geq 50$  kg or are HF due to the increasing popularity of pasture-based dairying in the UK. The aim of this study was to compare the pre and post-weaning performance of spring-born bull calves classified as either HF or HF and Jersey crossbred calves of  $\geq 50$  kg and  $< 50$  kg starting live weight (SLW) at 7 to 10 d of age.

**Material and methods** A total of 60 dairy bull calves were allocated, at 10 ( $\pm 3$ ) d of age, to one three treatments according to breed and LW ( $n=20$  calves/ treatment): Holstein / NZ Friesian (HF: 50  $\pm$  0.9 kg SLW); HF x Jersey higher (HF x Jersey: 51  $\pm$  0.9 kg SLW) and lower (HF x Jersey: 44  $\pm$  0.9 kg SLW) SLW. Calves were offered ad libitum access to stored colostrum until 10 ( $\pm 3$ ) d of age, followed by which calves were housed in pairs and individually offered milk replacer (MR) at  $< 10$  L/d (150 g MR/L) along with ad libitum access to water and calf starter (16 % CP and 12.4 MJ ME /kg DM). Calves were weaned at 12 weeks of age, followed by which they were offered ad libitum access to a mixed ration (grass and maize silage; hay; ground barley) up until finishing (12.8 ME; 14% CP; Starch 36.7%). Live weight, growth rate, carcass weight, grade, fat class and days to finishing were normally distributed and analysed using General Linear Mixed Models procedure (GLM ANOVA), applying breed type/LW group (HF, HF x J-H, HF x J-L) as a fixed effect and animal as a random effect in the model. Differences were assessed by Tukey's test and reported at  $P < 0.05$ .

**Results** HF and HF x Jersey bull calves, of higher and lower SLW at 10 ( $\pm 3$ ) d of age had similar pre and post-weaning growth rates (Table 1), carcass grade and fat class. HF x Jersey crossed calves that had a mean SLW 44 kg required 16 d longer to finish compared with HF of a  $\geq 50$  kg SLW.

**Table 1** Mean live-weight gain, live weight, carcass grade, fat class and age at the finishing of Holstein Friesian (HF) and Jersey HF crossbred dairy bull calves with a mean starting live weight (SLW) 50 kg and 44 kg at 10 ( $\pm 3$ ) d of age

	HF	HF and Jersey cross		SEM	P-value
	50 kg	50 kg	44 kg		
Pre-weaning gain, kg/d	1.01	0.97	0.91	0.031	0.069
Post-weaning gain, kg/d	1.10	1.09	1.10	0.011	0.842
Live weight at finishing, kg	560	567	570	7.3	0.713
Cold carcass, kg	283	288	292	4.8	0.644
Grade (EUROPE)	0	0	0	-	-
Fat class	- 2.5	- 3	- 3	-	-
Mean fat class (1 to 15)	7	7	7	0.5	0.797
Time to finishing, d	468	479	484	4.2	0.038

**Conclusion** Pre and post-weaning growth rate, carcass weight, class and grades were similar for HF and HF and Jersey crossbred dairy bull calves of  $\geq$  and  $\leq$  50 kg SLW. Crossbred calves with a mean SLW of 44 kg took (16 d) longer to finish at a 10 kg greater LW compared with HF calves  $\geq$  50 kg SLW, but similar amounts of time compared with HF Jersey crossbred dairy bull calves that had a mean SLW of 50 kg.

**Acknowledgements** To Suputo (formerly Dairy Crest) and Denkavit for funding of this research.

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# Predicting dairy cow behaviour using computer vision

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**Application** Image analysis software for digital camera systems allows real time 24-hour automated monitoring of dairy cows and their behaviours.

**Introduction** Recent technological advances in the field of computer vision based on the technique of deep learning have emerged which now makes automated monitoring of video feeds feasible. Deep neural networks can be used for a number of animal monitoring tasks such as recognising the type of animals and detecting where the animals are located in the image. Furthermore, adaptations of neural networks for analysing video can be used for a number of high-level analysis tasks such as recognition of specific animal behaviours (e.g. standing, lying, walking, eating, drinking) (Cangar et al., 2008). The aim of this study was to assess the use of computer vision software to track dairy cow behaviours.

**Material and methods** Video cameras (Hikvision HD Bullet; Hangzhou, China) were used to record Holstein Friesian dairy cows at the Nottingham University Dairy Centre (Sutton Bonington, Leicestershire, UK) during parturition. Three calving pens with two surveillance cameras looking into each pen were used to provide 24-hour video surveillance. Both cameras are placed on the same side of each pen, at opposite ends, with approximately a 45-degree angle looking into the pen. Several days prior to calving, each cow is moved into one of the three calving pens so that the entire calving process can be monitored. We recorded 30 individual cows that gave birth between April and June 2018. The video and behaviour of each cow was annotated from 10 hours prior to giving birth until 5 hours afterwards. Seven cow behaviours (Table 1) were recorded with video footage being used for training or validation of the computer vision model.

**Table 1** Number of video clips for each behaviour class in the model training and validation datasets.

Label	Behaviour	Training	Validation	Total
1	Stand	357	90	447
2	Lie	333	90	423
3	Walk	320	87	407
4	Shuffle	343	90	433
5	Contractions	300	90	390
6	Eating	204	75	279
7	Drinking	102	38	140
	<b>Totals</b>	<b>2,052</b>	<b>587</b>	<b>2,639</b>

For each video clip a bounding box was added around the cow of interest to focus and train the computer vision model to identify the selected objective (i.e. cow) and its behaviour. The computer vision model consisted of a non-local network proposed by Wang et al. (2018) with a ResNet-50 backbone architecture (He et al., 2015). The behaviour recognition model was trained on 2-GPU's with an effective mini-batch size of 16 video clips (8 clips per GPU). The model was trained for a total of 8,000 iterations before validation testing on an independent set of cow video clips. The accuracy of the model was assessed by its ability to correctly classify a behaviour.

**Results** The model developed was 70% or more accurate at correctly classifying the cow behaviours studied (Table 2). The most accurately detected behaviour was eating followed by contractions, which in itself is sufficient enough to predict the birth of a calf as a cow will generally start contractions approximately 1 to 2 hours prior to giving birth. Standing, lying, eating and drinking behaviours all scored greater than 70% and can also help with estimating the current parturition state. Plus, they can also be used to identify other rare behaviour patterns.

**Table 2** Evaluation of model predictions against validation dataset.

	Stand	Lie	Walk	Shuffle	Contractions	Eating	Drinking
Target	90	90	87	90	90	75	38
Output	70	71	63	63	74	65	27
Percentage	78	79	72	70	82	87	71

1 The target row shows how many video clips were tested for each behaviour.

2 Output row shows how many behaviour video clips the model classified correctly.

3 The percentage of target behaviour video clips correctly classified.

**Conclusion** We show that automated monitoring of an individual cow is possible and the detection of its behaviours, which can assist a stockperson by continuous monitoring when housed. This approach could be used for early detection of abnormal behaviour, birth events and the need for assistance. This will enhance animal welfare.

**Acknowledgements** The authors are grateful to the Douglas Bomford Trust, EPSRC and BBSRC for funding this work.

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# Effect of regrouping lactating dairy cows on behavioural variability

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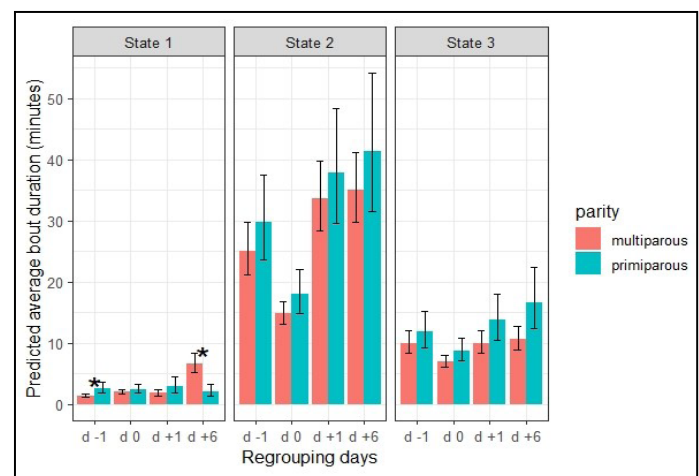
**Application** Regrouping and parity had a significant impact on the behaviour of lactating dairy cows. Primiparous spent less time, while multiparous cows spent more time engaged with other cows days after regrouping.

**Introduction** Regrouping of animals is a common animal husbandry practice in the commercial dairy industry. It can induce negative effects on cow's behaviour and physiology due to changes in the social environment (Zelena, Haller et al. 1999). Animals have to learn to interact with new individuals as well as a new environment and it may contribute to stress. In the current study, we expected cows to change their behavioural phenotype in order to cope in their new environment under this practice (regrouping). Therefore, the objective of this study was to assess whether regrouping influence altered cows' activities.

**Material & methods** The study was carried out on a commercial dairy farm in Aberdeenshire, Scotland. The cows were loose-housed in a cubicle shed and milked voluntarily via an robotic automatic milking machine. A total of 52 lactating cows (n =17 primiparous; 35 multiparous) were observed in an established group continuously for 4 hours/day and observations were sampled at 2.5 minutes intervals for 4-time blocks (day-prior (d -1), day-of regrouping (d 0), day-after (d +1) and 6-days after (d +6) regrouping) without interfering with the daily farm routines and cows' normal behaviour. Scan sampling every 2.5 mins of all individuals took place from the aerial walkway over the pens to minimise the impact that the observations had on the cows' behaviour. We sampled 1-h scanning bouts each day and observations were classified as one of three mutually exclusive and cumulatively inclusive activity states: with others (state 1); alone (state 2) and feeding (state 3). We modelled behavioural dynamics using a time discrete hidden Markov model using msm package in R to determine whether regrouping and parity affected cow's activity dynamics. Models were fitted to describe the behavioural phenotypic changes over the three states and the transition intensity matrix of the best model (AIC model selection) was used to estimate the activity budgets and average state bout duration.

**Results** Regrouping had significant impact on the behavioural dynamics and activity budgets between the activity states depending on the parity group (goodness-of-fit,  $-2 \times LL = 333$ ,  $df=42$ ,  $p<0.0001$ ).

Primiparous cows were less likely to be engaged with others on d +1 and d +6 after regrouping than d -1 compared with multiparous. Moreover, The results of the model (Figure 1) showed that there was no significant differences in average bout duration (minutes) observed between activity states 2 and 3 for both primiparous and multiparous. However, the average bout duration spent was significantly higher in state 1 for primiparous ( $2.67 \pm 0.463$  vs  $1.46 \pm 0.151$ ) on d -1 and for multiparous ( $6.61 \pm 0.835$  vs  $2.13 \pm 0.469$ ) on d +6. Similarly, multiparous cows spent significantly more total time (minutes) ( $9.67$ ; 95%-CI: 7.6917, 11.9166) engaged in state 1 on d +6 than did the primiparous ( $3.43$ ; 95%-CI: 2.3790, 5.1155), whereas no significant difference were observed between state 2 and 3. However, primiparous cows were more likely to be engaged in state 3 days following regrouping than multiparous, although the differences were not significant.



**Figure 1:** Estimated average bout duration (mean sojourn time) spent between activity states. Bars represent the parity group (see legend) whereas error bars represent 95% confidence intervals. Statistically significant are indicated in asterisk (\*). **Conclusion** The results of this study show that days following regrouping and parity had a significant impact on the animal behavioural changes. Primiparous cows had significant higher average bout duration in state 1 on d -1 compared to their multiparous counterparts. This is an indication negative impact of the regrouping on social interactions due to changes in the social environment and may lead to social instability. Multiparous cows may benefit from previous regrouping experiences.

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# Lying preference of dairy cows: a trade-off between unrestricted space and preferred surface

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**Application** When lying down, an unrestricted lying space was more important than the lying surface to Holstein dairy cattle, which could be applied to make cow housing more comfortable in the future.

**Introduction** Dairy cows are being housed for longer in the UK, which may reduce animal welfare as they prefer pasture access when given the choice. Pasture preference is not influenced by herbage mass when cows also have access to a total mixed ration and cows have a higher motivation to access pasture at night, when they primarily lie down, both suggesting that cows primarily value pasture access for lying down rather than for grazing. Pasture and cubicles differ in two main aspects for lying down; the surface type and the restriction of lying space. The aim of the present study was to assess which of these two qualities are more important to a cow when choosing where to lie down.

**Material and Methods** Twenty-four pregnant Holstein dairy cows in mid to late lactation (271 ± 14.8 DIM, mean ± SEM; range 142 to 412 DIM) with a milk yield between 12.2 and 29.4 kg/d (mean 20.8 ± 0.79 kg/d) were selected for the study. Cows were allocated to one of six experimental periods (n = 4 × 6) and housed in individual pens (6m × 15.2m). Each pen had three lying surfaces (2.4 × 2.4m): deep-bedded sand (SA), mattress (M; Pasture Mat) and deep-bedded straw (ST). These were designed to allow a cubicle to be fitted over the middle and removed again. Lying behaviour and location was recorded using video cameras. Each experimental period lasted a total of 20 d over three stages with a change-over day between Stages 1 and 2; Stage 1 (8 d): cubicles were fitted for each surface. Cows were given 2 days training on each surface, to experience lying on all surface types, before having a 2 day free choice phase with access to all surfaces. Stage 2 (9 d): cubicles removed. Cows were given 2 days training on each surface, followed by a 2 day choice phase with access to all surfaces. Stage 3 (3 d): a cubicle was fitted onto each cow's most preferred surface, determined by Stage 2, with the other two surfaces remaining without a cubicle, and the cows were given a final 3 day choice phase. This created a trade-off between preferred surface (P1) with restricted space (P1+Cubicle), second most preferred surface (P2) with unrestricted space (P2+Open) and third preferred

surface (P3) with unrestricted space (P3+Open). To analyse cow surface preference during Stage 1 and 2, a REML algorithm was applied to the percentage of total time spent lying on each of the three surfaces during choice days. For Stage 3, lying time in minutes was analysed using a series of Wilcoxon matched pair sign ranks test, paired for each cow, for a.) P1+Cubicle against P2+Open and b.) P1+Cubicle against P3+Open. Six cows had no preference between P2 and P3 during Stage 2 and either one of the two surfaces could have been preferred in principal. All possible combinations were considered and the Wilcoxon test was applied 64 times (2<sup>6</sup> = 64) to each possible combination to test for a. and b., with the mean values of these two series compiled.

**Results** Percentage of time spent lying on each surface is summarised in Table 1. For Stage 1, cows lay down longer on ST and M than on SA (P = 0.02). During Stage 2, cows spent longer lying on ST than on SA or M (P = 0.027). During Stage 3, cows lay down for longer on P2+Open than P1+Cubicle (P = 0.023) but there was no difference in lying times between P1+Cubicle and P3+Open (P = 0.73).

**Table 1** Percentage of time (mean ± SEM) spent lying on each surface during Stage 1, 2, and 3.

	Sand	Straw	Mattress
Stage 1 (Cubicle)	5.8 ± 1.5%	29.9 ± 4.9%	30.2 ± 5.3%
Stage 2 (Open)	9 ± 3.8%	45.8 ± 5.1%	16.5 ± 4.7%
	P1+Cubicle	P2+Open	P3+Open
Stage 3 (Trade-Off)	13.9 ± 3.9%	44.8 ± 4.7%	9.5 ± 2.5%

**Conclusion** When presented with a trade-off between lying on their preferred surface or another surface with unrestricted space, the majority of cows chose unrestricted space. These results suggest that when choosing where to lie down, cows value unrestricted space over their preferred surface type and may help to explain cow preference and motivation for pasture access.

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# The behaviour of dairy cattle in late gestation: Effects of parity and dystocia

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**Application** Farmers rely on expected calving dates to manage cows around calving, and direct observations to identify calving cows. Automated monitoring of cow behaviour has the potential to provide farmers with a more accurate indicator of the day and time of calving when compared to the expected calving date.

**Introduction** Two common issues at calving are calving difficulty (dystocia) and perinatal mortality, and therefore intensive management of cattle in late pregnancy is critical to ensure neonatal and maternal survival, health and welfare (Mee, 2004). As the availability of labour decreases, there is a need to apply automated technology to facilitate herd management. This study involved automated detection of lying behaviour and activity of dairy cattle in late gestation for animals that calved without assistance, and for animals that calved with assistance.

**Material and Methods** Data were collected from 32 multiparous and 12 primiparous Holstein dairy cattle to describe normal calving behaviour and parity differences. To quantify behaviour related to calving difficulty, the data from 14 animals that had dystocia at calving were matched to cows that had an eutocic calving based on parity, locomotion score, calf breed, calf sex, month, and year of calving. An IceQube (IceRobotics Ltd., UK) was fitted to the right hind leg of cows 4 wk prior to their expected calving date. Data for lying time, standing time, number of steps, motion index (total motion), and the total number of standing and lying bouts (postural transitions) were automatically collected and summed into 15-minute blocks. Behavioural variables were summarized into 2h periods and 24h periods prior to analyses. Mixed-effect models were used to analyze cow behaviour in the last 4 d before calving (d -4 to d -1), and on the day of calving.

**Results** In the 4 d prior to calving, there was an effect of parity on all behavioural variables. When compared to multiparous cows, primiparous cows lay down an average 2.8h/d less, had 9.1 more postural transitions/d ( $37.7 \pm 1.2$  vs.  $27.6 \pm 0.7$ ), walked 172 more steps/d, and had a higher motion index ( $2673.2$  unit/d vs  $1981.5$  unit/d). While the lying time of multiparous cows remained constant in the 4 d before the day of calving, the lying time of primiparous cows decreased by 25 min/d ( $13.0 \pm 0.4$  h to  $11.6 \pm 0.6$  h;  $P = 0.009$ ).

On the day of calving, there was an effect of 2h period on all behavioral variables. Step count linearly increased from an average of 32.3 steps/2 h at -22 h to 78.8 steps/2 h just before calving, and motion index increased from an average of 139.5 unit/2 h at -22 to 437.5 unit/2 h just before calving. Breakpoint analysis concluded that the number of postural transitions started to increase -6.3h before calving ( $P < 0.001$ ), and primiparous cows had 0.24 more postural transitions per 2/h when compared to multiparous cows ( $P < 0.05$ ). The change in lying time on the day of calving for primiparous and multiparous cows was best described by a polynomial pattern, with a decline from 65.3 min/2 h at -22 h to a low of 50.6 min/2 h at -12 h, increasing to an average of 66.8 min/2 h just before birth ( $P < 0.001$ ). No indicator of calving difficulty was found on the day of calving, nor the days leading up to calving.

**Conclusion** This study found important differences in the behaviour of primiparous cows and multiparous cows, which suggest that parity must be considered when predicting the day of calving. The behavior of dairy cattle undergoes numerous changes on the day of calving. Although there were parity differences in the 4 days preceding calving, there were no differences in parity on the day of calving for duration of lying time, step count, or motion index. Although no indicator of calving difficulty was identified by this research, the ability to identify calving cows and to predict the time of calving would allow farmers to monitor the progression of calving and intervene where necessary.

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# Routine prediction of bovine tuberculosis status of dairy cows from milk mid-infrared spectral data

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**Application** Prediction of tuberculosis status from milk spectral data alone could be deployed as part of routine milk recording enabling the early identification of potentially affected cows.

**Introduction** Bovine tuberculosis (bTB) is a chronic zoonotic disease of cattle which is of great economic, welfare and societal importance. Mid-infrared (MIR) analysis of milk is used routinely to predict fat and protein concentration but has also been shown to provide a robust predictor of several other economically important phenotypes such as individual fatty acids (Soyeurt et al., 2011) to body energy (McParland et al., 2011; Smith et al., 2019). The objective of the present study was to use phenotypic reference data combined with concurrent MIR spectral data to generate prediction equations for bTB status at the individual cow level.

**Material and methods** Cows (n= 1,678,165) were from national herds involved in routine milk recording with National Milk Records (NMR) and were distributed across the UK. Cows were Holstein-Friesian (81%), Belted Galloway (9%), Jersey (3%), Ayrshire (1%), Brown Swiss (0.8%), Swedish Red & White (0.8%), and Guernsey (0.7%). The data also included small numbers of other dairy breed and crosses (< 3.7%). Bovine TB data were made available by the Animal and Plant Health Agency (APHA) and were collected via the UK's national bTB testing program. These data provided information from over 40,500 bTB breakdowns. Data relating to cattle births, movements, and deaths were supplied by the British Cattle Movements Service (BCMS). Data relating to SICCT skin test result, culture, slaughter status, presence of lesions were combined to create a binary bTB phenotype; labelled 0 to represent non-responders (i.e., healthy cows) and 1 to represent responders (i.e., bTB-affected cows). Concurrent milk MIR spectral data were matched to bTB status on sample date ( $\pm 15$ d). Deep learning, a sub-branch of machine learning (Lecun et al., 2015) was used to train prediction equations. Spectra were converted to 53x20 px images then used to train a deep convolutional neural network (CNN). Additional training data were synthesised using SMOTE (Chawla et al., 2002) to balance data in each class.

**Results** Deep CNNs resulted in a prediction accuracy of 71% (Table 1) with a low validation loss (0.71) and moderate sensitivity and specificity (0.79 and 0.65, respectively). Accuracy increased to 95% when synthesised data were included in training and validation loss was minimised (0.26). Sensitivity and Specificity also seen an increase (0.96 and 0.94, respectively).

**Table 1** Summary of model performance

	Raw data	+ synthesised data
Animal test-dates	4,257	15,824
Training epochs	278	295
Validation loss	0.71	0.26
Accuracy	0.71	0.95
Sensitivity	0.79	0.96
Specificity	0.65	0.94

**Conclusions** Predicting bTB from milk MIR spectra and deep learning is promising. Further external validation is required in order to make suggestions regarding point of infection. Additional research is required to maximise accuracy, minimise loss, and highlight practicality to dairy industry.

**Acknowledgements** The authors gratefully acknowledge funding from Biotechnology and Biological Sciences Research Council (BBSRC); and data from National Milk Records (NMR), Animal & Plant Health Agency (APHA), and British Cattle Movement Service (BCMS).

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# Meta-analysis of the probiotic effect of yeast (*Saccharomyces cerevisiae*) on feed efficiency in broiler chickens

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**Application** The results on the use of probiotic-yeast as an alternative to in-feed antibiotics to enhance feed efficiency in broiler chickens have been inconclusive among authors. Our result has established the effectiveness of probiotic-yeast to improve feed efficiency in broiler chickens.

**Introduction** Variable probiotic effects of yeast on feed efficiency in broiler chickens have been reported by many authors. Factors such as study country, broiler strain, bird's age, yeast supplementation levels and duration may be responsible for inconsistent outcomes observed. Meta-analysis is an inferential statistical method that enables all published data to be combined and analysed to draw quantitative conclusions, which cannot be obtained from individual studies or narrative reviews (Sauvant et al. 2005). The aim of this study was to determine the probiotic effect of yeast on feed efficiency in broiler chickens using a meta-analysis.

**Material and Methods** Peer-reviewed papers published in English were retrieved from Pubmed, Scopus and Google Scholar databases. To guarantee quality and reduce bias, the authors designed the following condition each paper must meet to be included in the study. Studies were included if they have randomized and control trial, reported feed efficiency (FE) and a measure of dispersion such as standard error (SE), p-value and standard deviation (SD) for each effect size. From 64 studies published between 1999 and 2017, 15 studies (with 59 effect sizes and 4874 birds) met the inclusion criteria, from which data on the surname of the first author, the year the article was published, duration of yeast feeding, chicken strains, inclusion level, measures of variance (SE, SD or p-value) and FE were extracted. Lack of randomization, study not reported on FE and data duplications were the basis for the rejection of the other 49 studies. Data generated were analysed using the OpenMEE

software. The overall effect estimate is considered significant when the confidence interval (CI) has no contact with no effect line (Koricheva et al. 2013). DerSimonian and the Laird test (Q - statistic) and Inconsistency index (I<sup>2</sup>) were employed to compute heterogeneity.

**Results** The result of pooled estimate revealed that yeast intervention significantly improved feed efficiency in broiler chickens [Standardized Mean Difference; SMD = -0.354; 95% CI = -0.517 to -0.190; P < 0.001]. I<sup>2</sup> - statistics found that studies used in the analysis were not homogenous (I<sup>2</sup> = 87.27%, P < 0.001). Restricted subgroup analysis (Table 1) revealed that duration, inclusion level and broiler's strain had positive and significant influence on feed efficiency. Meta-regression results revealed that yeast inclusion level (QB = 10.81, df = 2, p = 0.005) and broiler's strain (QB = 10.80, df = 6, p = 0.094) explained most of the heterogeneity observed on feed efficiency results.

**Conclusion** It was concluded that yeast could be used to improve egg production in hens and may replace antibiotics to counteract the negative effects of their widespread use.

**Acknowledgements** The authors acknowledged the UNISA Library for their technical support

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**Table 1** Restricted subgroup analysis of the moderators

Parameter	subgroup	n	Pooled estimate	Confidence interval		SE	p
				Lower	Upper		
Duration (days)	21	18	-0.404	-0.659	-0.149	0.130	0.002
	28	8	-0.239	-0.731	0.253	0.251	0.340
	35	4	-1.140	-1.384	-0.896	0.125	<0.001
	42	25	-0.260	-0.506	-0.015	0.125	0.038
	56	4	-0.189	-1.047	0.669	0.438	0.665
	Overall	59	-0.354	-0.517	-0.190	0.083	<0.001
Inclusion (g/kg)	<10	37	-0.610	-0.791	-0.429	0.092	<0.001
	>10	16	0.218	-0.051	0.487	0.137	0.113
	10	6	-0.284	-0.633	0.065	0.178	0.111
	Overall	59	-0.354	-0.517	-0.190	0.083	<
Broiler's strain	Ross	21	-0.516	-0.833	-0.199	0.162	0.001
	Arbor	9	-0.474	-0.790	-0.159	0.161	0.003
	Hubbard	9	-0.221	-0.689	-0.246	0.238	0.354
	Cobb	3	-0.657	-0.990	-0.323	0.170	<0.001
	Overall	44	-0.359	-0.529	-0.189	0.083	<0.001

# The relationship between supplementary milk usage and piglet performance and usage behaviour

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**Application** An understanding of the relationship of piglet weight, duration of visit to milk feed stations and day of lactation with supplementary milk consumption facilitates an estimation of the quantity of supplementary milk consumed. This is potentially of use to animal scientists assessing supplementary milk intake of piglets on an individual basis. This information may be applied by farmers as a management tool to estimate milk intake of litters and so optimise provision.

**Introduction** In the UK, the indoor average number of piglets born alive/litter has increased from 11.6 in 2010 to 13.6 in 2019, with the top 10 % of herds currently exceeding 15 born alive/litter. (AHDB, 2019). Supplementary milk products offered to piglets in farrowing pens have been shown to assist the sow in rearing large litters, with the benefit that excess piglets do not need removing from the sow (Stewart et al., 2010). Technology is now available to predict supplementary milk consumption at the individual piglet level. The aim of this study was to determine relationships between litter supplementary milk intake and individual piglet milk usage, feeding behaviour and growth performance.

**Materials and methods** Litters were selected from sows (JSR 9T) with above herd-average numbers of viable piglets at birth. Fostering was only performed within the first 48 hours after birth, and was restricted to removing piglets to ensure that there were no more than two piglets in excess of the number of functional teats of the sow. Within 24 hours of birth all piglets were given an electronic identification (EID) tag, weighed and standard management procedure of teeth-grinding, iron injection and tail-docking applied. Piglets were allowed to suckle normally until weaning. Supplementary milk (reconstituted according to manufacturer's instructions; Volac, UK) was provided to litters ad libitum through a bowl situated in the farrowing pen, connected to a bucket to allow recording of litter daily milk intake. The PigTrack<sup>®</sup> monitoring system (Asserva, France) was utilised to record each visit to the milk bowl. Piglets were individually weighed on days 4, 11 and 18 after birth, and at weaning (day 26). Litters were video-recorded to assess the accuracy of the monitoring equipment. From watching these, it was determined that any visit shorter than 7 s was unlikely to result in supplementary milk consumption. When visits were longer than 70 s, the additional time was spent interacting with

the feeding station, or lying inside it. Due to this, only visits 7 – 70 s were included for data analysis. Multiple linear regression (GenStat 18th edition) was performed to determine the effect of performance and behaviour parameters measured with litter supplementary milk intake. Terms included were day of lactation, average daily litter weight, average daily duration of visits, litter size and  $\sum wti \cdot dui$ . The term  $\sum wti \cdot dui$  was calculated using the daily predicted weight ( $wti$ ; kg) of each piglet, (calculated using average daily gain from weekly weighing), multiplied by the daily duration of visit ( $dui$ ; s) for that piglet, and totalled for the litter.

**Results** The model with best fit (Table 1) included  $\sum wti \cdot dui$  ( $P < 0.001$ ) and day of lactation ( $P < 0.001$ ), where  $r^2 = 0.772$ . The equation was: milk disappearance =  $0.000227 \cdot \sum wti \cdot dui - 0.115 \cdot \text{day of lactation} + 0.801$ .

**Table 1:** The effect of  $\sum wti \cdot dui$  and day of lactation on supplementary milk intake of piglets from day 4 of age to weaning

Parameter	Estimate	P-value	r <sup>2</sup>
Constant	0.801	0.014	0.772
$\sum wti \cdot dui$	0.000227	<.001	
Day of lactation	-0.115	<.001	

**Conclusion** Supplementary milk intake can be estimated with 77.2 % variance accounted for as a function of individual piglet weight and duration of visit, and including day of lactation. **Acknowledgements** The authors gratefully acknowledge funding from AHDB Pork, Primary Diets Ltd, and JSR Genetics Ltd.

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# Egg production and shell assessment of hens offered varying dietary calcium concentrations and phytase in the presence of a supplementary limestone grit

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**Application** This experiment reports on the influence of dietary Ca concentrations and phytase inclusion on the role of voluntary limestone grit intake as a supplementary mineral for laying hens which may be helpful for nutritionists and egg producers

**Introduction** Laying hens require substantial quantities of calcium (Ca) to maintain skeletal health, egg output and quality. However high dietary limestone may impede nutrient availability, including that of other minerals (Selle et al. 2009). Poultry including commercial broilers can select dietary components based on nutrient or energy requirements (Wilkinson 2011). Exogenous phytase can increase luminal nutrient availability including that of phytate-bound Ca which may influence the appetite for Ca. It was hypothesised that providing limestone grit would ensure optimum Ca intake of hens offered diet containing suboptimal Ca concentrations. Furthermore, the impact of dietary phytase at a 'superdosing' inclusion rate on the voluntary consumption of limestone grit was evaluated.

**Material and methods** Ethical approval was obtained from the University of Sydney Animal Ethics Committee (2016/945). One hundred and forty-four individually caged, early-lay ISA Brown hens were assigned to 1 of 6 dietary treatments in a 3 x 2 factorial

design comprising 3 dietary Ca levels (40, 30 & 20g/kg) and  $\pm$  dietary phytase (3500 IU / kg diet). A trio of adjacent hens were considered one statistical unit (n=8/treatment). Diets were formulated predominately of wheat and soybean meal to provide nutrients and energy as suggested for the breed and age. Non-phytate phosphorus levels were constant across treatments at 2.5g/kg. Basal diets contained 114 IU phytase activity / kg diet while exogenous phytase supplemented diets contained 3695 IU / kg diet. Limestone grit (3.4 mm  $\pm$ 1) was provided ad libitum to all hens in a separate, adjacent container. Hens were offered ad libitum access to diets which were in mash form. Daily egg production, weekly feed and limestone grit disappearance, and weekly egg shell assessments were conducted over a 6-week collection period. Eggshell assessments were made as described by Akter et al., (2018). Data was analysed as a 2 x 3 factorial arrangement of treatments using the GLM procedure (SAS Inst. Inc., Cary, NC). The statistical model investigated the main effects of phytase inclusion, calcium level and associated two-way interactions. Differences were reported as significant if  $P < 0.05$ .

**Results** Results are presented in Table 1. Ca levels of the diet failed to affect voluntary limestone grit intake significantly, resulting in reduced shell thickness, shell breaking force and lower egg weight in hens fed the 20 g/kg Ca diet. Dietary Ca influenced several variables with birds offered 20g Ca/kg failing to maintain egg production and shell quality when compared with 40g Ca/kg. Birds offered 30 g Ca/kg had intermediate values. There was no effect of dietary phytase or interaction between phytase and Ca on bodyweight changes, feed or grit disappearance. Rate of lay, egg weight, feed conversion ratio (FCR) and shell thickness and breaking force were similarly unaffected by dietary phytase.

**Conclusion** Birds offered 20g Ca/kg diet had worsened egg output and shell quality when compared with those offered 40g Ca/kg suggesting supplementary Ca intake was not sufficient. Birds offered 30 g Ca/kg diet had an intermediate egg performance and shell variables response and the lowest FCR and merit further comparisons with Ca recommendations.

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**Table 1** Egg production and shell assessment of hens offered varying dietary Ca and phytase and all offered limestone grit

	Bodyweight (g; final)	Feed intake (g/day)	Limestone intake (g/day)	Total intake (g/day)	Ca intake (g; combined)	Rate of lay (%)	Egg weight (g)	FCR	Shell breaking force (g)	Shell thickness (mm)
Ca 40 g/kg	1937b	115	4.82	119	7.47b	93	58ab	1.99	4763b	0.402c
Ca 30 g/kg	1925b	116	6.56	123	6.49ab	96	61b	1.95	4485ab	0.386b
Ca 20 g/kg	1879a	113	6.63	120	5.93a	91	56a	2.03	4158a	0.340a
SEM	11.1	1.87	1.03	2.1	0.42	1.36	0.97	0.025	126	0.004
No Phytase	1913	114.3	6.49	121	6.61	93	58	2.0	4442	0.376
Phytase	1914	114.9	5.52	120	6.65	94	59	1.99	4495	0.376
SEM	9.06	1.52	0.843	1.72	0.345	0.25	0.79	0.02	97.9	0.003
Phytase	0.936	0.792	0.418	0.869	0.931	0.252	0.405	0.954	0.010	0.001
Ca	0.002	0.577	0.380	0.511	0.044	0.053	0.010	0.083	0.705	0.889
Ca x Phytase	0.552	0.820	0.465	0.904	0.507	0.608	0.374	0.702	0.917	0.756

# Effect of phytase supplementation of soyabean meal on endogenous phosphorus loss and true total tract retention of phosphorus in broiler chickens

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**Application** The poultry specie lacks the ability to secrete endogenous phytase to break down phytate-phosphorus complex. This deficiency results in relatively poor utilization of phosphorus (P) from oil seed meals.

**Introduction** Poor utilization of phosphorus (P) from oil seed meals by poultry is a significant problem of poultry production. Therefore, to improve utilization of dietary P in monogastric animals, there is the need to assess the bioavailability of P in feed ingredients (Rodehutsord, 2009). However, research findings on true ileal phosphorus digestibility (TIPD) of some oil seed meals have largely been on phytase supplementation of soyabean meal (SBM) in pigs, with little attention given to chicken. Therefore, a study was conducted to investigate the effect of phytase supplementation of soya bean meal (SBM) on endogenous phosphorus losses (EPL), TIPD and true phosphorus retention (TPR) in broiler chickens.

**Material and methods** A total of 288, one-day-old Abor acre chicks were randomly allotted to six diets with 6 replicate cages/diet and 8 birds/ replicate in 2x3 factorial arrangement: two levels of phytase (0 or 1000FTU/kg diet) and three levels of total phosphorus (1.45, 2.79 or 4.12g /kg diet). The dietary P content was obtained solely due to the increasing dietary inclusion level of soya bean meal (SBM). Limestone was used to maintain similar calcium:total P and chromic oxide (5g/kg diet) was added as an indigestible marker. Feed intake and body weight gain (BWG) of birds were recorded. Data were analysed using regression and ANOVA at 0.05. From the regression graphs, EPL, TIPD and TPR for diets were estimated for without or with phytase using the index method by Dilgner and Adeola (2006). Calculations of phosphorus digestibility and retention estimates were based on analysed dietary P concentrations.

**Results and discussion** The health status of the chicks were not compromised during the 7-day assay period and no mortality or leg problems were recorded. In this study, the analysed total phosphorus concentration of SBM was lower than values documented by Akinmusire and Adeola (2009). Supplementation of SBM with phytase revealed strong linear relationship ( $P < 0.05$ ) between digested P, retained P and P intake. As presented in table 1, the EPL estimated at the ileal section for birds fed diets without or with phytase were 165.5 and 98.3mg/kgDMI, respectively and at the total tract section 492.2 and 326.0mg/kg DMI, respectively. Supplementation of SBM with 1000FTU phytase/kg diet led to 40.1% reduction in EPL at ileal section and 33.8% at total tract section with corresponding increase in TIPD and TRP by 9.46% and 7.06%, respectively.

**Conclusion** It can be concluded from the study that supplementation of soya bean meal with 1000FTU/kg diet reduced endogenous phosphorus loss, improved true ileal phosphorus digestibility and true phosphorus retention in birds.

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**Table 1.** Linear relationship between dietary P intake, digested P and retained P in broilers1 fed SBM

Section/Item	Regression <sup>2</sup> Equation	SE of the slope	SE of the intercept	r <sup>2</sup>	EPL (g/kg DMI)	TIPD/TRP (%)
<b>Ileal section</b>						
SBM + Phytase, 0 FTU/kg	Y=0.7093X - 0.1655	0.15	0.13	0.96	0.1655 <sup>a</sup>	70.93 <sup>a</sup>
SBM + Phytase,1000FTU/kg	Y=0.7764X - 0.0983	0.29	0.25	0.89	0.0983 <sup>b</sup>	77.64 <sup>b</sup>
<b>Total tract section</b>						
SBM + Phytase, 0 FTU/kg	Y=0.859X - 0.4922	0.25	0.25	0.91	0.4922 <sup>a</sup>	85.90 <sup>a</sup>
SBM + Phytase,1000FTU/kg	Y=0.9197X - 0.3260	0.26	0.19	0.93	0.3260 <sup>b</sup>	91.97 <sup>b</sup>

# Effect of rice husk supplemented with phytase and vitamin D3 on ileal phosphorus digestibility in broiler chickens fed diets low in calcium and phosphorus

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**Application** supplementation of poultry diets with phytase and vitamin D3 will not only minimize eutrophication but decrease excessive dependence on inorganic phosphates in poultry.

**Introduction** Supplementation of non ruminant diets with microbial phytase and vitamin D3 has been shown to improve calcium (Ca), copper, phosphorus (P) and zinc utilization, and consequently reduce the excretion of these minerals in the faeces (Adeola, 1995). The level of Ca in the diet may also affect the utilization of phytic acid-P through the formation of insoluble calcium phytate and/or reduction of phytase activity. Cholecalciferol (vitamin D3) plays a role in Ca and P absorption, and therefore influences their utilization. Calcium to phosphorus ratio and phosphorus content of diets also influence the inorganic P release from phytate by phytase (Poulsen et al., 2010). Inclusion of the appropriate amount of RH will provide information on the amount of phosphorus digestible in broiler diets. Hence the objective of this study is to determine the effect of supplementing low P diets containing RH with vit D3 and phytase on ileal P digestibility in broiler chickens.

**Materials and methods** One hundred and twenty five 1-day-old Abor Acre broiler chickens was fed starter diet till day 20. A Positive control (PC) diet with required NPP (4.84 g/kg diet) and a Negative control (NC) diet in which the NPP is reduced by 65% (i.e. from 4.84 to 1.69 g/kg diet for RH) was formulated. Three other diets each for RH in which the NC diet is supplemented with either 1000 FTU phytase/kg diet, 10,000 IU. Vit D3/kg diet or both was formulated, making a total of 5 semi-purified diets. The statistical analysis used was a completely randomized design.

**Table 1** Ileal, digested and apparent phosphorus digestibility of 28-day-old broiler fed rice husk-based diets

	PC	NC	NC+Vit D3	NC+Phy	NC+Phy+Vit D3
Measurements	T1	T2	T3	T4	T5
Ileal phosphorus					
(g/kg DMI) Digested phosphorus	0.11	0.13	0.14	0.14	0.21
(g/kg DMI) Apparent phosphorus	0.46 <sup>c</sup>	1.07 <sup>a</sup>	0.63 <sup>b</sup>	0.45 <sup>c</sup>	0.44 <sup>c</sup>
Digestibility (%)	80.78 <sup>ab</sup>	89.53 <sup>a</sup>	81.88 <sup>ab</sup>	75.97 <sup>ab</sup>	67.45 <sup>b</sup>

In this study, ileal P was not significantly ( $P>0.05$ ) affected by the assayed diets but there was an increase in digestible P for birds fed a combination of the NC and vit D3 (T3), percentage apparent P digestibility (APD) improved with the supplementation of vit D3 and phytase. These reports agree with the work of Mohammed et al. (1991), these authors reported that cholecalciferol supplementation of poultry diets may improve phytic acid-P utilization. Due to the increase in digestible and APD it can be concluded that phytic acid was effectively hydrolysed. Phytase supplementation has been reported to improve the amount of digestible P in plant feedstuffs and consequently reduce P loss from feed ingredient (Akinmusire and Adeola, 2009) as seen from the results of this study. There may also be an indirect effect of dietary Ca on P digestibility because vitamin D is activated at low Ca concentration, which may enhance both Ca and P absorption (Poulsen et al., 2010), and therefore increase P digestibility.

**Conclusion** Based on the results from this study, supplementation of rice husk with Natuphos<sup>®</sup> phytase at 1000 units/kg of diet and vitamin D3 at 10000 IU/kg of diet improved digested P and apparent P digestibility.

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# Effect of the number of growing-finisher pigs per pen using wet-dry feeders on growth performance and body lesions

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**Application** Wet-dry feeders are one of the most common feeders in commercial pig farms. However, number of pigs per pen fed with the same feeder space is a key factor to maximize performance and welfare.

**Introduction** Most swine production systems in Europe use pens of 10 to 14 pigs with one wet-dry feeder per pen in the growing-finisher facilities. The same pen design is often used in farms with a different number of pigs. A higher number of pigs in the same pen causes a reduction on the space allowance (SA) and on the feeder space per animal. Thus, it may result in a reduction in average daily feed intake (ADFI) followed by a reduction on average daily gain (ADG) (Thomas et al. 2018). Moreover, lower SA increases animal welfare concerns because it can lead to adverse social behaviours directed to pen mates, resulting in skin lesions, lameness, and tail biting (Vermeer et al. 2017). These lesions may be a good indicator of an excess number of pigs even before growth is affected. Therefore, the aim of the study was to investigate and quantify the effect of the number of pigs per pen on growth performance and body lesions (BL) induced by aggression during the growing-finisher stage.

**Material and Methods** A total of 216 pigs were assigned to three different SA; 0.96 m<sup>2</sup>/pig (n=10 pigs/pen), 0.84 m<sup>2</sup>/pig (n=12 pigs/pen) and 0.72 m<sup>2</sup>/pig (n=14 pigs/pen), in a randomized design. Pigs were housed in mixed sex pens using a total of 6 pens per group. Pens were fully slatted concrete floor (2.4 x 4.2m) containing a wet-dry feeder (330 x 370mm), one nipple drinker and enriched with a larch wood post. Pigs were weighed every two weeks from 10 weeks (26.3 ± 2.25kg) until 20 weeks (101.3 ± 6.43kg) of age, when pigs started to go to slaughter. Feed intake was recorded daily at a pen level and ADG, ADFI and feed conversion ratio (FCR) were calculated during the grow-finisher period. Body lesions were counted when pigs were 19 weeks (90.8 ± 5.8kg) and 20 weeks (101.3 ± 6.43kg) of age. Data was considered as a unique measure for the statistical analysis. For BL scoring, the body of the pigs was divided into

anterior, mid and posterior part. All red lesions in each location (scratches) were counted and recorded individually. Data were analysed using SAS v9.4. Pen was used as the experimental unit. Growth performance data were analysed using ANOVA. The model included SA and initial body weight (BW) as fixed effects. Body lesions data were analysed using a generalized linear mixed model which included SA as fixed effect, pen as random effect using a Poisson distribution. Alpha for determination of significance was 0.05.

**Results** Growth performance was not affected by SA (P>0.05; Table 1) although pigs that had 0.96 m<sup>2</sup>/pig were heavier and had a lower FCR than those in 0.84 and 0.72 m<sup>2</sup>/pig by the end of the trial. Nevertheless, BL were increased in lower SA. Pigs with 0.72 m<sup>2</sup>/pig had 52.4% more scratches than those in 0.84 and 0.96 m<sup>2</sup>/pig on the anterior body region (P<0.001). There was no difference on the mid region between treatments (P>0.05). On the hind region, pigs with a 0.72 m<sup>2</sup>/pig had 53.1% more scratches than pigs with 0.96 m<sup>2</sup>/pig (P<0.05), although no differences were observed between pigs with 0.84 m<sup>2</sup>/pig and the other groups (P>0.05). In total, pigs with 0.72 m<sup>2</sup>/pig had 46% more scratches than those in 0.84 and 0.96 m<sup>2</sup>/pig (P<0.01).

**Table 1:** Effect of SA on initial BW (Means ± Standard deviation), final BW, ADG, ADFI, FCR and BL from 216 growing-finisher pigs (6 pens/treatment; Least square means ± Standard error) from 10 to 20 weeks of age.

	Space allowance, m <sup>2</sup> /pig			p-value
	0.96	0.84	0.72	
Initial BW, kg	27.6 ± 1.29	26.8 ± 3.06	24.7 ± 1.45	
Final BW, kg	103.3 ± 2.05	100.5 ± 1.94	100.1 ± 2.15	0.534
ADG, kg	1.09 ± 0.03	1.06 ± 0.03	1.03 ± 0.03	0.447
ADFI, kg	2.31 ± 0.06	2.25 ± 0.06	2.30 ± 0.07	0.783
FCR	2.08 ± 0.05	2.14 ± 0.05	2.16 ± 0.05	0.548
BL wk19-20				
Anterior	2.0 ± 0.30 <sup>b</sup>	2.3 ± 0.33 <sup>b</sup>	4.1 ± 0.57 <sup>a</sup>	< 0.001
Mid	1.0 ± 0.23	1.0 ± 0.22	1.6 ± 0.33	0.263
Hind	0.8 ± 0.17 <sup>b</sup>	1.1 ± 0.20 <sup>ab</sup>	1.7 ± 0.32 <sup>a</sup>	0.021
Total	3.8 ± 0.58 <sup>b</sup>	4.3 ± 0.65 <sup>b</sup>	7.5 ± 1.12 <sup>a</sup>	0.003

a,b Within rows, significant differences between groups (P<0.05).

**Conclusion** Pigs with a 0.72 m<sup>2</sup>/pig SA have a huge increase on BL albeit no differences in productive performance are observed. Although 0.72 m<sup>2</sup>/pig SA is legal, a SA equal or higher than 0.84 m<sup>2</sup>/pig should be considered to improve animal welfare.

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# Slow growing pigs show a response on feed conversion ratio to high levels of standard ileal digestive lysine

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**Application** Slow growing pigs (SG) in conventional pig farms increase weight heterogeneity within batch and reduce farm's profitability. High levels of standard ileal digestible (SID) lysine (Lys) could improve productive performance of SG resulting in economic benefits for pig farmers.

**Introduction** In conventional pig farms operating on a batch system, 10 to 15% of pigs within batch are SG. These pigs need more time to reach the abattoir, increase the occupation time of the facilities and are a limiting factor for the All-In/All-Out swine production systems (Patience et al. 2004). Previous research has studied different strategies to improve performance of SG and some feeding management strategies have shown improvement on growth performance of growing-finishing pigs (López-Vergé et al. 2018). Standard ileal digestible (SID) lysine (Lys) requirements have been previously established for the growing-finishing pigs (NRC, 2012), however there is no information regarding specific SID Lys requirements for the SG. Thus, the aim of the study was to compare the growth performance of SG and fast growing pigs (FG) to different SID Lys dietary levels.

**Material and Methods** A total of 84 pigs out of 421 pigs born within a week were selected based on bodyweight (BW) and moved to the finisher accommodation at 11 weeks of age. Pigs were individually weighed, ear tagged and classified as SG (n = 48; 24.1 ± 1.38 kg) or FG (n = 36; 42.7 ± 1.63 kg). Pigs were

housed in 6 mixed sex pens (n = 14 pigs per pen; 0.81 m<sup>2</sup> / pig) with fully slatted concrete floor equipped with individual feeding stations to record daily individual feed intake. At 15 weeks of age, pigs were assigned to three different dietary treatments in a factorial randomized block design. Diet was considered as fixed factor, growth rate (SG or FG) as block factor and pig as the experimental unit. Each diet was assigned to two pens, each pen containing 8 SG (40.1 ± 1.72 kg) and 6 FG (63.2 ± 2.04 kg) pigs. Diets were formulated by increasing the level of SID Lys (0.92%, 1.18% and 1.45%) and ideal protein. Pigs were weighed every two weeks until they reached the target slaughter BW, 110 kg. Feed intake was recorded daily and individually. Therefore, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for the whole trial period. Data were analysed by general linear mixed model in SAS v9.4. Model included diet, growth rate group and their interaction, and sex as fixed effects and pig as random effect. Alpha for determination of significance was 0.05.

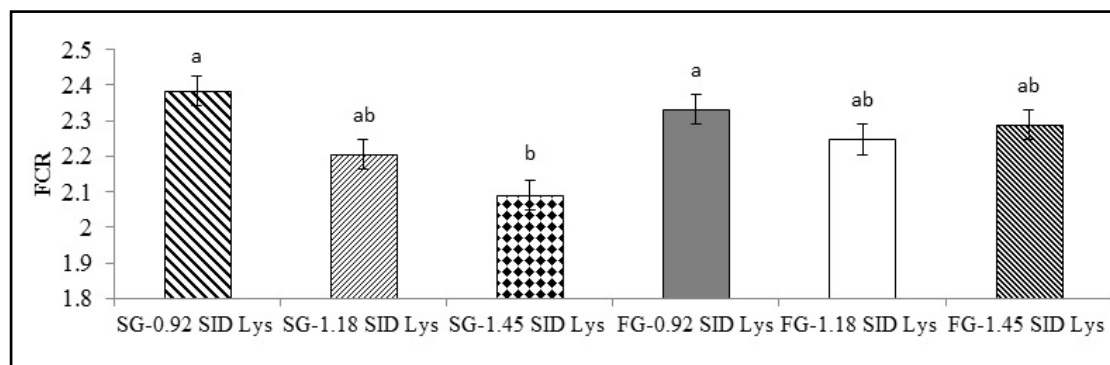
**Results** For final BW, ADG and ADFI no interaction and no diet effect were observed (P > 0.05). Nonetheless, FG had 33.7 kg BW higher than SG at the end of the trial (P < 0.001). FG gained 255 g/day more than SG in all SID Lys levels (P < 0.001). ADFI was 625.5 g per day higher for FG versus SG (P < 0.001). FCR showed an interaction between SID Lys levels and growth rate group. FCR was 0.3 lower for pigs fed 1.45% SID Lys compared to those fed 0.92% SID Lys within the SG group (P < 0.05; Figure 1), but was not different for the FG group.

**Conclusion** Slow growing pigs show a response on FCR when SID Lys levels are increased from 0.92 up to 1.45% SID Lys. Such a response is not present in FG. Although SG present a better productive performance at 1.45 SID Lys dietary level, a further economic analysis should be done to evaluate its economic benefit.

**Acknowledgements** The authors gratefully acknowledge funding from the Walsh fellowships programme.

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**Figure 1.** FCR performance from 84 growing-finisher pigs grouped by dietary treatments 0.92, 1.18 and 1.45% SID Lys and classified as SG and FG, from 15 to 21 weeks of age. a,b Significant differences between groups (P < 0.05).

# Comparison of performance in commercial hybrid broilers and off-sex chickens under different energy and nutrients levels

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**Application** Performance of commercial broiler chickens were significantly better than off-sex chickens however, off-sex chickens can be used in some circumstances as their performance was not much lower than the commercial ones.

**Introduction** Commercial broiler chickens are produced by breeder farms. Breeders (parent male and female) originated from grandparent flocks. Parts of male and female breeders are used to produce commercial hybrids of broiler chickens and the rest omitted as off-sex. The aim of this research is to evaluate the potential performance of day old male off-sex chickens in compare to commercial male broiler chickens under different energy levels.

**Material and methods** Ross male broiler chickens (600,1-d-old) and related off- sex male chickens (600,1-d-old) were randomly assigned to a completely randomized design experiment with 6×2 factorial arrangements with 4 replicates, 6 levels of metabolisable energy contents in diets number 1 to 6 diets (3060, 3010, 2960, 2910, 2860 and 2810 Kcal/Kg for starter (0-10 days) period; 3225, 3175, 3125, 3075, 3025 and 2975 Kcal/Kg for grower (11-28 days) period and 3275, 3225, 3175, 3075 and 3025 Kcal/Kg for finisher (29-49 days) period), respectively and two different genotypes (commercial broiler chickens vs.

off-sex chickens). Diet 2 was formulated based on Ross broiler recommendations; diet 1 was higher and diets 3 to 6 were lower than recommendations. The energy nutrients ratios were the same for all diets. The experiment was used to evaluate the individual and combined effects of these factors. The birds had 24-h access to feed, water and light. Ingredients samples were analyzed for dry matter, crude protein, ether extract, crude fiber, nitrogen free extract and ash. Metabolisable energy calculated base on NRC 1994 equation. Growth performance include body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) and carcass properties data were recorded. Data were analysed as a two-way factorial arrangement of treatments by using the GLM procedure of SAS. Significant differences among treatments (Duncan's multiple range test) were accepted if  $P \leq 0.05$ .

**Results** BWG, FI and FCR data for the experiment are given in Table 1. BWG and FCR in hybrids were better in comparison to off-sex. These traits were improved significantly when diets with higher levels of energy were consumed. This effect resulted from the reduced intake of chickens fed the highest level of energy. There was no interaction among diets and genotype in all traits.

Dressing percentage, breast and thigh meats as a percentage of carcass weight were unaffected by energy level of the diet, and these traits were not affected by the genotype. Percent abdominal fat was decreased in chicks fed diet with the lowest energy level.

**Conclusion** According to the performance data BWG, FI and FCR in commercial broiler chickens were significantly better than off-sex chickens. However BWG, FI and FCR of off-sex chickens were 97.3, 98 and 1.09 percent of commercial hybrid, respectively. The findings of this research suggest that off-sex chickens can be used in some circumstances.

## References

National Research Council, 1994. Nutrients Requirements of Poultry. National Academy Press, Washington, DC.

**Table 1** Mean body weight gain (BWG; g), feed intake (FI; g) and feed conversion ratio (FCR; 0-49 days)

	Diet						Genotype	
	1	2	3	4	5	6	Hybrid	Off-sex
BWG	2505a	2451ab	2404b	2399b	2328c	2307c	2431a	2366b
FI	4485c	4517bc	4530b	4521bc	4546ab	4571a	4576a	4489b
FCR	1.79c	1.84bc	1.89ab	1.88ab	1.95ab	1.98a	1.80b	1.97a

# Evaluation of an emulsifier blend on growth performance, nutrient digestibility, blood lipid profiles, and fecal microbial in growing pigs fed low energy density diet

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**Application** Energy is one of the major cost components in swine diets. Exogenous emulsifiers could support emulsification processing in gastrointestinal tract, thereby enhancing the digestion of lipids.

**Introduction** Fats and oils are ingredients commonly added to swine diets nowadays. They are the most concentrated sources of energy in addition to cereal grains and have positive effects on productivity in swine nutrition. However, because of their water insoluble chemical property, the digestion of lipid in gastrointestinal tract needs to be emulsified by bile salts and pancreatic lipase. The principal objective of this study was to determine the effect of dietary supplementation of emulsifier blend (10.21% GPGR(Glycerol Polyethylene glycol) and 2.2% LPL(lysophospholipids); GLB(Blend) in low energy density diets on growth performance, nutrient digestibility, blood lipid profiles, and fecal microbial in growing pigs.

**Material and methods** A total of 135 female and castrated male healthy growing pigs [(Yorkshire × Landrace) × Duroc] with an average initial BW of 25.07 ± 1.76 kg (54 days of age) were used in a 6 week experiment. Pigs were randomly allotted to 1 of 3 treatment diets according to initial BW and sex in a randomized block design. There were 9 replicated pens per treatment with 5 pigs (3 barrows and two gilts) per pen. Dietary treatments were: 1) CON: basal diet, 2) RET: reduced energy treatment (50 kcal/kg), 3) RET5, RET + 0.05% GLB. The energy was reduction by fat in this experiment. The diets were formulated to meet or exceed the recommendation of NRC (2012) for growing-finishing

pigs. All pigs were housed in an environmentally controlled room with a slatted plastic floor. Each pen was provided with a one-side self-feeder and a nipple drinker. Pigs were weighed on a pen basis on d 0 and d 42, and feed consumption was recorded throughout the experiment. ADG, ADFI and G/F were then calculated. During the last week of experiment, pigs were fed diets mixed with 0.2% chromic oxide as an indigestible marker to determine apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and crude fat (CF). At the end of the experiment on d 42, blood samples were taken from all the pigs by jugular venipuncture using vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). After collection, the serum samples from vacuum tubes were centrifuged (2,000 × g) for 30 min at 4°C and stored until further analysis. Blood lipid profiles, including total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride were analyzed using an automatic biochemistry analyzer with a commercial kit (Sigma Diagnostics, MO, USA). At the same time, fecal samples were collected from the pigs by via rectal massage. One gram fecal sample from each pen for fecal microbial flora was diluted with 9 ml of 1% peptone broth (Becton, Dickinson) and then homogenized. Viable count of bacteria in the fecal samples was then conducted by plating serial 10-fold dilution (in 1% peptone solution) onto MacConkey agar plates and lactobacilli medium III agar plates to isolate *E. coli* and *Lactobacillus* respectively. All data were subjected to statistical using the General Linear Model procedure of the SAS software (SAS Inst. Inc., Cary, NC). The pen was used as the experimental unit. Orthogonal contrasts used to separate treatment means were: CON vs. RET, CON vs. RET5 and RET vs. RET5. A P-value less than 0.05 was considered as statistically significant.

**Results** At the end of the experiment, pigs fed RET5 diet had significantly ( $P < 0.05$ ) increased BW, average daily gain (ADG), dry matter (DM) and crude fat (CF) digestibility, serum HDL-C and triglyceride concentrations, and fecal *Lactobacillus* counts compared with those fed RET diet. Moreover, pigs in RET5 group also showed significant ( $P < 0.05$ ) increase in DM and serum triglyceride concentration compared with CON groups.

**Conclusion** Supplementation of an emulsifier blend consisting of GPGR and LGP has significant improvement on growth performance, ATTD of DM and CF, serum HDL-C concentration, and fecal *Lactobacillus* population in growing pigs fed a low energy diet. The findings of the current study provided a positive evidence of the use of emulsifiers in reduced energy diets, which improves economic returns for producers.

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**Table 1.** Effects of emulsifier blend supplementation on growth performance and nutrient digestibility in growing pigs<sup>a,c</sup>

Items <sup>c</sup>	CON <sup>c</sup>	RET <sup>c</sup>	RET5 <sup>c</sup>	SEM <sup>c</sup>	P-Value <sup>c</sup>		
					CON vs. RET <sup>c</sup>	RET vs. RET5 <sup>c</sup>	CON vs. RET5 <sup>c</sup>
Final BW, kg <sup>c</sup>	56.13 <sup>c</sup>	55.57 <sup>c</sup>	57.24 <sup>c</sup>	0.50 <sup>c</sup>	0.4355 <sup>c</sup>	0.0311 <sup>c</sup>	0.1373 <sup>c</sup>
ADG, g <sup>c</sup>	740 <sup>c</sup>	726 <sup>c</sup>	766 <sup>c</sup>	11.88 <sup>c</sup>	0.4366 <sup>c</sup>	0.0308 <sup>c</sup>	0.1372 <sup>c</sup>
ADFI, g <sup>c</sup>	1,653 <sup>c</sup>	1,647 <sup>c</sup>	1,648 <sup>c</sup>	26.71 <sup>c</sup>	0.8654 <sup>c</sup>	0.9648 <sup>c</sup>	0.9002 <sup>c</sup>
G/F <sup>c</sup>	0.45 <sup>c</sup>	0.44 <sup>c</sup>	0.47 <sup>c</sup>	0.01 <sup>c</sup>	0.5904 <sup>c</sup>	0.0644 <sup>c</sup>	0.1700 <sup>c</sup>



# Animal Scientists and Cell-Cultured Meats: Learning from Past Science Communication Failures

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Misinformation around agriculture abounds. Like professionals dealing with other controversial scientific topics such as vaccine efficacy and safety, agricultural scientists have tried to counter fallacies and distortions with peer-reviewed evidence, data, and logic. As suggested by the science communication literature, such an approach is ineffective at changing people's perceptions.

Enter 2020, and the building of the first cell-cultured meat plant in Israel. Meat grown in a bioreactor from stem cells that will purportedly reduce carbon emissions, the land and water used for animal protein production, and reduce or eliminate farm animal suffering. And perhaps, in the opinion of Silicon Valley JUST CEO, "end world hunger". Rarely do such hubristic claims emanate from scientists, but rather from small companies in their efforts to raise venture capital investment. Such bold pronouncements are reminiscent of the early days of the biotechnology industry. There too, finance followed aspirations for a "tomorrow without hunger". Such promises opened up the biotechnology industry to attack by activist groups, who effectively created fear around genetically modified (GM) food by suggesting it was unnatural, and therefore dangerous to consumers and the environment. And while the target of their campaigns was large multinational companies, the impact was to preclude global access of academic researchers, small companies, and developing countries to the use of biotechnology in food production.

Many of these same groups, and sometimes even the same individuals, have since moved their "Frankenfood" sights towards cell-cultured and alternative meats. All of this leaves animal scientists in an awkward position. Much of the rationale behind cell-cultured meats invokes an overly simplistic narrative, especially as it relates to greenhouse gas (GHG) emissions, the nutritional attributes and dietary importance of animal-sourced foods, and the ecosystem services of grazing ruminant production systems. Some schadenfreude might seem justified when anti-GMO activists target the alternative meat industry, and perhaps it is tempting to augment the "unnaturalness" message of animal cell culture.

However, it bears repeating that the anti-GMO movement has employed such fear-based tactics to effectively turn public opinion against the use of genetic engineering to introduce sustainability traits like disease-resistance and nutrient fortification into global genetic improvement programs. It is hard to overestimate the deleterious environmental and human health impacts that have resulted from the demonization of this useful breeding method.

So what is the appropriate role for scientists in cell-cultured meat discussions, and what can we learn from past mistakes? The first is that countering the misinformation about animal agriculture that often accompanies the cultured meat discussion with facts alone, is unlikely to alter the narrative. However, framing the discussion around how nutritionists, geneticists, and veterinarians have collectively worked to also dramatically reduce the environmental footprint of animal-sourced food touches on shared values of reducing the environmental footprint of food production.

Likewise, the importance of grazing ruminants to non-arable working landscapes and the preservation of biodiversity is another story that resonates with many consumers. Storytelling is rarely invoked in science communication, and yet it is a powerful means of communication. Perhaps there is an opportunity to tell a story about how both cultured- and conventional meat producers are both working to address the global protein demand challenge. And that jointly we need to tell compelling stories to counteract the possibility that misinformation, fear, and false narratives will inhibit the adoption of safe innovations in global food production systems, to the detriment of all.



# Evidence about the sustainability of beef production

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**Application** Some say that the production of beef, and red meat in general, is not sustainable. The evidence quoted concerns only certain aspects of sustainability and refers mainly to feedlot system data. Here systems are compared and beef production evaluated taking account of a wide range of sustainability factors.

**Introduction** A system or procedure is sustainable if it is acceptable now and if its expected future effects are acceptable, in particular in relation to resource availability, consequences of functioning, and morality of action. A production system might be unsustainable because of inefficient usage of world food resources; adverse effects on human welfare, including health; poor welfare of production animals; harmful environmental effects, such as greenhouse gas production, low biodiversity or insufficient conservation; unacceptable genetic modification; not being "fair trade"; or damage to rural communities.

**Material and methods** Methodologies used in life cycle analysis and in measurement of system externalities<sup>1</sup> are used here to assess sustainability. The usage of land and conserved water are estimated for four methods of beef production from birth to slaughter in production and breeding animals<sup>2</sup>. Usage is for the production of plants that the animals eat directly and imported feed, mainly grain. Representative figures were obtained for beef production and density of animals in each system in an area where data for land and water use exist. Production and water consumption figures were adjusted to 27°C. Data on greenhouse gas production, human welfare, cattle welfare, biodiversity and cell-cultured cattle meat were obtained from published papers.

**Results** Beef produced without grain-feeding uses world resources more efficiently than if grain is fed and more efficiently than chicken or pork production unless these animals are fed on waste products. Cattle fed on pasture plus leaves of high protein shrubs and trees are 1.5 to 4 times more productive than cattle fed on fertilised pasture only<sup>3</sup>. The land area to produce one tonne of beef (hot carcass weight) was: 27ha for extensive unmodified pasture, 20.7ha for feedlots (pre-feedlot conditions extensive), 8.9ha for feedlots (pre-feedlot conditions irrigated pasture), 10.0ha for fertilised irrigated pasture, and 2.2ha for semi-intensive silvopastoral systems<sup>2</sup>. The conserved water usage in l. kg beef<sup>-1</sup> was: 673 l. for feedlots (pre-feedlot conditions irrigated pasture), 553 l. for feedlots (pre-feedlot

conditions extensive), 411 l. for fertilised irrigated pasture, 155 l. for extensive unmodified pasture, and 87 l. for semi-intensive silvopastoral system<sup>2</sup>. Human health varies with the amount of beef consumed and the proportion of saturated fat. The welfare of beef cattle is: best on semi-intensive silvopastoral systems because of shade, lower disease incidence, opportunities to hide and food selection opportunities; good on extensive pasture if food supply is good and shade is available; and worst in feedlots. Biodiversity is best on silvopastoral systems and worst in feedlots. Greenhouse gas production varies greatly with system and cattle diet. Tannins and other methanogen suppressors can greatly reduce methane production. High producing systems, such as feedlots and semi-intensive silvopastoral systems, produce less greenhouse gas per unit of beef<sup>3</sup>. Data show that cell-cultured cattle meat may prove sustainable in the future.

**Conclusion** Lack of sustainability is causing consumers to stop buying beef. Beef production is sustainable if the best systems are used but the use of feedlots with grain feeding is not sustainable. Where they can be used, semi-intensive silvopastoral systems are the best beef production systems for all aspects of sustainability. Well-managed pasture-fed beef from areas where crop production is uneconomic is also sustainable.

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# Where to invest first? Sensitivity of beef carbon footprints to emission factors under two evaluation methods

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**Application** The current debate surrounding roles of livestock farming in sustainable agriculture often overlooks discussions on R&D priorities. This paper addresses this issue through a novel uncertainty analysis.

**Introduction** Agriculture, and in particular pasture-based ruminant production, has high degrees of uncertainties associated with natural processes that underpin production (McAuliffe et al., 2018). In the presence of these uncertainties, point-estimates provided by life cycle assessment (LCA) models may not be sufficiently informative to offer robust policy implications. Furthermore, most LCA methods currently used to evaluate climate impacts use a single emission metric to aggregate multiple greenhouse gases, which can obscure some important dynamics (Lynch, 2019). This paper explores the effect of emission factor uncertainty on emissions reported using global warming potential (GWP100) and global temperature potential (GTP100), two alternative emission metrics which have a significant impact on the valuation of methane relative to nitrous oxide, resulting from their differing atmospheric lifespans of around 12 and 114 years, respectively.

**Materials and methods:** The study was carried out at the permanent pasture beef enterprise on the North Wyke Farm Platform (NWFP), a BBSRC National Capability grazing trial in Devon, UK, from 2016-2017. The system boundary included both breeding and finishing herds. The functional unit was set as 1 kg liveweight (LW) departing the farmgate. On-farm emissions were calculated using a modified IPCC Tier 2 approach (McAuliffe et al., 2018). A 9 × 10 factorial virtual experiment

was designed to include various combinations of gross energy methane conversion factors ( $Y_m$ : 4.5-8.5 %) and nitrous oxide emission factors (e.g. losses of nitrous oxide from nitrogen-applied) for faeces and dung deposited on pasture (EF3: 0.4-4.0 %), of which values have previously been shown to be the most important drivers of emissions uncertainty (Takahashi et al., 2019). Carbon footprints were calculated for each scenario under GWP100 and GTP100, using IPCC 5th Assessment Report values (GWP100 used 28 for methane and 265 for nitrous oxide; GTP100 used 4 for methane and 234 for nitrous oxide).

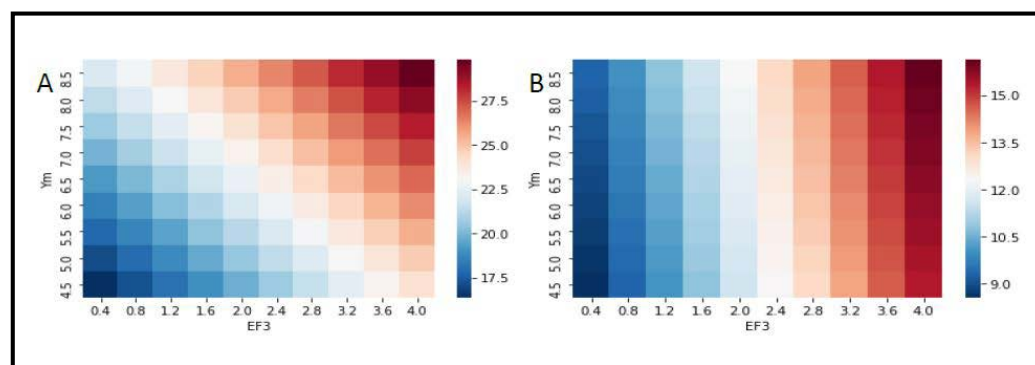
**Results** Model assumptions and choice of impact method were both shown to affect reported mid-point climate impacts considerably (Figure 1). Under GWP100, methane arising from enteric fermentation accounted for 39.9% of total emissions in the default scenario, whilst under GTP100, it only accounted for 9.1%. Under GWP100, reductions in emission factors equally resulted in lower mid-point impacts; under GTP100, however, a smaller  $Y_m$  value does not produce notable improvement in mid-point impacts. Different nitrous oxide emission factors had a major impact under both emission metrics.

**Conclusion** While methane receives considerable attention in livestock sustainability assessments due to its dominance in carbon footprints using GWP100, the most common emission metric, its relative importance decreases if using GTP100, which emphasizes different climate behaviours. Meanwhile, nitrous oxide plays a key role in determining emissions intensities under both metrics and therefore R&D priorities might be better placed in this area for grazing livestock. Although end-point (physical) climate modelling is required to fully evaluate the relative superiority of different LCA methods, exploring different emission metrics can provide insight into important climate dynamics. This finding suggests that focusing solely on methane may be a suboptimal intervention strategy to mitigate climate impacts associated with ruminant production systems.

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**Figure 1.** Heatmaps of GWP100 (A) and GTP100 (B) for the 90 scenarios. Values represented as kg CO<sub>2</sub>-eq/kg liveweight.

# Reducing enteric methane emissions from dairy-beef steers with a blend of essential oils

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**Application** Supplementing diets of Holstein steers with a blend of essential oils reduces the volume of methane they emit but does not affect the conversion of feed into animal growth.

**Introduction** Reducing the environmental impact of beef production is of increasing importance to the industry. Various feed additives have been proposed to reduce enteric methane (CH<sub>4</sub>) emissions from cattle. Beef from dairy is an increasingly important contributor to the UK beef industry, thus the objectives of this study were to investigate the efficacy of a commercial blend of essential oils (Agolin Ruminant, containing coriander oil, geranyl acetate and eugenol) to reduce CH<sub>4</sub> emissions and/or increase feed efficiency of dairy steers.

**Material and Methods** Holstein steers (n = 36) were separated into six blocks (balanced for body weight and farm of origin), and then paired by body weight. One of each pair was randomly assigned to either the control (C) or treatment (T) group and allocated to one of 6 respiration chambers (one animal from each block, and 3 C and 3 T animals per chamber). Three CH<sub>4</sub> emission measurements were made per animal in indirect open-

circuit respiration chambers (pre-treatment baseline, 6 weeks and 16 weeks after treatment introduction). The basal diet was a 50:50 forage:concentrate (DM basis) total mixed ration (TMR), with the T group receiving the Agolin Ruminant at a rate of 1.1 ± 0.1 ml/head/day (applied to concentrates prior to TMR mixing). Dry matter intake (DMI) of individual animals was monitored throughout the trial. Feed conversion ratio (FCR) was calculated between the 2nd and 3rd CH<sub>4</sub> emission measurements. The data was analysed by ANOVA using the car and lme4 packages in R.

**Results** After accounting for block and chamber effects, and including baseline CH<sub>4</sub> emissions as a co-variate, there were significant differences between the T and C groups at 6 weeks after introduction (C: 24.7 ± 4.01 gCH<sub>4</sub>/Kg DMI, T: 22.8 ± 3.42 gCH<sub>4</sub>/Kg DMI, p < 0.05) and 16 weeks after introduction (C: 25.5 ± 3.77 gCH<sub>4</sub>/Kg DMI, T: 22.7 ± 3.40 gCH<sub>4</sub>/Kg DMI, p < 0.01) (Table 1). This equates to an 8-9% reduction in CH<sub>4</sub> emissions. There was no significant difference in FCR between the T and C groups.

**Conclusion** Our results show that supplementing feed with a blend of essential oils (coriander oil, geranyl acetate and eugenol) is an effective means of reducing enteric CH<sub>4</sub> emissions from Holstein steers.

## Acknowledgements

This work was funded by the Scottish Government's Strategic Research Programme and Agolin SA.

Measurement time point	Treatment group mean	Control group mean	Significant difference between group means (p)	Variable significance (p)			
				Block	Chamber	Treatment	Baseline
Baseline	22.2 (3.02)	23.1 (4.27)	ns	-	-	-	-
6 weeks after introduction	22.8 (3.42)	24.7 (4.01)	<0.01	<0.01	<0.05	<0.05	ns
16 weeks after introduction	22.7 (3.40)	25.5 (3.77)	<0.01	<0.05	ns	<0.01	ns

**Table 1:** Treatment and control group means (standard deviations in parenthesis) for methane emissions (g CH<sub>4</sub> / kg DMI) at each measurement point. Significant differences between group means (ANOVA) and significance of variables (ANOVA) included in linear models for methane measurements made at 6 weeks and 16 weeks after introduction of the treatment.

# The effect of fibre type and dietary fat addition on finishing pig performance and odour and ammonia emissions

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**Application** Diets high in soluble fibre require additional fat to maintain performance and ammonia emissions were variable between pigs but appeared to be lowered with high levels of fibre.

**Introduction** Dietary fibre and fat have been reported to influence the microbiota of the intestinal tract thus positively impacting on odour and ammonia output (Agyekum et al. 2017), but there has been limited research conducted on the interactive effect of fibre and fat in diets. Fibre has been reported to reduce performance through decreased intake and digestibility (Gao et al. 2015) but the effect of the type of fibre (soluble or insoluble) within feed ingredients has not been well established. The aim of this study was to investigate the effect of fibre type with and without fat addition and increasing dietary fibre content on finishing pig performance and odour and ammonia emissions.

**Materials and methods:** Boar pigs (n=36) were individually housed in batches of six, from 10 weeks of age and offered a standard diets until 14 weeks of age (185g/kg crude protein (CP), 15.0MJ/kg digestible energy (DE)). At 14 weeks of age, pigs were assigned to one of six treatment diets; 1) low fibre, low fat (CP 150g/kg, DE 13.8MJ/kg, fibre 34.2g/kg, oil A 20.9g/kg), 2) low fibre, high fat (CP 147g/kg, DE 14.3MJ/kg, fibre 33.5g/kg, oil A 39.9g/kg), 3) high insoluble fibre, low fat (CP 152g/kg, DE 13.3MJ/kg, fibre 60.5g/kg, oil A 29.3g/kg), 4) high insoluble fibre, high fat (CP 150g/kg, DE 13.8MJ/kg, fibre 60.0g/kg, oil A 48.4g/kg), 5) high soluble fibre, low fat (CP 151g/kg, DE 13.4MJ/kg, fibre 60.3g/kg, oil A 21.2g/kg) and 6) high soluble fibre, high fat (CP 150g/kg, DE 13.8MJ/kg, fibre 60.0g/kg, oil A 35.2g/kg). Soluble sources of fibres were sugar beet pulp and soya hulls. Insoluble sources of fibre were rapeseed, dried distillers grains soluble and wheat bran. Individual pig performance (daily feed intake (DFI), average daily gain (ADG) and feed conversion ratio (FCR)) were determined for three weeks.

**Table 1.** The effect of fibre level, fibre type and fat level on performance and odour and ammonia emissions

	Low fat			High fat			Effect of fibre		Effect of fat		Interaction	
	Low fibre	High I fibre	High S fibre	Low fibre	High I fibre	High S fibre	SEM	P	SEM	P	SEM	P
DFI (g/d)	2.68a	3.02bc	2.85ab	3.18c	2.82ab	3.00bc	0.067	NS	0.054	NS	0.096	<0.01
ADG (g/d)	1.24a	1.33ab	1.19a	1.41b	1.23a	1.40b	0.031	NS	0.038	NS	0.055	<0.05
FCR	2.16	2.29	2.39	2.27	2.31	2.15	0.059	NS	0.048	NS	0.085	NS
Ammonia emission (mg/hr)	191.5	86.4	127.6	208.5	108.8	106.7	36.04	NS	29.97	NS	50.30	NS
Odour emission (Oue/Sec)	1.62	1.39	1.49	1.72	1.71	1.33	0.185	NS	0.154	NS	0.258	NS
Water intake (L/day)	6.40	5.37	6.95	4.86	5.37	4.82	0.526	NS	0.437	NS	0.734	NS
Slurry output (L/day)	2.32	2.30	3.20	2.10	2.02	1.62	0.309	NS	0.257	NS	0.526	NS

After three weeks on the diets, pigs were transferred to individual calorimetry and after ~24 hrs in the chambers, the analysers recorded ammonia production for a total of ~73 hrs. At ~28 hours of recording, odour offensiveness was measured using dynamic olfactometry (BSEN13725:2003). Dry matter intake (DMI) was recorded. Pigs were dissected and samples of the gastrointestinal tract taken for microbiota profiling (not presented). Data were analysed using a general analysis of variance as a three (fibre level) x two (fat level) factorial design. Start weight was applied as a covariate for the performance data and DMI was applied as a covariate for the ammonia and odour emission data. Interactive and main effects were tested for.

**Results** There was an interactive effect ( $P<0.05$ ) of fibre level/source and fat addition on DFI and ADG (Table 1). DFI of pigs offered the low fibre diet with additional fat was increased whereas, fat addition had no significant effect on DFI of pigs offered diets high in soluble or insoluble fibre although, there was a tendency for DFI to be increased when fat was added to diets high in soluble fibre. ADG was increased when fat was added to low fibre and high soluble fibre ( $P<0.05$ ) but not when fat was added to diets high in insoluble fibre. FCR was not significantly different across treatments but there was a wide variance (2.15 to 2.39). There was no significant effect of fibre level/source or fat addition on ammonia or odour emissions or on water intake or slurry output (Table 1). However, there was a tendency for water intake and slurry output to be lower for pigs offered diets containing higher levels of fat. Ammonia emissions were highly variable across treatments but on average, were higher (NS) for pigs offered a low fibre diet (200mg/hr) than for pigs offered the other diets. Diets high in insoluble fibre resulted in the lowest (NS) ammonia emissions (97mg/hr). Odour emissions followed the same overall trend.

**Conclusions:** The effects on performance suggest that varying fibre level/source and fat level impacts on feed intake and ADG. It is difficult to conclude if the differences observed were intake driven or as a result of improved efficiency as there were wide variance in FCR which were non-significant. However, the results suggest that high insoluble fibre may reduce performance and that diets high in soluble fibre require additional fat to maintain intake and ADG in finishing. The results also indicate that diets low in fibre should contain high fat addition which is reflective of current industry practice. Higher levels of fibre resulted in reductions in ammonia and odour output but the differences were not significant due to individual pig variability. A possible shift in gut microbiota due to fibre level is suggested. This will be confirmed when the results of the gastrointestinal microbiota are analysed.

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# Effects of phosphorus sources and sowing methods on the fibre fractions of *Stylosanthes guianensis*

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**Application** The understanding of better source of fertilizers and sowing methods will help producers anticipate and plan for changes in forage quality

**Introduction** Ruminant animals play an important role in the farming systems in the production of milk, meat, draught power, manure as well as hides and skins (Ojo et al., 2016). Natural grazing lands constitute the major feed resources in the developing countries, especially in Africa countries by providing more than 90% of animal feed requirements (Lulseged, 1985; Kitaba and Tamir, 2007). The low availability of phosphorus nutrition in soils has become the "limiting factor" for plant and root growth (Borch et al., 1999; Kanako et al., 2004) especially after plants have gained sufficient nitrogen nutrition (Woolmanse and Duncan, 1980). The concentration of chemical components in the forage varies depending on many variables such as plant species (Ramirez et al., 2004), fertilization (Türk et al., 2007). Therefore, forage quality is determined by the content of different nutrients.

**Materials and methods:** The field work was carried out at the Pasture unit of the Directorate of University Farms, while the chemical analysis was carried out at the Department of Pasture and Range Management laboratory, Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. The land was cleared, stumps were removed on the total area of 589 m<sup>2</sup> (31 m x 19 m) and pegged, having each plot measured (4 m x 4 m). A boundary of 1 m was made between the sub-plots and 2 m between replicates. Soil samples were collected using a soil auger and were taken for analysis to determine the pre-sowing nutrient status of the soil (Table 1). Fertilizers used were poultry manure, Single super phosphate (SSP) and Aleshinloye organo-mineral fertilizer. Sub samples from each fertilizer was taken to the laboratory to determine the nutrient composition (i.e. phosphorus content). The rate of application for the fertilizers was 80kg P/ha. The quantity of each manure applied was calculated based on the nutrient composition (i.e. phosphorus) content of the fertilizers. The study was a 4 x 2 factorial arrangement laid out as a split plot design. The fertilizer sources were assigned to the main plot while the methods of sowing were assigned to the sub plots. Samples were harvested at 10 weeks after sowing at 10 cm above ground level and oven dried at 65°C to constant weight. The dried samples were milled and analyzed for Fibre fraction analysis according to Van Soest et al., 1991. Data collected was analyzed using two-way analysis of variance (ANOVA) and treatment means was separated using Duncan's Multiple Range Test of the SAS (2000) package.

**Results** The phosphorus sources were significant ( $P < 0.05$ ) only on the NDF content of *Stylosanthes guianensis* (Table 2). The differences in the NDF, ADF, ADL, Hemicellulose and cellulose values were not significant ( $P > 0.05$ ). The NDF content significantly decreased with poultry manure fertilization (52.33%) compared to the unfertilized *Stylosanthes guianensis* (61.33%). The NDF content

recorded for the *S. guianensis* fertilized with the other sources of phosphorus used did not differ significantly from the control. There were no significant changes in the fibre content (i.e. NDF, ADF, ADL, hemicellulose, cellulose) with the sowing methods. The interaction between fertilizers and sowing methods significantly affected the NDF content only as shown in Table 3. Values for NDF ranged from 46.00% for drilled Stylo fertilized with poultry manure to 63.33% for broadcasted unfertilized Stylo and drilled Stylo fertilized with single super phosphate (SSP).

**Conclusion** The study shows that decline in the NDF content of *S. guianensis* with phosphorus fertilization was more pronounced with poultry manure while adopted sowing methods did not influence the fibre fractions of *S. guianensis*.

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**Table 1:** Selected mineral content of used phosphorus sources

Element	Poultry manure (%)	Aleshinloye organo-mineral fertilizer (%)	SSP (%)
Nitrogen	1.56	3.00	-----
Phosphorus	3.27	2.50	20
Potassium	17.53	1.50	-----

**Table 2:** Effect of phosphorus source and sowing method on the fibre fractions of *Stylosanthes guianensis*

	NDF (%)	ADF (%)	ADL (%)	Hemicellulose (%)	Cellulose (%)
<b>Phosphorus sources</b>					
Control	61.33 <sup>a</sup>	21.67	6.33	39.67	15.33
AOF	59.33 <sup>ab</sup>	27.00	7.00	32.33	20.00
Poultry manure	52.33 <sup>a</sup>	21.00	5.17	31.33	15.83
SSP	58.67 <sup>ab</sup>	26.00	6.17	32.67	19.83
SEM	2.95	3.00	1.41	3.18	2.64
<b>Sowing Methods</b>					
Broadcast	59.50	25.00	6.50	34.50	18.50
Drilling	56.33	22.83	5.83	33.50	17.00
SEM	2.17	2.18	1.00	2.38	1.82

ab : means on the same column with different superscript were significantly ( $P < 0.05$ ) different. S.E.M: Standard Error of Mean; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin; P. sources: Phosphorus sources; AOF: Aleshinloye Organo-mineral Fertilizer.

**Table 3:** Interaction between phosphorus source and sowing method on the fibre composition of *Stylosanthes guianensis*.

		NDF (%)	ADF (%)	ADL (%)	Hemicellulose (%)	Cellulose (%)
<b>Phosphorus sources</b>						
Control	Broadcast	63.33 <sup>a</sup>	24.00	5.33	39.33	18.67
	Drilling	59.33 <sup>a</sup>	19.33	7.33	40.00	12.00
AOF	Broadcast	62.00 <sup>a</sup>	29.33	10.00	32.67	19.33
	Drilling	56.67 <sup>ab</sup>	24.67	4.00	32.00	20.67
Poultry manure	Broadcast	58.67 <sup>a</sup>	23.33	5.33	35.33	18.00
	Drilling	46.00 <sup>b</sup>	18.67	5.00	27.33	13.67
SSP	Broadcast	54.00 <sup>ab</sup>	23.33	5.33	30.67	18.00
	Drilling	63.33 <sup>a</sup>	28.67	7.00	34.67	21.67
<b>SEM</b>		3.33	4.37	1.93	4.68	3.60

ab : means on the same column with different superscript were significantly ( $P < 0.05$ ) different. S.E.M: Standard Error of Mean; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin; P. sources: Phosphorus sources; AOF: Aleshinloye Organo-mineral Fertilizer



# Per area and per-animal performance of lambs grazing contrasting swards submitted to cell or continuous grazing

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**Application** Combining floristically diverse swards with intensive grazing strategies can be used to increase land productivity and biodiversity with no detrimental effect on individual animal performance in grazing systems.

**Introduction** Plant diversity has often been shown to increase productivity and stability in natural grasslands (Tilman 1996). However, grazing strategies that aim to maximize per-area animal performance have been shown to compromise the individual performance of grazing livestock (Macdonald et al., 2008). Our aim was to compare per-area and per-animal performance of swards with different levels of plant diversity submitted to contrasting grazing strategies, i.e., cell grazing vs. continuous stocking with sheep.

**Material and methods** The twelve plots at Rowden site (Devon, UK) had two levels of drainage (D, enhanced, standard), with six plots each. These plots were randomly assigned to six factorial treatments: two grazing strategies (G, cell grazing, CELL; continuous stocking, CONT) and three sward types (S, permanent pasture, PP; grass mixture, GM; multispecies mix, MS; reseeding in autumn 2017). Inorganic N applied (kg/ha) during April-June was: 40 for GM-CELL, 80 for GM-CONT, 40 for PP-CONT and 65 for PP-CELL plots (no N added to MS plots). At lambing (April 2019) 15 ewes (average LW 73 ± 10.7 kg) and their 26 lambs were allocated to each plot. The CELL plots were divided into 60 equal cells and six cells were allocated for grazing every 2-d (20-d rotation). In CONT sheep had access to graze the entire plot. Lambs were weighed from birth periodically. At weaning, ewes were removed from the plots. Following this, only lambs grazed the swards. Weekly estimations of the herbage mass (HM) were performed with a plate-meter (F400, Farmworks, NZ) using the equation 'kg DM/ha = 125 x + 640' (individual equations are being developed for the different swards). Modifying the number of grazing animals was used as a management tool to match forage availability and animals' demand. The allocation of treatments was orthogonal to D, and the allocation allowed for a number of

constraints associated with the spatial arrangement of the plots (a constrained randomisation procedure). The design approach allowed analysis within ANOVA to consider the main effects of all three factors – D, S, G – and the two 2-factor interactions – G x S and D x S using Genstat® 18th edition. Data reported here relates to assessments until late August 2019 only (lambs had to be temporarily removed from the plots for sanitary treatment).

**Results.** Lambs' average daily gain was similar among groups (F pr > 0.05), averaging 0.259 kg/d per lamb over a 20-w period. Given the interaction between S and G (F pf < 0.05), total lamb LW produced per ha after 20-w from lambing was similar between MS and PP under the CG and both were greater than the GM. However, MS had the greatest value under the CS, followed by PP and the lowest value was in the GM (Table 1). Average HM was 19% greater in the MS and PP compared with the GM swards (F pr < 0.05), and 18% greater in CELL than CONT plots (Table 1). Despite differences, the percentage of lambs finished after 20-w did not vary significantly with the swards or grazing options (F pr > 0.05), averaging 55.8 % (Table 1). Note, eight ewes and their 12 lambs were removed from the two GM-CONT plots after 4-w due to a low average HM (<1800 kg DM/ha) to reduce grazing pressure (similar to the PP's).

## Figure 1

**Table 1** Average daily gain (ADG), live-weight (LW) gained per hectare, percentage of lambs finished after 20 weeks, and average herbage mass from grazing plots combining three sward types (permanent pasture, PP; grass mixture, GM; multispecies mix, MS) submitted to two grazing managements (cell grazing, CELL; continuous stocking, CONT).<sup>1</sup>

	Cell grazing			Continuous stocking			F pr (residual df = 3)			
	GM	MS	PP	GM	MS	PP	LSD	S (df = 2)	G (df = 1)	S x G (df = 2)
ADG (g/d)	240	269	273	273	262	237	40.5	0.548	0.692	0.071
LW gained (kg/ha)	809	857	878	705	879	803	67.6	0.010	0.024	0.049
Lambs finished at 20 weeks (%)	42.3	73.1	67.3	51.0	61.5	40.4	3.6	0.165	0.223	0.227
Average herbage mass (kg/ha)	2296	2940	2384	1974	2379	2097	397.2	0.019	0.012	0.366

<sup>1</sup>Neither the drainage factor (D) nor the interaction D x S were significant (F<sub>pr</sub> > 0.05); Df: degrees of freedom

**Conclusion** Over the first 20 weeks following lambing, CELL increased productivity per hectare without showing detrimental effects on individual animal performance when compared to CONT management practices. MS swards were as productive as PP, although both surpassed GM swards. However, to understand forage persistency and resilience the effect of sward type and grazing strategy on the nutritional quality of the herbage should be assessed in the long-term.

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# Use of Near Infrared Spectroscopy (NIRS) for nutritional quality analysis of haylage

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**Application** Analysis obtained by NIRS is used to inform decisions based on forage quality. An inaccurate prediction for key nutritional analytes could have both an economic impact and implications for animal health.

**Introduction** Assessing the quality of forage before feeding equids is routine for optimisation of nutrition and subsequently animal health. Commonly, samples are sent for analysis to commercial laboratories that scan fresh samples using NIRS and obtain results by using spectral data fitted to a prediction model. Results are obtained quickly with minimal sample processing thereby increasing the number of samples that can be processed compared with chemical methods. Here we compared the results gained from fresh scanning NIRS and industry standard wet chemistry to determine the level of accuracy of NIRS from a commercial facility.

**Material and Methods** Samples from 66 bales of haylage across seven holdings in Devon and Dorset (Donkey Sanctuary) were analysed by NIRS and wet chemistry. Samples were obtained by taking 6 cores/bale, placing in a large bowl and mixing the samples by hand to provide a single composite sample per bale. The composite sample was immediately subsampled into 2. Both subsamples were immediately vacuum packed and posted for fresh NIRS analysis following instructions from the commercial laboratory, the second was analysed for dry matter (DM) (oven dry method), ash (loss on ignition method), water

soluble carbohydrate (WSC) (Luffschoorl method), Neutral detergent fibre (NDF) (Ankom method) and crude protein (Kjeldahl N method) by standard wet chemical methodologies at an ISO/IEC17025:2005 accredited laboratory.

**Statistical analyses** Data obtained by wet chemistry and NIRS for each sample were compared. For each analyte, Pearson correlation coefficients were calculated and used to generate R<sup>2</sup> values. Bland-Altman analysis was used to determine agreement between the two methods and to test if there was an overall bias in one method over the other by calculating the average difference of all values in the dataset.

**Results** The results obtained for DM (Table 1) show a good agreement between NIRS predicted and oven drying with an R<sup>2</sup> of 0.95. However, the results for water soluble carbohydrates, NDF, ash and crude protein showed poor agreement between the samples with R<sup>2</sup> ranging between 0.053 and 6E-05 and average bias ranging from 111.63 to 24.64 g/kg. The poorest predictions were for WSC where the average bias was 24.64 g/kg with upper and lower differences of 133.18 and -75.76 g/kg for samples that had wet chemistry results of 213.88 and 24.23 g/kg respectively.

**Conclusion** NIRS provides rapid assessment of forage for nutritional quality parameters. However, some analytes were very poorly predicted compared to the wet chemistry. Of particular concern for equids would be the inaccurate prediction of WSC as this is a key nutritional factor implicated in laminitis in equids. In addition, ash which provides a gauge of soil contamination and therefore an indicator of the risk of contamination from members of the Clostridium genus which are also a significant health risk to equids. This work highlights the need for continually updated calibrations to allow for seasonal variation in commercial calibrations that are being used for prediction on forage. Therefore, it is not recommended to make decisions based on dietary requirements or hygienic quality using results that have been obtained by fresh NIRS prediction.

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**Table 1.** Mean, SD and range of wet chemistry values and R<sup>2</sup>, average bias between methods and SD of bias values for five key analytes measured by NIRS and wet chemistry for 66 samples of haylage.

Analyte	Mean of wet chemistry	SD of wet chemistry	Range (Min-max) wet chemistry	R <sup>2</sup> value	Average bias between methods	SD of bias between methods
Dry matter g/kg	668.47	142.81	321.27-921.93	0.9467	39.23	43.145
Water Soluble carbohydrates g/kg	111.31	49.06	24.23-229.42	6E-05	24.6456	50.107
NDF g/kg	630.57	41.66	525.85-697.12	0.0087	111.63	47.962
Ash g/kg	76.81	13.69	53.00-113.50	0.053	7.42	14.448
Crude Protein g/kg	88.97	17.84	47.31-129.01	0.0314	-22.08	20.142

# Can commercial enzymes be used to enhance the nutritional composition of *Tenebrio molitor* (yellow mealworm) as an alternative protein source

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**Application** Exogenous phytase has the potential to be used in wheat bran based diets to improve mineral status of *Tenebrio molitor* (yellow mealworm) further enhancing it as component for animal feeds

**Introduction** The ever increasing global population is creating growing demand for meat (Bedford et al. 2017) putting additional pressure on traditional feed ingredients (e.g. soyabean meal). *Tenebrio molitor* larvae (TM) have a similar nutrient composition and could act as a partial replacement of these ingredients. Wheat bran is used as the main feed for TM, however it contains high levels of phytate which decreases feed digestibility and reduces mineral absorption. In other production species exogenous phytase is added to feeds to digest the phytate and reduce the associated anti-nutritional effects thereby releasing bound minerals. The aim of this study was to compare the effect of adding exogenous phytase to wheat bran based diets on mineral composition of TM.

**Materials and Methods** Wheat bran (WB) was treated by soaking in either water, water + low dose (500FTU/L) phytase or water + high dose (5000FTU/L) phytase for 6 hours, then dried until a constant weight. TM (mini form) were obtained from Monkfield Nutrition Ltd and acclimatised on a WB diet for 3 days. At day 0, TM were transferred to a plastic dish (n=200/dish) and allocated to one of four treatment groups (4 dishes per treatment): Control (WB only), Water (WB soaked in water), Low (WB soaked in water + 500FTU/L phytase) and High (WB soaked in water + 5000FTU/L phytase). TM were kept in a dark warm (25±5°C) environment and water was supplied through cotton wool. TM were fed *ad libitum* for 14 days, with feed and water consumption measured and refreshed on days 4, 7 and 11, and any dead removed. At day 14, any dead were removed and final measurements taken before TM were killed by cold exposure and stored at -20°C. Samples were freeze dried then analysed by inductive-coupled plasma mass spectrometry (ICPMS) to determine mineral content. Data were analysed using one way ANOVA (Genstat 19th Edition), when significant (P<0.05) a Bonfferoni post hoc test was carried out.

**Results** Compared to controls, any treatment of wheat bran (water, low or high phytase) resulted in significant increases in calcium, magnesium, iron, and phosphorus contents of mealworms, but a decrease in selenium content (all P<0.05, Table 1). Inclusion of low dose phytase unexpectedly reduced the phosphorus content compared to water only, while high dose phytase significantly increased the zinc, sodium and manganese contents of mealworms compared to the other treatments (Table 1).

**Table 1:** Effects of treatment of wheat bran (water, low or high phytase) on mineral composition of remaining live TM on day 14.

	Control	Water	Low	High	SED	P
Calcium (g/kg)	0.47 <sup>a</sup>	0.64 <sup>bc</sup>	0.58 <sup>b</sup>	0.67 <sup>c</sup>	0.02	<.001
Magnesium (g/kg)	2.90 <sup>a</sup>	3.54 <sup>bc</sup>	3.11 <sup>ab</sup>	3.65 <sup>c</sup>	0.15	<.001
Iron (mg/kg)	47.15 <sup>a</sup>	73.18 <sup>b</sup>	67.69 <sup>b</sup>	80.48 <sup>b</sup>	4.19	<.001
Zinc (g/kg)	0.12 <sup>a</sup>	0.16 <sup>ab</sup>	0.18 <sup>b</sup>	0.22 <sup>c</sup>	0.01	<.001
Sodium (g/kg)	0.95 <sup>a</sup>	1.15 <sup>b</sup>	1.10 <sup>b</sup>	1.28 <sup>c</sup>	0.03	<.001
Manganese (mg/kg)	12.19 <sup>a</sup>	18.36 <sup>b</sup>	16.64 <sup>ab</sup>	24.29 <sup>c</sup>	1.59	<.001
Phosphorus (g/kg)	8.69 <sup>a</sup>	9.91 <sup>b</sup>	8.58 <sup>a</sup>	9.85 <sup>b</sup>	0.29	<.001
Selenium (mg/kg)	0.25 <sup>a</sup>	0.20 <sup>b</sup>	0.18 <sup>b</sup>	0.21 <sup>b</sup>	0.01	0.003

**Conclusion** Pre-treatment of wheat bran with water or with water and exogenous phytase affected the mineral content of mealworms. Water soaking alone induced some changes with no added effect of exogenous phytase for calcium, magnesium, iron, and phosphorus, potentially due to endogenous phytase in the wheat bran being activated by the water. The lack of effect of Low dose phytase (compared to Control) on phosphorus and magnesium contents was unexpected, but variability in the original mealworm mineral contents before treatment could be the cause of this. The High dose of phytase significantly increased the content of zinc, sodium and manganese contents. The greatest differences were in zinc levels, which might be expected since phytate is known to preferentially chelate zinc thereby making it unavailable. Further work is needed to investigate the variability of mealworm mineral contents and to refine the use of different doses of exogenous phytase in mealworm diets.

**Acknowledgements** The work was gratefully supported by funding from AB Agri, AB Vista and BBSRC.

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# Effect of dietary crude protein concentration and brown seaweed type on ruminal fermentation parameters in vitro using the rumen simulation technique (RUSITEC)

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Effect of dietary crude protein concentration and brown seaweed type on ruminal fermentation parameters in vitro using the rumen simulation technique (RUSITEC)

Application Nitrogen loss and methane emissions from ruminant systems represent environmental concerns. A combination of brown seaweed and lower crude protein concentration displayed potential to reduce nitrogen and methane emissions.

**Introduction** Nitrogen utilisation in beef cattle is low 10–20% (Cole & Todd, 2009) which is responsible for 80% of the ammonia emissions in Ireland. Among all seaweeds, brown seaweeds (BS) are the only ones able to produce high levels of Phlorotannins (PT). The major tannins found in terrestrial plants are condensed tannins and hydrolysable tannins which have been shown to decrease microbial utilisation of plant nutrients by direct inhibition of microbial activity and indirectly by forming complexes with the nutrients (Min et al., 2003). It's been previously reported that *Ascophyllum nodosum* promoted a decrease in N degradability (~24%) (Belanche et al., 2016) with similar results found with PT from *Laminaria digitata* (Vissers et al., 2017). Therefore, the objective of this study was to evaluate the effects of supplementing diets with differing crude protein

(CP) levels with different brown seaweeds, on the manipulation of rumen fermentation in vitro using artificial Rumen Simulation Technique (RUSITEC).

**Material and Methods** The experiment consisted of a single incubation period using three RUSITEC systems (8 vessels/system). Each vessel was considered an experimental unit, in a completely randomized design with a 2 x 4 factorial arrangement in which a lower 12% (LP) and higher 16% (HP) CP levels and BS species at 50 g kg DM-1 of; no seaweed CO, *Alaria esculenta* ALE *Ascophyllum nodosum* ASC, *Himanthalia elongata* HIM, were compared. The in vitro basal diet consisted 50:50 grass silage: concentrate on a dry matter (DM) basis. Concentrates in the LP diets consisted of maize meal while the concentrate in the HP diets consisted of maize meal and maize distillers' grains. Rumen inoculum was sourced from four rumen-cannulated dairy cows. Data was found to be normally distributed and analysed using Proc Mixed procedure in SAS. Significant differences were considered when  $P < 0.05$ , while  $P < 0.1$  was considered as a tendency towards significance.

**Results** There was a tendency towards an interaction ( $P < 0.10$ ) between CP level and BS addition on  $\text{NH}_3$  concentration. Independent of CP %, the addition of BS reduced  $\text{NH}_3$  concentrations on overflow vessels ( $P < 0.01$ ). However, there was an interaction between CP % and BS addition on fluid pH, individual volatile fatty acids and total fatty acids ( $P < 0.01$ ) concentrations. The addition of BS had no effect on methane production, but the higher 16 % CP resulted in greater methane production ( $P < 0.05$ ).

**Conclusion** Under in vitro conditions, the addition of BS reduced  $\text{NH}_3$ , while the lower 12% CP level produced less methane. Acknowledgements This project was funded by DAFM under research stimulus fund (RSF) project no. 13/S/430.

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**Table 1** Effect of supplementing different crude protein diets with three different brown seaweeds on ruminal fermentation parameters in vitro using the rumen simulation technique (RUSITEC).

CP level	HP				LP				SEM	P-value <sup>1</sup>		
	CO	ALE	ASC	HIM	CO	ALE	ASC	HIM		CP	S	CP x S
pH	6.64 <sup>ac</sup>	6.28 <sup>b</sup>	6.60 <sup>abc</sup>	6.57 <sup>c</sup>	6.46 <sup>ac</sup>	6.49 <sup>b</sup>	6.39 <sup>abc</sup>	6.47 <sup>c</sup>	0.043	0.274	0.0001	0.001
Methane <sup>2</sup>	13.60	15.03	15.28	13.61	12.55	11.38	11.49	11.85	1.348	0.0082	0.9688	0.6709
<b>Concentration mmol L<sup>-1</sup></b>												
$\text{NH}_3$	3.23 <sup>a</sup>	2.33 <sup>b</sup>	2.80 <sup>b</sup>	2.93 <sup>b</sup>	3.34 <sup>a</sup>	2.99 <sup>b</sup>	2.53 <sup>b</sup>	2.51 <sup>b</sup>	0.213	0.8897	0.01	0.055
Acetic	76.31	96.93	78.99	79.77	84.37	74.65	93.02	90.50	4.087	0.3636	0.4784	0.0001
Propionic	20.43	25.11	21.70	22.05	22.88	20.92	23.32	23.93	0.916	0.4939	0.4224	0.0008
Butyric	13.38	16.11	13.81	13.80	14.25	13.75	15.22	14.39	0.574	0.7469	0.231	0.0053
Total VFA <sup>3</sup>	117.87	146.47	123.00	123.70	129.33	117.14	139.43	136.46	5.287	0.4516	0.3957	0.001



# Nutrient digestibility of rabbits fed varying levels of sugarcane rind meal (SCR)

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**Application** Decrease production cost in rabbit production without compromising animal performance; improve income and make animal protein available at a lower cost using sugarcane rind, which is waste thus reducing carbon emissions.

**Introduction** In rabbit production feeding accounts for 60-80 % of the total cost. Sugarcane rind is a by-product with appreciable levels of crude fibre and not utilised by humans. Rabbit production is a source of food and skin for the resource poor farmer and its production also helps in improving food security. The study therefore is aimed at determining the varying levels of sugarcane rind meal (SCR) in the diet on the nutrient digestibility of growing rabbits.

**Material and methods** A total of twenty one (21) male growing rabbits of mixed breeds with average initial weight of  $649 \pm 28.18$ g were used for the study in a completely randomized design feeding trial over a four week period. There were 3 dietary treatments with 7 replicates per treatment. The treatments were designated T1, 0% sugarcane rind meal, T2, 10% sugarcane rind meal and T3 with 20% sugarcane rind meal. Feed and water were provided ad libitum throughout the experiment. The pens, feed and water troughs were cleaned daily prior to provision of the assigned feed and water. Fresh, clean drinking water was provided daily to the animals. The rabbits were treated with antibiotics to clear any pending infections, anti-stress and deworming medications were provided for the first five days. All feed were formulated for 15% crude protein and 3000kcal kg<sup>-1</sup> ME. Feed intake was measured by weighing feed offered and feed refusals weekly. On the feed intake measurement days, all the faeces voided daily for each animal were collected, weighed, mixed and sub samples taken for analysis. The faecal samples were oven dried at 60°C for 48h and the dried samples analysed for DM. All proximate analysis was regulated following the procedures described by AOAC (1980). The values obtained for all parameters measured were subjected to ANOVA in a Completely Randomized Design using the General Linear Model Procedure and pair-wise difference was used to separate significant means (SAS, 1987).

**Results** Digestibility of crude fibre increased with increasing level of sugarcane rind in the diet. Nworgu et al. (2001) also reported increase in crude fibre digestibility in the diets of rabbits when a mixture of leaf meals and concentrates were fed to growing rabbits. The digestibility values obtained for crude fibre in this study were lower than those from the other nutrients and these were in line with the observation of Nworgu et al. (2001). This may be due to the fact that rabbits are less efficient at digesting fibre than ruminants as reported by Joyce et al. (1971). Cheeke et al. (1986) also observed that dietary fibre has a low digestibility in rabbits, because the digestive strategy of a rabbit is to first separate the larger fibrous particles from smaller non-fibrous particles in the hindgut hence excreting the fibre in their faeces.

## Nutrient Digestibility of Growing Rabbits fed Varying Levels of SCR (%)

Means in the same row with different superscript are significantly (P<0.05) different.

**Conclusion** It can be concluded that inclusion of Sugarcane Rind Meal in the diet of growing rabbits positively affected nutrient digestibility.

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**Table 1** Nutrient Digestibility of Growing Rabbits fed Varying Levels of SCR (%)

Nutrient (%)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P-value
Dry matter	86.48 <sup>a</sup>	76.23 <sup>b</sup>	73.67 <sup>c</sup>	0.00
Crude protein	14.40 <sup>a</sup>	12.65 <sup>b</sup>	10.41 <sup>c</sup>	0.00
Ether extract	8.79 <sup>a</sup>	9.14 <sup>a</sup>	7.76 <sup>b</sup>	0.00
Crude fibre	0.38 <sup>c</sup>	0.96 <sup>b</sup>	1.99 <sup>a</sup>	0.00
Ash	7.23 <sup>a</sup>	4.90 <sup>b</sup>	4.00 <sup>c</sup>	0.00
Nitrogen free extract	69.20 <sup>c</sup>	72.53 <sup>b</sup>	75.84 <sup>a</sup>	0.00

Means in the same row with different superscript are significantly (P<0.05) different



# An investigation into the interactions between perennial ryegrass varieties, sown as mixtures, for herbage production and grazing performance

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**Application** Mixtures of Perennial ryegrass (*Lolium perenne* L.; PRG) varieties performed as the mean of the component varieties for production and grazing traits.

**Introduction** Farmers selecting varieties to re-sow into pasture typically choose mixtures of three or four varieties. PRG varieties within Ireland are evaluated within monoculture plots under a mechanical harvesting (cutting) protocol. The composition of a seed mixture is known at sowing but afterwards the interaction between varieties and each individual varietal contribution to the performance of the sward is unknown. The objective of this study was to examine the interaction between PRG varieties for production and grazing traits. The performance of the mixtures for grazing traits was of particular interest as these traits are not measured within National/Recommended List testing of varieties.

**Material and methods** Two plot studies were conducted over 2 years. Plots within each study contained one of four sowing variety treatments: monoculture, equal proportions of two varieties, equal proportions of three varieties and equal proportions of four varieties. Study 1 contained varieties with similar (late) heading dates and study 2 contained varieties with differing heading dates (intermediates and lates). Ploidy was balanced within each study. Three replicates were conducted within each study. Grazing of the plots and measurement of pre-grazing herbage mass, pre-grazing sward height, post-grazing height and herbage grown was conducted as in Tubritt et al., (2018). Herbage density was calculated as (Pre-grazing herbage mass)/pre-grazing sward height - 3.5 cm). Proportion of herbage consumed was calculated as (Pre-grazing sward height - post-grazing sward height)\*herbage density/pre-grazing herbage mass.

**Results** Within both studies ploidy had a significant effect ( $P < 0.001$ ) on both herbage yield, pre/post-grazing sward height, herbage grown and proportion of herbage consumed (see table 1.). Plots containing a greater proportion of diploid (D) varieties had higher herbage yields than tetraploid (T) dominant plots. Post-grazing sward height fell and proportion of herbage consumed increased as the proportion of T varieties increased, indicating that they were more suited to grazing. Plots with equal proportion of T and D performed to the component varietal mean. Difference between heading groups in study 2 for the measured traits were insignificant.

**Table 1.** Mean results of both Study 1 and 2 for the measured variables for diploid and tetraploid dominant swards and swards of equal ploidy

Ploidy	Pre-grazing sward height (cm)	Post-grazing sward height (cm)	Herbage grown (kg DM ha <sup>-1</sup> )	Proportion of herbage consumed
Diploid	10.77	4.18	11,513	0.91
Equal	10.66	4.08	11,803	0.93
Tetraploid	10.02	3.80	10,672	0.97

**Conclusion** In agreement with Tubritt et al., (2018), both studies found that T varieties had superior grazing efficiency than D indicating that that monoculture performance is an accurate measure of variety performance within mixtures for grazing traits. Griffith et al., (2015) found that mixtures of varieties perform to the varietal mean for herbage production under cutting protocols. As in Tubritt et al., (2018) assessment of herbage yield under grazing was biased due to differing post-grazing residuals at each grazing which had subsequent effects on herbage regrowth.

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# Effect of supplementing soybean meal (SBM) with hydrolysed fish protein collagen (HFPC) on the body composition and growth performance of zebrafish (*Danio rerio*)

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**Application** Plant protein-based diets fortified with feed additives enhanced fish feed intake, and growth performance, suggesting that it could serve as an alternative protein source for fishmeal (FM) in aquafeeds.

**Introduction** The focus of recent fish nutrition research is to increase the dietary inclusion of alternative protein sources to replace FM by adding feed additives to enhance the nutritional values and acceptance of fish diets (Chatzifotis et al., 2009). Despite various studies involving the use of additives and attractants in fish culture, there is a limited information about the use of these chemical substances in the diets of zebrafish and the consequent effect of such substances on their body composition. The study evaluated the efficacy of SBM fortified with different inclusion level of HFPC on the body composition and growth parameters of zebrafish (*Danio rerio*).

**Material and methods** The study was approved by Newcastle University's Animal Welfare and Ethics Review Board (AWERB –ID 401). Four diets containing 40% crude protein (isonitrogenous) and 17 MJ GE/kg (iso-calorific) were formulated. Dietary treatments included a control diet free of SBM and HFPC (diet 1); SBM + 10 g/kg HFPC (diet 2); SBM + 20 g/kg HFPC (diet 3), and SBM + 30 g/kg HFPC (diet 4). Each dietary treatment was tested with 80 fish, which were distributed equally in five tanks as replicates for the completely randomised experiment. Forty-two days post fertilisation (dpf) fish were fed three times daily for twelve weeks. Morphometric measurements, water quality and feed allowances were determined on a weekly basis. After the experiment (126 dpf), fish were euthanised using tricaine methanesulphonate MS-222 (Sigma Aldrich) following Schedule 1 (Animal Scientific Procedures Act 1986). The length-weight relationship, condition factor and growth parameters of the fish were determined. The euthanized fish were frozen at -20°C and later freeze-dried until processed for various chemical analysis. Representative samples of feeds and fish were analysed for proximate composition (AOAC 2000). The data were analysed using ANOVA in Minitab 18. Tukey's post-hoc test was used to compare means to observe significance at  $p < 0.05$ .

**Results** Table 1 shows the mean and the standard error of the proximate composition of zebrafish fed the various diets. There were no significant differences ( $p > 0.05$ ) in the proximate composition of the fish. However, the crude protein, fat and the nitrogen-free extract contents were numerically highest in the fish fed SBM plus 10 g/kg of HFPC. Similarly, Table 2 shows the mean and standard error of the growth parameters of the fish. There were no significant differences ( $p > 0.05$ ) in the mean weight gain (MWG), feed conversion ratio (FCR), specific growth rates (SGR), protein efficiency ratio (PER) and the condition factor (CF) of the fish fed the diets. The weight gain, feed intake and protein intake reduced as HFPC increased in the diets.

**Table 1.** The proximate composition (g/kg) of the fish fed various diets.

Parameters	FM Diet (Control)	SBM + 10 g/kg HFPC	SBM + 20 g/kg HFPC	SBM + 30 g/kg HFPC	PSEM	p-value
Dry matter	299.19	312.67	300.47	270.02	1.974	0.582
Crude protein	167.22	175.55	161.34	160.05	1.599	0.852
Fat	74.95	90.76	81.01	70.07	0.819	0.269
Ash	25.89	24.66	22.91	23.35	0.208	0.657
NFE	40.05	46.79	41.04	25.37	0.757	0.202

**Table 2.** The growth performance indices of the fish fed various diets.

Growth indices	FM Diet (Control)	SBM + 10 g/kg HFPC	SBM + 20 g/kg HFPC	SBM + 30 g/kg HFPC	PSEM	P-value
Initial Weight (g)	0.756	0.746	0.564	0.664	0.076	0.383
Final. Wt (g)	2.516a	2.384ab	2.032ab	1.976b	0.145	0.031
MWG (g)	1.760	1.638	1.468	1.312	0.158	0.329
Feed Intake (g)	0.096a	0.081ab	0.074ab	0.070b	0.007	0.035
Feed Conv. Ratio	0.055	0.051	0.051	0.059	0.005	0.710
SGR (%)	1.455	1.408	1.529	1.347	0.161	0.906
Protein Intake (g)	0.837a	0.675ab	0.625b	0.638b	0.058	0.046
Protein Eff. Ratio	2.111	2.486	2.415	2.033	0.225	0.520
GPCR	0.117a	0.094ab	0.088b	0.089b	0.008	0.046
Condition Factor	1.154	1.183	1.130	1.132	0.005	0.291

**Conclusion** Fish fed SBM supplemented with 10 g/kg of HFPC showed an excellent growth performance as compared with those fed FM based diet. However, the fish fed SBM plus 20 g/kg of HFPC produced comparable results in terms of fish survival, growth performance and body composition. Increasing HFPC above 20g/kg in the SBM diet did not cause a significant impact on the overall performance of the fish in terms of the food intake, weight gain and protein efficiency ratio in zebrafish.

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# Influence of postovulatory luteal function on early pregnancy in the horse

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In horses, conceptus loss until the beginning of placentation, i.e. approximately day 40 after ovulation frequently occurs. Until formation of the placenta, nourishment of the conceptus solely depends on endometrial histotroph. Early postluteal progesterone concentration, histotroph composition and conceptus development are closely associated in ruminants and pigs (Bazer and Spencer 2009), but also in horses (Willmann et al. 2011, Beyer et al. 2019). In this species, it is, however, still unclear if and to what extent low early postluteal progesterone concentration contributes to conceptus loss.

In the present study, early postovulatory progesterone concentration was experimentally reduced in Haflinger mares (n=11) that had been inseminated until spontaneous ovulation. Mares received the prostaglandin F<sub>2</sub> agonist cloprostenol (PGF<sub>2</sub>; 125 µg; CLO) once daily on days 0 to 3 after ovulation or left untreated as control (CON). From day 10 after ovulation, the occurrence and development of the conceptus was determined by daily transrectal ultrasound until the conceptus and its fetal membranes were recovered by transcervical uterine lavage on day 34. From seven mares, intact conceptuses could be collected from both, treatment (CLO) and control (CON) pregnancies. The material was weighed, measured, and conceptuses underwent microcomputed tomography (micro-CT). Statistical comparison for differences between control and treatment pregnancies was done by non-parametric Wilcoxon test or Chi<sup>2</sup> analysis.

Plasma progesterone concentration was lower in CLO compared to CON pregnancies until day 14 after ovulation (treatment  $p < 0.01$ , treatment x time  $p = 0.05$ ). There was no difference between treatments in first visualization of the embryonic vesicle (treatment day  $10.8 \pm 0.3$ , control day  $10.2 \pm 0.1$ ), the embryo proper (treatment day  $18.8 \pm 0.5$ , control day  $19.0 \pm 0.4$ ) and fetal heartbeat (treatment day  $21.6 \pm 0.5$ , control day  $20.5 \pm 0.4$ ). After CLO and CON treatment, conceptus fixation occurred on day  $19.3 \pm 0.5$  and  $16.7 \pm 0.4$ , respectively ( $p < 0.05$ ). On 34 days of gestation, the conceptuses from CLO pregnancies were smaller than CON conceptuses when measured with ultrasound callipers in vivo and after recovery ( $p < 0.05$  for both measurements). Micro-CT images revealed differences in organ development between CLO and CON conceptuses with regard to presence of the footpad (CON 6/7, CLO 0/7,  $p < 0.01$ ), the auricular hillocks (CON 7/7, CLO 3/7,  $p < 0.05$ ), development of the trachea (CON 7/7, CLO 4/7,  $p < 0.05$ ) and incomplete closure of the interventricular foramen (CON 1/7, CLO 5/7,  $p < 0.05$ ). Volume of lungs, liver and primordial pituitary differed between

treatments and was always smaller in CLO compared to CON pregnancies ( $p < 0.05$ ). Volume of mesonephros, metanephric blastema, primordial gonads, heart and nervous system did not differ between treatments.

In conclusion, low postovulatory progesterone concentration due to induced partial luteolysis is detrimental to conceptus development before placentation as reflected in external and internal conceptus morphology and may result in pregnancy loss at subsequent stages.

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# Antepartum endocrine and cardiac changes of the mare and her foetus differ between small, medium and large breed horses

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**Application** Understanding of breed differences in prepartum endocrine and cardiac changes

Introduction While in cattle, breed related endocrine and metabolic changes in the peripartum period are well known, breed differences are often times neglected in horses. We hypothesised that during late gestation parameters related to foetal and maternal well-being differ between small, medium and large-size horses. To evaluate breed related differences in horses, weight of mares, foals and placenta, progesterin and cortisol concentration, heart rate (HR) and heart rate variability (HRV) were studied in Shetland, Haflinger and Warmblood mares and their foetuses during the last three weeks of gestation.

**Material and methods** Late pregnant Shetland (n=7; age: 10.6±2.2; weight: 175±7 kg), Haflinger (n=8; age: 9.0±1.1; weight: 464±9 kg) and Warmblood mares (n=9; age: 8.8±1.6; weight: 563±16kg) were included. Mares did not differ with regard to age and parity but differed in weight (p<0.001). During the last three weeks before the expected day of parturition, blood samples from mares were taken for analysis of progesterin and cortisol concentration and a one-hour foetomaternal electrocardiogram (ECG) recording was made once daily. Retrospectively calculated from the actual day of parturition, ECG recordings from days -20, -15, -10, -5, -3 and -1 before foaling were used for analysis. From the recorded beat-to-beat (RR) intervals, a five-minute interval was taken to determine HR and the HRV variable standard deviation of the RR interval (SDRR) in mares and foetuses. For analysis of maternal plasma progesterins and cortisol concentration, blood samples from days -10, -8, -6, -4, -3, -2 and -1 before foaling were evaluated. Concentration of progesterin and cortisol was determined by a commercially available enzyme immunoassay (progesterin: ADI-900-011, Assay Designs, Ann Arbor, MI, USA; cortisol: Demeditec Diagnostics, Kiel-Wellsee, Germany) as described and validated for equine plasma in our laboratory. After passage of foetal membranes, weight of placenta was recorded and on day one after birth of the foal, all mares and neonatal foals were weighed.

**Statistical analyses** Statistical analysis were made with the SPSS statistics software (version 25.0; SPSS-IBM, Armonck, NY, USA). Data were tested for normal distribution by Kolmogorov-Smirnov test. Data for weight of foal, mare and placenta were normally distributed and further analysis was made by Kruskal-Wallis-Test and Mann-Whitney-Test. Data for HR, SDRR, cortisol and progesterins were not normally distributed and log transformed before analysis. Comparison were made by ANOVA with a general linear model (GLM) for repeated measures with time as within subject factor and group as between subject factor. A p-value <0.05 was considered significant.

**Results** While foal weight relative to the mares weight was about 10 % and did not differ among horse breeds, relative placenta weight and foal weight in relation to placenta weight was both highest in Warmbloods (p<0.05). Progesterin concentration decreased (p<0.001) towards foaling in horses of all breeds but was at all times highest in Warmblood mares (p<0.01; e.g., d -1: Warmblood 202.4±17.0, Haflinger 118.2±22.6, Shetland: 102.6±17.6 ng/mL). Cortisol concentration in maternal plasma increased (p<0.001) before parturition without any breed differences. Heart rate in mares increased towards foaling (p<0.001) and this increase was most pronounced in Shetlands (group p<0.001, time x group p<0.001; Warmblood: d -20: 50±1, d -1: 53±1 beats/min; Haflinger: d -20: 54±2, d -1: 64±3; Shetland: d -20: 57±3, d -1: 77±4 beats/min). In contrast to this, maternal HRV variable SDRR decreased (p<0.05) towards foaling with the most pronounced decrease in Shetlands (group p<0.05, time x group p<0.05). Fetal HR was almost identical in all breeds three weeks before birth and decreased thereafter (p<0.001) with the most pronounced decrease in Warmblood foetuses (p<0.01).

**Conclusion** Clear breed related differences among Shetland, Haflinger and Warmblood mares exist in characteristic cardiac and endocrine changes that precede parturition in horses. Breed related differences, especially in cardiac parameters are only marginal during late gestation, but as parturition approaches differences become more obvious and are increasingly related to the size of the horse. A stress-like situation before parturition is only present in Shetland mares and do not occur in mares from larger-size breeds. Thus, breed related normal values for HR and HRV must be taken into account when foetal and maternal well-being needs to be evaluated.



# An investigation of the use of blue light therapy to extend photoperiod in pregnant mares and its influence on gestation length on commercial breeding farms

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**Application** Pre-partum administration of artificially extended photoperiod through head-worn blue light masks may prevent prolonged gestation periods in mares and improve breeding efficiency on commercial farms.

**Introduction** Foals born earlier in the year have an advantage of maturity by the time they reach competition age (Langlois and Blouin, 1997) and mares can be bred again sooner, meaning economic profitability and sustainability of breeding is maximised (TenBroeck, 2016). However, multiple studies have shown that gestation lengths decrease as the natural foaling season progresses and the daily length of daylight gets longer (Hintz et al., 1979; Perez et al, 2003). Previously, it was shown that artificially extended photoperiod administered to pre-partum mares, from early December, significantly shortened gestation lengths (Hodge et al., 1982). The aim of this study was to investigate whether treatment with blue-light therapy, used to artificially extend photoperiod, correlates with a decrease in gestation periods on commercial farms in multiple geographical locations.

**Material and Methods** For this project, ethics approval was given by the Social Science Research Ethical Review Board at the Royal Veterinary College under reference number SR2019-0119. Data on gestation lengths were collected from three commercial breeding farms and 22 Thoroughbred mares, aged between 4 and 10 years old, for two consecutive years. In year 1, mares were exposed to natural photoperiod length throughout gestation. In year 2 mares were fitted with Equilume Light Masks from Dec 1st to extend photoperiod during the last trimester. Farms 1 and 2 were located in England, and Farm 3 in Japan. Sample sizes differed between farms, with 9, 5 and 8 mares on Farms 1, 2 and 3 respectively. No data on sires or foaling due dates was made available. During data collection periods, mares on Farm 1 were maintained at pasture and only stabled for 2h daily until Jan 15th, after which they were stabled each day, from 3pm, overnight. Mares on Farms 2 and 3 were turned out from 9am to 3pm daily and housed individually overnight. Barn lighting was switched off at 4pm. Mares on Farms 1 and 2 grazed ad libitum in their pasture, and on Farm

3, were supplied ad libitum forage whilst turned out, as grazing was limited. Concentrate feed was supplemented twice a day. Water and hay were provided ad libitum while stabled. In year 2, blue-light therapy masks (Equilume) that administered 50 lux short wavelength blue light (465 nm) to the right eye were fitted on Dec 1st until parturition. These masks were programmed to deliver blue-light into one of the mare's eyes from 4pm (dusk) until 11pm nightly. This provided an initial day length of approximately 14 hours, which increased with the progression of the seasons, to just over 16 hours by the end of the breeding season. Two-way repeated measures ANOVA followed by Sidak's multiple comparisons tests were performed using GraphPad Prism (Version 8.0). Data are presented as means +/- SEM and a P value of <0.05 was considered significant.

**Results** There was no farm x year interaction or main effect of farm on mare gestation lengths observed. There was a strong overall effect of year ( $P < 0.0001$ ) with a mean gestation reduction across the three farms of 13.5 days in year 2 (Figure 1). Similarly, multiple comparisons analyses revealed significant reductions in gestation lengths within each farm in Year 2 (15.3, 14.8 and 10.5 days respectively, Table 1)

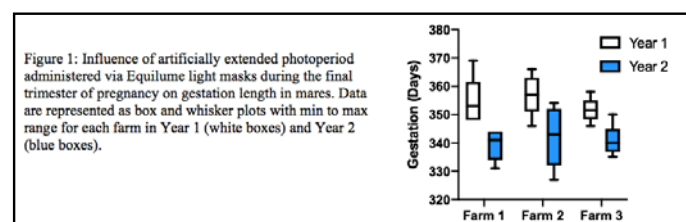
**Conclusion** The results support the hypothesis, that administration of extended photoperiod via blue light masks to pre-partum mares during the final trimester reduces gestation length. Future studies should attempt to ascertain the minimum duration of treatment required to induce this effect as data on treatment length prior to parturition was not available in these studies. Further studies using larger sample sizes should account for other factors which influence gestation length, such as sire and parity, to further confirm the relationship.

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**Table 1:** Descriptive statistics for gestation length (mean +/- SEM) in days for mares from each of three farms and the compiled data in Year 1 (control) and Year 2 (Treatment with Equilume Light Masks)

Year	Farm 1		Farm 2		Farm 3		Compiled	
	1	2	1	2	1	2	1	2
Gestation length (days)	354.8+/-7.6	339.4+/-5.1	357+/-7.3	342.2+/-10.7	351.5+/-4	341+/-5	354.4+/-1.6	340.9+/-0.8
Difference	15.3+/-2.3	14.8+/-3.1	10.5+/-2.5	13.5+/-1.5				
P value	<0.0001	0.0004	0.0013	<0.0001				





# A customised LED lighting system utilising daytime polychromatic white light and night-time red light influences testosterone levels and semen parameters in Sport Horse stallions

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**Application** Sport Horse stallions are used for breeding purposes throughout the year, but are often busiest between Jan and Apr. The lighting regime evaluated in this study improved fertility parameters by increasing Total Sperm Number and testosterone levels early in the breeding season and could represent an effective management tool for stallion management.

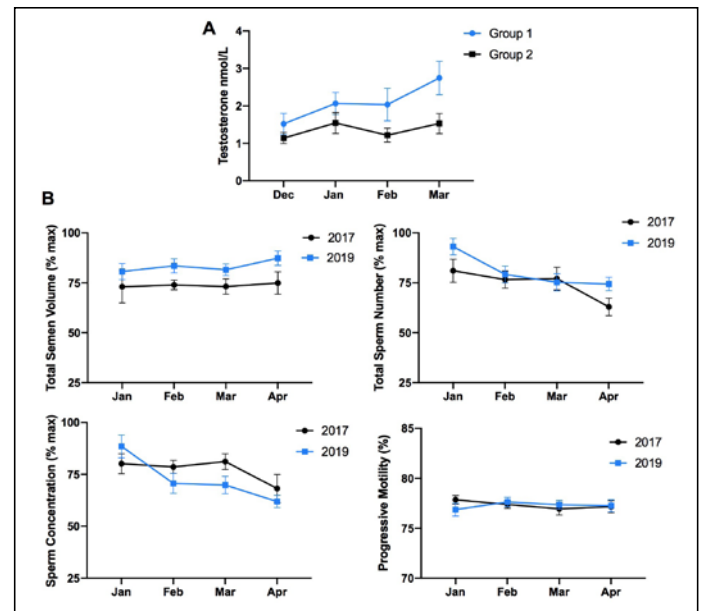
**Introduction** In horses, retinal photoreception records the annual cycle in day length and synchronises peak reproductive activity with the lengthened photoperiods of late spring and summer via modulation of daily pineal melatonin production. Short-wavelength blue light effectively suppresses melatonin and can be used to manipulate seasonal reproduction in mares (Murphy et al., 2014). Reproductive and behavioural characteristics in stallions also undergo seasonal variations related to photoperiod length (Pickett et al., 1976; Clay et al., 1987). The present study aimed to assess the effects of a customised LED lighting system on fertility parameters in stallions between Dec and Apr.

**Material and methods** 22 warmblood stallions from a commercial stud were blocked for age and randomly allocated to one of two groups: Group 1 (Treatment; n=11; mean age = 10.5±5.8yr) were housed under a customised LED lighting system and received 17.5h of blue-enriched polychromatic white light by day (peak wavelength 454 nm, >200 lux), 6.5h of dim red light by night (625 nm, 10 lux) with 20 min gradual light transitions at dawn (6am) and dusk (11.30pm) from Oct 28th, 2018. Group 2 (Control; n=11; mean age = 9.6±5.4yr) received 12h of standard fluorescent light by day, no light at night and abrupt transitions at dawn (6am) and dusk (6pm). At the start of each month from Dec 2018 until Apr 2019, a single morning blood sample was taken from each stallion and analysed for testosterone (SYNLAB, Germany) using the LCMS method (Wang et al., 2014). Semen ejaculates were evaluated from Group 1 stallions from Jan to Apr 2019 and monthly means for Total Semen Volume (TSV), Sperm Concentration (SC), % Progressive Motility (PM) and Total Sperm Number (TSN) were calculated and compared to those of Group 1 stallions during the same time period in 2017 when housed under control conditions. Recording of semen data began once sperm viability >50% had been determined using a NucleoCounter device (SP-100, Chemometec). TSV (mL) was determined by weighing the ejaculate on a calibrated scale (1g = 1mL). %PM was analysed visually under a microscope by an experienced technician. SC (million/mL) was determined using the NucleoCounter. TSN (billion) was determined by multiplying SC and TSV. To account for individual variability, values for TSV, TSN and SC were normalized within stallion as a % of maximum. Data for all parameters were found to be normally distributed using the Shapiro-Wilk test for normality. Data were analysed using a mixed effects analysis with repeated measures in Prism Version 8.01 for macOS (Graph Pad Software, USA). For testosterone; month, treatment and month x treatment were considered fixed effects and stallion within group was

considered a random effect. For semen parameters; month, year and month x year were considered fixed effects and stallion, stallion by month and stallion by year were considered random effects. Sidak's multiple comparisons tests were performed where appropriate and P<0.05 was considered significant.

**Results** An effect of month (P<0.05) and treatment (P<0.05), but no month x treatment interaction was observed for testosterone levels with Group 1 stallions exhibiting higher levels across all months. There was an effect of year on TSV (P<0.05) with higher levels in 2019, but no effect of month or month x year interaction. There was an effect of month on TSN (P<0.01) and an effect that approached significance for year (P=0.055) and month x year interaction (P=0.054). Sidak's multiple comparisons test revealed higher values in Jan compared to Apr in 2019 (P<0.05). An effect of month only (P<0.01) for SC was observed. There was no effect of month, year or month x year interaction for % PM. Results are described in Figure 1 and data represented as means +/- SEM.

**Figure 1** A) Serum testosterone levels in stallions exposed to 17.5h daily blue-enriched polychromatic LED light (Group 1; n=11) or 12h fluorescent light (Group 2; n=11) from Oct 28th. B) Semen parameters for TSV, TSN, SC and %PM in Group 1 stallions during the months Jan-Apr 2017 and 2019. All data are presented as means+/-SEM.



**Conclusions** A customised LED lighting system providing extended daily blue-enriched polychromatic light and red light at night increased mean testosterone levels in treated stallions compared to age-matched control animals between Dec and Apr. Marked increases in TSV and TSN were observed in treated stallions compared to previous historical data from the same animals. Modifying the method of light exposure may represent a simple management tool for improving fertility parameters in breeding stallions.

**Acknowledgements** The authors gratefully acknowledge funding from Equilume Ltd.

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# Effect of Disturbance on the Time of Foaling Before or After Midnight

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**Implications** Knowledge at what time of night a mare is likely to foal would be helpful to mare owners

**Introduction** Many owners, particularly those with single mares, often spend sleepless nights watching for their mare to foal so that no birth is unattended. The degree of this observance can vary from, popping their head over the stable door every hour, to actually sleeping in the stable. It was observed in a previous study of two foaling units where one (B), in which there was constant disturbance until late into the night, experienced the majority of their mares foaling after midnight, whilst mares in the other unit (A), in which any disturbance was kept to a minimum, experienced the reverse with only a minority of mares not having foaled by midnight. However, a difference in breed and location could not be ruled out as an explanation. More recently Internet monitored CCTV has meant that disturbance before foaling can be reduced to a minimum.

**Material and Methods** Recently, the 'late' foaling unit (B) has changed its observance to in-stall cameras, with an internet connection to a distant mobile phone. Foaling times of 68 mares were recorded to the nearest 15 minutes for every mare which foaled between 18.00h and 6.00h. 6 mares foaled during the day and were excluded from the data. Recent data was also available from the original thoroughbred farm (A) of 151 mares foaling between January and July. Using the same CCTV/ internet system of observance, data was available whether they foaled before or after midnight. Mares were last fed around 4-5 pm and thereafter human disturbance after about 6 pm on both units was minimal or none. Install lighting was provided sufficient for the CCTV and stalls remained lit until daylight.

**Results** Data was compared on farm A as either foaling before or after midnight by month and on farm B by time and month (Table 1) and for comparison, times of sunset (Table 2)

On farm A, the majority of mares foaled before midnight as had been previously shown (Newcombe and Nout 1998). However over four times as many in May and June foaled after midnight than during January to April. Similarly on farm B, where previously the majority foaled after midnight, since the introduction of CCTV, 56% foaled before midnight at a mean time of 23.57. However as in Farm A, during the period May 21st to July, almost more than twice as many foaled after midnight (mean time 00.43) as during the period April to May 20th

**Discussion** The question had been previously posed do most mares foal before or after midnight with Rossdale and Short (1969) finding 52.6% before while Bain and Howey (1975) found 52.2% after. It had been shown previously (Newcombe and Nout) that disturbance of the mare immediately before foaling may delay the onset, but it was not realised that the onset of darkness also had a major effect. This study shows that at both farms, those mares foaling later in the season, foaled later in the evening, at a mean time of approximately four hours after sunset.

**Conclusions** The vast majority of mares if undisturbed foal either side of midnight. Although the time between individuals is extremely variable, there is a tendency for foaling to occur at approximately four hours after sunset.

## References

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**Table 1**

Farm	n	After Midnight	%	Mean time Foaling
A	151	17	11.3	ND
Jan-April	114	7	6.4	ND
May-June	37	10	27	ND
B	68	30	44.1	23.57pm
April - May 20th	37	10	27	22.53pm
May 21 - July	31	20	64.5	0.43am

**Table 2**

Sunset on	Times of Sunset	
	GMT	BST
March 1st	17.45	17.45
March 30th	18.37	18.37
April 1st	19.41	18.41
May 1st	20.33	19.33
June 1st	20.20	20.20
July 1st	21.34	20.34

# Influence of *Bacillus subtilis* GCB-13-001 on growth performance, nutrient digestibility, blood characteristics, fecal microbiota, and fecal score in weanling pigs

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**Application** Weaning is critical to piglet growth. Supplementing probiotics in the diet may help manipulate the composition of the microbiota and its metabolic activities to overcome the stress of the weanling pigs.

**Introduction** In recent years, probiotics as growth promoters have been suggested as a desirable antibiotic substitute for pigs. Also, the results of pigs supplemented with probiotics may be inconsistent due to different combination of selected bacterial genera, their doses, and feed composition. The aim of the present study was to assess the potential of *B. subtilis* probiotic on growth performance, nutrient digestibility, blood profile, faecal microbiota, and faecal score in weanling pigs.

**Material and methods** The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University. A total of 120 weanling pigs [(Landrace × Yorkshire) × Duroc; 7.73 ± 0.75 kg (28 days of age)] were randomly allotted into three treatments according to their initial body weight (BW) and gender in a 6-week experiment. There were 8 replication pens in each treatment, with 5 pigs/pen. Dietary treatment groups were as follows: (i) basal diet (CON), (ii) CON + 0.1% *B. subtilis* GCB-13-001 1 × 10<sup>8</sup> CFU/kg (T1), (iii) CON + 0.1% *B. subtilis* GCB-13-001 1 × 10<sup>9</sup> CFU/kg (T2). The diets were formulated to meet or exceed the NRC (2012) nutrient requirements. All pigs were housed in an environmental controlled room with slatted plastic floor and the piglets had free access to feed and water. Individual pig BW and feed disappearance were measured initially and on d 7, d 21 and 42 of the experiment. The ADG, ADFI, and feed:gain (F:G) ratio were also calculated. During the last week, chromic oxide

(2 g/kg) was added to all the diets as an indigestible marker for the determination of apparent nutrient digestibility of dry matter, nitrogen and energy. At the end of experiment, two pigs were randomly selected from each pen (n=16) per treatment and blood samples were collected via jugular venipuncture. The blood samples were collected into both nonheparinized and K3EDTA vacuum tubes in order to obtain the serum and whole blood, respectively. Serum total protein, immunoglobulin G (IgG), and albumin were determined using an automatic biochemistry analyzer (HITACHI 747, Japan). The white blood cells, red blood cells and lymphocyte counts in the whole blood were analyzed using an automatic blood analyzer (ADVIA 120, Bayer, NY). At d 42, faecal samples were collected via massaging the rectum. The composite faecal sample (1 g) from each pen was diluted with 9 ml of 1% peptone broth and homogenized. Viable counts of bacteria in the faecal samples were then determined by plating serial 10-fold dilutions onto MacConkey agar plates and lactobacilli medium III agar plates to isolate *E. coli* and *Lactobacillus*, respectively.

**Results** Days 1 to 7, the BW and ADG with T2 treatment were higher (P = 0.017, P = 0.02) than CON treatment, as well as F:G showed trends in linear reduction (P = 0.058). Days 8 to 21, the BW and ADG were improved (P = 0.001, P = 0.007) in pigs offered T1 and T2 diets compared with CON. Days 22 to 42, BW and ADG were higher (P < 0.0001, P = 0.004) in pigs fed T2 diet than CON and T1 diets, and the pigs fed T1 diet had higher BW than CON. Overall, the ADG with the T2 treatment was higher (P < 0.0001) than that with the T1 and CON treatments. Moreover, F:G were significantly decreased (P = 0.012) by T2 treatment compared with CON. The faecal *Lactobacillus* counts were improved (P = 0.011) and *E. coli* counts were reduced (P = 0.041) in pigs fed T2 diet compared with CON (Table 1).

**Conclusion** Supplementation with probiotics containing 0.1% *B. subtilis* GCB-13-001 1 × 10<sup>9</sup> CFU/kg has shown a beneficial effect in improving BW, increase ADG, decrease F:G ratio, and improve faecal *Lactobacillus* and reduce *E. coli* counts. Thus, *B. subtilis* GCB-13-001 is a promising growth promoter as a feed additive for piglet diets.

**Acknowledgements** This work was supported by the Technology Development Program (C0507791) funded by the Ministry of SMEs and Startups (MSS, Korea).

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**Table 1.** Effect of dietary supplementation probiotics on fecal microbial flora in weanling pigs<sup>†</sup>

Items <sup>‡</sup>	CON <sup>‡</sup>	T1 <sup>‡</sup>	T2 <sup>‡</sup>	SE <sup>‡</sup>	P-value <sup>‡</sup>		
					Anova <sup>‡</sup>	Linear <sup>‡</sup>	Quadratic <sup>‡</sup>
<i>Lactobacillus</i> , log <sup>10</sup> cfu/g <sup>‡</sup>	7.30 <sup>ab</sup> <sup>‡</sup>	7.37 <sup>ab</sup> <sup>‡</sup>	7.49 <sup>b</sup> <sup>‡</sup>	0.05 <sup>‡</sup>	0.014 <sup>‡</sup>	0.011 <sup>‡</sup>	0.587 <sup>‡</sup>
<i>E. coli</i> , log <sup>10</sup> cfu/g <sup>‡</sup>	6.54 <sup>a</sup> <sup>‡</sup>	6.43 <sup>ab</sup> <sup>‡</sup>	6.31 <sup>b</sup> <sup>‡</sup>	0.07 <sup>‡</sup>	0.038 <sup>‡</sup>	0.041 <sup>‡</sup>	0.942 <sup>‡</sup>

# Comparison of pig gut and faecal microbiota compositions using MG-RAST and QIIME2 pipelines

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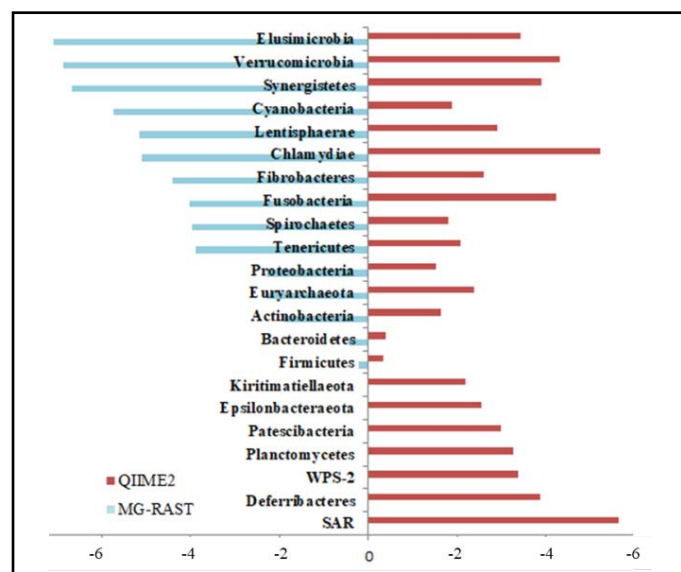
**Application** The results from the comparison of pipelines MG-RAST and QIIME2 will enable users to improve their application and the interpretation of the obtained microbial community compositions.

**Introduction** Studies focused on sequencing the microbiota from specific environments such as different gut locations rely on the existence and development of bioinformatics tools that are affordable, fast and accurate in the characterisation of the microbiota based on rRNA amplicon sequences. The aim of the present study was to compare the taxonomic compositions obtained from processing 16S rRNA amplicon sequences using two pipelines: MetaGenome Rapid Annotation using Subsystem Technology (MG-RAST) and Quantitative Insights Into Microbial Ecology (QIIME2).

**Material and methods** Briefly, 188 samples including faecal and gastrointestinal microbiota (colon and caecum, post-mortem) were obtained from 38 intact male pigs. The V4 hypervariable region of the 16S rRNA gene was amplified using primers 515F and 806R, resulting in a total of 376 fasta.gz files. The 16S rRNA amplicon sequences obtained from Illumina MiSeq were processed using 2 pipelines: MG-RAST (4.0.3) and QIIME2 (2019.1). Taxonomic compositions obtained were based on the SILVA database (SILVA SSU in MG-RAST and "silva-132-99-515-806-nb-classifier.qza", a Bayesian naïve algorithm pre-trained in SILVA database in QIIME2). In QIIME2, trimming thresholds are selected by the user, based on the reads' quality (Q-scores), filtering out poor quality data. For this study, this was set at 153 and 157 bases for forward and reverse reads, respectively. Chimera detection and removal was not actively included in this pipeline, because it uses the DADA2 algorithm; DADA2 is an amplicon sequence variant (ASV)-based method that models and corrects errors in Illumina sequenced amplicons, implementing a workflow that includes filtering, dereplication, sample inference, chimera identification and merging of paired-end sequences. In MG-RAST, read quality is assessed through SolexaQA, allowing for the user to select a threshold Q-score for trimming and filtering of the sequences (in this study, Q=25). Chimera detection and removal was not performed, as it is not included in the MG-RAST pipeline. MG-RAST works as an operational taxonomic unit (OTU)-based method that first compares the query sequences against a reduced database (M5RNA\_90) to identify rRNA features (at 70% of similarity); in the second step, sequences are clustered at 97% similarity and each cluster representative sequence is used as query against a non-redundant database that includes SILVA, Greengenes and RDP (M5nra). The taxonomic annotation was here performed using SILVA SSU identifiers. The user can then select several quality control parameters, i.e. the maximum e-value and minimum abundance threshold (both left as default here), the minimum identity cut-off and the minimum alignment length (here set at 80% and 100, respectively). Taxonomic compositions obtained from both pipelines

were compared using paired t-tests assuming differing variances. Significance was assumed at  $P < 0.05$ .

**Figure 1** – Log-transformed relative abundance of phyla composition of samples using MG-RAST and QIIME2



**Results** Average relative abundances of Eukaryota and Bacteria were, respectively, significantly higher and lower in MG-RAST than in QIIME2. Based on 16S rRNA, only Bacteria and Archaea can be identified so the presence of Eukaryota is a misclassification and should be considered as a disadvantage of MG-RAST. At phylum level, all 15 taxa reported by MG-RAST were shared by QIIME2, whereas QIIME2 reported 7 exclusive phyla. The pairwise t-tests showed that 13 of the 15 shared phyla had significantly different relative abundances (Figure 1). Adjusted Shannon and Simpson and Bray-Curtis dissimilarity indices calculated for the family-level compositions showed that QIIME2 resulted in significantly higher diversity and dissimilarity than MG-RAST. The 5 most abundant genera identified by MG-RAST and QIIME2 showed substantial differences, but in both, Prevotella was the genus with highest relative abundance (Table 1).

**Table 1** - The 5 genera with highest average relative abundance using MG-RAST and QIIME2. Shaded cells represent genera shared in both top 5 results.

QIIME2	(%)	MG-RAST	(%)
Prevotella	32.99	Prevotella	37.08
Lactobacillus	5.01	Faecalibacterium	12.27
Faecalibacterium	4.44	Clostridium	8.57
Alloprevotella	4.33	Eubacterium	6.49
Subdoligranulum	3.86	Selenomonas	5.32

**Conclusion** The use of different bioinformatics tools for the processing of taxonomic annotation of microbiota samples led to different results, with potential consequences such as mischaracterisation of environmental microbial composition and erroneous diversity assessments.

**Acknowledgements** This project was funded by EU H2020-MSCA-RISE-2015 (MetaPlat project) and the Scottish Government.

**References** Bolyen E. et al. 2019, Nature Biotechnology, 37, 848-857; Meyer, F. et al. 2019 Briefings in Bioinformatics, 20, 1151-1159; Callahan BJ. et al. 2016. Nature Methods, 13, 581-583.



# The power of metagenome-assembled genomes (MAGs) to discover rumen uncultured microorganisms and their roles in methane emissions

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**Application** Identification of metagenome-assembled genomes (MAGs) from the rumen microbiome will improve prediction of methane (CH<sub>4</sub>) emissions in cattle from different breeds and diets and help to develop nutrition strategies against methanogenesis.

**Introduction** Diets with a higher content of concentrate (e.g., grain) compared to a forage diet (e.g., grass and silages) are known to produce lower methane emissions. In addition, diets have a stronger impact on microbial community composition than breeds. However, conflicting results have been obtained when correlating microbial abundance patterns such as the main ruminant methanogen genus *Methanobrevibacter* or one of the main plant fibre degraders and also hydrogen (H<sub>2</sub>) producers (like Ruminococcaceae) with CH<sub>4</sub> emissions (Auffret et al., 2018). The aim of this study was the identification of microbial taxa by comparing MAGs with Kraken identification and to explain most of the variability in CH<sub>4</sub> emissions.

**Material and methods** The data in this study were obtained from samples collected in four independent experiments balanced for breed type (cross breeds Aberdeen Angus, Charolais and Limousin and pure breed Luing) and selected for whole metagenome sequencing. The study used 282 animals offered a medium- (FOR) or high-concentrate (CONC) diet (50% or 92% on dry matter basis). Methane emissions were measured individually during 48 h in respiration chambers (Rooke et al. 2014). Post-mortem rumen digesta samples collected at the abattoir were used and total DNA was extracted prior to metagenomics analysis. For phylogenetic annotation (genus level), the genomic reads were aligned to a customized Kraken database (Stewart et al., 2018; Wood and Salzberg, 2014). The same protocols applied in Stewart et al. (2019) were used for the generation of metagenome-assembled genomes phylogenetically identified using a combination of databases. Partial least squares analysis (PLS, Version 9.1, SAS Institute Inc., USA) was used to identify the most correlated MAGs, families or genera associated with CH<sub>4</sub> emissions using Variable Importance in Projection (VIP). A variable with a VIP-value > 0.8 is considered significantly correlated with CH<sub>4</sub> emissions.

Furthermore, the regression coefficient indicates whether the variable is positively or negatively correlated.

**Results** In this study, the PLS results (including variables with VIP>0.80) revealed a 1.46 fold higher explained variation of CH<sub>4</sub> emissions using MAGs (on average 70%) compared to Kraken annotated taxa of different within diet analyses. In addition, different MAGs within *Methanobrevibacter* or Ruminococcaceae showed opposite effects on CH<sub>4</sub> emissions which could be one reason explaining the lack of significant PLS results for Ruminococcaceae family or *Methanobrevibacter* genus using Kraken (Table 1).

**Table 1** Examples of partial least squares analysis results comparing for CH<sub>4</sub> emissions.

DIET	Phylogenetic level	ID	VIP	Regression coefficient
CONC	Family	Ruminococcaceae	Not significant	
CONC	RUG14429	Ruminococcaceae bacterium	1.30	0.010
CONC	RUG11259	Ruminococcaceae bacterium	1.12	-0.004
FOR	Family	Ruminococcaceae	Not significant	
FOR	RUG10098	Ruminococcaceae bacterium	1.47	0.003
FOR	RUG14144	Ruminococcaceae bacterium	0.95	-0.003
CONC	Genus	<i>Methanobrevibacter</i>	Not significant	
CONC	RUG10188	Uncultured <i>Methanobrevibacter</i> sp.	0.98	0.008
FOR	Genus	<i>Methanobrevibacter</i>	Not significant	
FOR	RUG13115	uncultured <i>Methanobrevibacter</i> sp.	1.04	0.004
FOR	RUG12835	uncultured <i>Methanobrevibacter</i> sp.	0.97	-0.004

**Conclusion** This study demonstrated the advantage of generating MAGs over traditional phylogenetic annotation or enhanced Kraken annotation, providing additional insight in explaining CH<sub>4</sub> emissions in the rumen microbiome of beef cattle fed two different diets. In addition, the MAGs derived from the *Methanobrevibacter* and Ruminococcaceae appear to be completely novel, and some of the MAGs of the same taxonomy showed opposite correlations with CH<sub>4</sub> emissions depending on the diet. Therefore, such knowledge will help to develop new mitigation strategies based on diet intervention to reduce CH<sub>4</sub> emissions.

**Acknowledgements** This project was funded by BBSRC, the Scottish Government, AHDB and Defra.

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# Host genetics affects the rumen microbial gene abundances explaining feed efficiency traits in beef cattle

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**Application** Studying the host genetic control on the relative abundances of rumen microbial genes which affect feed efficiency traits will enable to integrate metagenomics in future breeding programs and to exploit novel sources of genetic variance in order to enhance feed efficiency in livestock production systems.

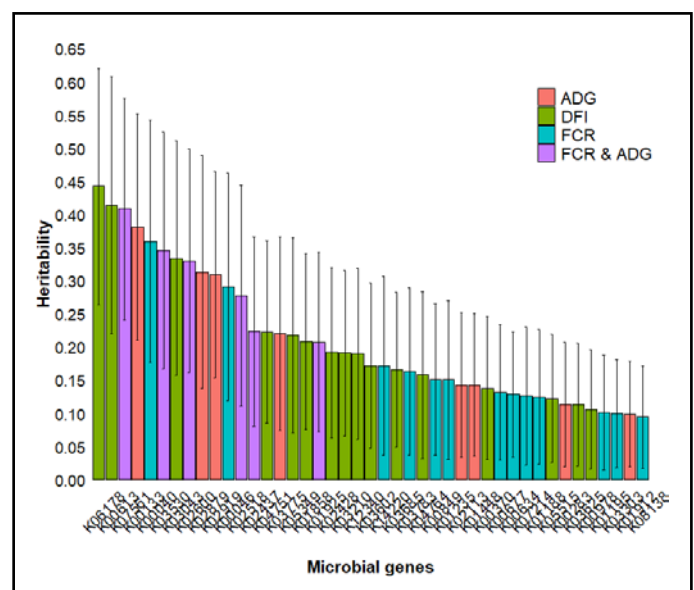
**Introduction** The variability on the relative abundance of specific microbial genes in the rumen are, at least partially, responsible for the efficiency of cattle to utilize feed (Lima et al., 2019). Feed efficiency that involves large recording cost could be alternatively predicted using these microbial genes as proxies. Several studies suggest a host genetic control of the rumen microbiota taxonomic composition (Roehe et al., 2016, Saborio-Montero et al., 2019), but there are no studies of the host genetic control of the rumen microbial gene abundances. If successfully demonstrated, this will open new opportunities for rumen microbial genes to be used as selection criteria in future breeding programs targeting feed efficiency in cattle. The aim of this study was to estimate the heritabilities ( $h^2$ ) of relative abundances (%) of microbial genes explaining feed conversion ratio (FCR), average daily gain (ADG) and daily feed intake (DFI) in beef cattle across different breeds, diets and independent animal trials.

**Material and methods** This study used phenotypic and metagenomics data from 359 steers from 5 different experiments using various breeds (rotational cross between Aberdeen Angus and Limousin, Charolais and Luig) and offered ad libitum two basal diets (forage and concentrate based). Data on DFI, ADG and FCR were available as described in detail by Lima et al., 2019. The rumen microbial gene abundances were determined using whole metagenomic sequencing of microbial DNA extracted from post-mortem rumen samples (fluid digesta) and by aligning the obtained reads to the KEGG database as described in detail by Roehe et al. (2016). In this study, we used 43 microbial genes previously identified as main proxies explaining FCR, DFI and ADG (Lima et al. 2019). To estimate  $h^2$  we fitted a univariate animal model on each microbial gene abundance. Pedigree comprised 3,392 animals from Aberdeen Angus and Limousin crosses, 927 from Luig and 650 from Charolais. The genetic model included a combination of breed, diet and experimental year as a fixed effect and a random genetic effect. Genetic effects and residuals were assumed to be independent and normally distributed as  $N(0, A)$  and  $N(0, I)$ , respectively,  $A$  being the additive genetic

relationship matrix based on the pedigree, the genetic variance and the residual variance. Bayesian inference was used (TM software, Legarra et al. 2008) with bounded flat priors for all unknowns. Results were based on Markov chain Monte Carlo chains consisting of 1,000,000 iterations, with a burn-in period of 200,000, only 1 of every 100 samples was saved for inferences. In all analyses, convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures.

**Results** To our knowledge, this is the first study reporting  $h^2$  for rumen microbial gene abundances in cattle (Fig. 1). Heritabilities of microbial genes explaining FCR, ADG and DFI traits ranged between 0.09 and 0.44. Most of the microbial genes involved in genetic processes such as translation, ribosomal structure and biogenesis, and transcription pathways (K06178, K07561, K03530, K02600, K02879, K02919, K02518), showed large  $h^2$  (> 0.28) independently of the trait. Other microbial genes with high  $h^2$  are involved in glycine (K00613,  $h^2=0.41$ ) and carbohydrate (K00040,  $h^2=0.36$ ) metabolisms. In general, microbial genes showing the largest  $h^2$  corresponded to those showing the largest genetic variances (results not shown), suggesting substantial potential for responses to selection.

**Figure 1.** Heritabilities (means and s.e.) of microbial genes explaining feed efficiency traits. Microbial genes are described in Lima et al., 2019



**Conclusion** Our results showed a host genetic effect on microbial gene abundances explaining feed efficiency traits, which open up new opportunities to integrate microbiome information into breeding programs targeting feed efficiency in cattle.

**Acknowledgements** This project was funded by Genus plc, BBSRC, the Scottish Government, Defra, AHDB and QMS. References Legarra et al., 2008 <http://snp.toulouse.inra.fr/~alegarra/manu-altm.pdf>; Lima J et al. 2019. *Front. Genet.* 10: 701; Saborio-Montero A. et al. 2019 *J Anim Breed Genet*, 1-13; Roehe R et al. 2016. *PLoS Genet.* 12, e1005846.

# Dynamic modelling of pig gut microbiota

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**Application** Manipulations of porcine gut microbiota could improve host health and performance. Mathematical modelling could forecast effects of such interventions prior to application.

**Introduction** The porcine gut microbiota (PM), composed of a large number of microbial species, has several levels of interaction with its host such as energy production from indigestible sources associated with the production of host-beneficial short chain fatty acids (SCFA). This makes PM manipulations a valid therapeutic strategy (Fouhse et al., 2016). Nevertheless, PM investigations are both ethically/technically demanding and expensive. Therefore, our aim was to develop and validate through in vitro experimentation, a mathematical model to predict SCFA and PM changes over time and test in silico interventions.

**Material and methods** An innovative mathematical model depicting the PM dynamics was developed through summarising the microbial community in ten functional groups (BFGs), based on a similar approach used for human colonic microbiota (Kettle et al., 2015). The model was initially parametrised through literature data comparison and finally through validation with an in vitro continuous fermentation. The in vitro experiment, mimicking the porcine proximal colon was carried out for 35 days, throughout which qPCR and 16S rRNA gene sequencing were used to quantify the BFGs and reveal qualitative insights of the bacterial populations. Gas chromatography was used to

assess SCFA concentration. The new set of data was then used to reparametrize the model.

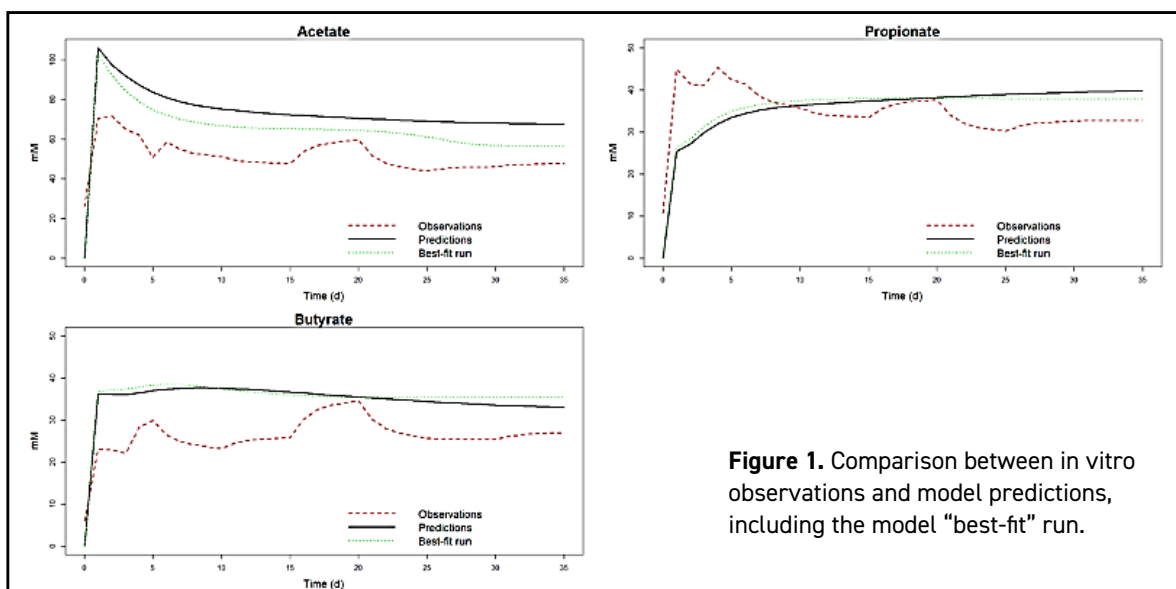
**Results** The PM model predicted rather optimally the dynamics of the major bacterial and archaeal communities and of the major SCFAs (i.e. acetate-propionate-butyrate) observed in vitro. Their modelled ratio was 48.1-28.4-23.6 %, whereas the ratio observed during the experiment was 44.5 -30.5-25.1 % (Figure 1). The closeness between observations and predictions was measured through root mean square error (RMSE) which decreased when parameterising the model using in vitro data, indicating an increase in model accuracy and reliability. The validated model was then used to simulate probiotic and prebiotic interventions targeting the BFGs. As a result, multiple scenarios were derived, for instance a combined therapy of non-starch polysaccharides (as prebiotic) and one BFG (as probiotic) showed to increase butyrate concentration in silico, suggesting a potential positive effect on the host well-being.

**Conclusions** The model presented here produced sufficiently accurate estimates of major SCFAs and bacterial communities, and thus represents a valuable tool in forecasting changes in the mentioned variables upon variations of the starting conditions, which could serve as a step preceding the necessary in vitro and ultimately in vivo investigations. The model was able to anticipate the dynamics of the main SCFAs and of the major BFGs. Although not meant to be a substitute for in vitro/in vivo experimentation it represents an adequate instrument to help anyone involved with the study of this biological ecosystem.

**Acknowledgments** The authors gratefully acknowledge SRUC, BioSS and the University of Edinburgh for supporting this work.

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**Figure 1.** Comparison between in vitro observations and model predictions, including the model "best-fit" run.

# Co-occurrence network analysis of rumen microbial genes and VFA in cattle based on mutual information

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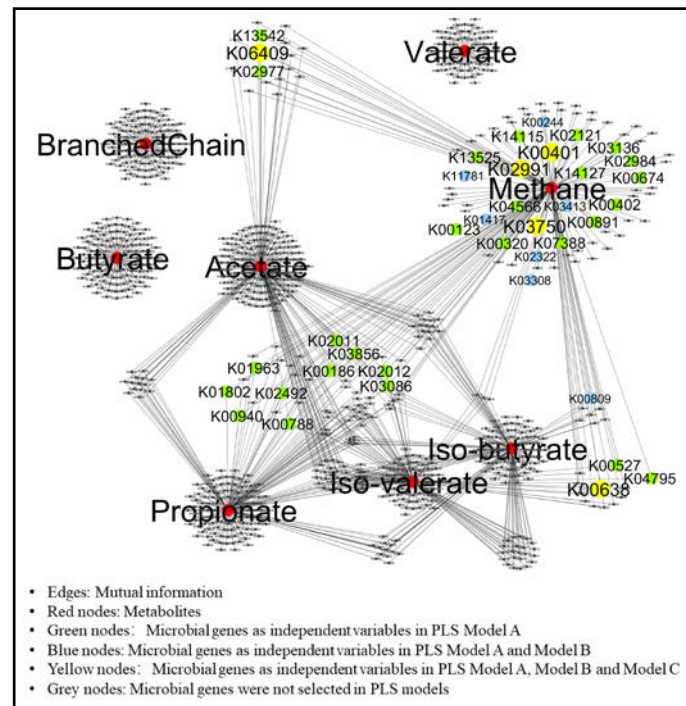
**Application** Rumen microbial genetic biomarkers for predicting methane emissions identified from this study will improve the knowledge of rumen microbial metabolism. This study could be applied to increase beef cattle productivity and economic efficiency while reducing greenhouse gas emissions, as well as improving meat quality (Omega-3 fatty acids) through nutritional interventions. Introduction Methane emissions from ruminants are among the most important greenhouse gases from animal agriculture. In addition, methane emissions cause losses of up to 12% of the total energy intake, depending on the composition of the diet and the digestibility of nutrients. In the rumen, feed is converted into acetate, propionate, butyrate and other fermentation acids by the microbes, and methane is subsequently synthesized by the methanogens. In this study, microbial gene biomarkers for methane emissions were inferred by a co-occurrence network based on mutual information between metabolites and microbial genes.

**Material and methods** This study was based on data from two-factorial design experiments which were performed using four bovine genotypes (purebred Luing and crossbred Charolais steers in 2013, Aberdeen Angus and Limousin rotational crossbred steers in 2014) and two basic diets (forage: concentrate ratios of 520:480 or 84:916 g/kg dry matter) with added nitrate, lipid or a combination. Methane emissions were recorded individually for 48h in respiration chambers (Rooke et al., 2014). A total of 42 animals balanced for breed type and diet were selected for whole metagenomic sequencing and high-performance liquid chromatography (HPLC). Details of the experiment were reported by Wallace et al., 2015 and Roehe et al., 2016. The associations between microbial KEGG (Kyoto Encyclopaedia of Genes and Genomes) genes and metabolites were investigated by a co-occurrence network based on mutual information, in which the significance of the association was calculated by Brown's method and corrected by the Benjamini-Hochberg method using the Cytoscape plugin CoNet. Significance was assessed at  $P < 0.05$ . Subsequently, the network was clustered by Markov clustering available at clusterMaker (<http://www.cgl.ucsf.edu/cytoscape/cluster/clusterMaker.shtml>). The partial least squares model (PLS) and the variable importance for projection (VIP) were calculated by Matlab.

**Results** Eight metabolites and 1461 microbial genes were analysed in the co-occurrence network, and a network of 905 nodes and 1414 mutual-information edges was finally formed. After the co-occurrence network was clustered, butyrate, valerate and branched-chain volatile fatty acids formed three separated clusters surrounded by the microbial genes that are significantly associated

with them. The interconnected metabolites and microbial genes in the central cluster of co-occurrence network were used in the PLS model for the prediction of methane. The PLS analysis repeatedly excluded variables with VIP values less than 0.8, then Model A could explain 94.99% of the methane variation was subsequently generated, which included acetate, propionate, iso-butyrate, iso-valerate and 39 microbial genes, which all have VIP greater than 0.8. Then Model B has been improved by setting the VIP screening threshold to 1, which can explain 84.75% of methane variation only with same four metabolites and 12 microbial genes. Then the selection has been repeated until got the final Model C with two acetate, propionate and five microbial genes, and the explanation of methane variation became 67.63%.

**Figure 1** Co-occurrence network



**Conclusion** The co-occurrence network combined PLS model with VIP values as a screening condition can help identify the key metabolites and microbial genes for the methane prediction. Although the explanation of methane variation decreased with excluding variables, it helped us identify the most possible biomarker for methane metabolism in rumen. Acetate and propionate are undoubtedly influencer for methane emissions, surpassing other metabolites and microbial genes in the results of this study. Followed by iso-butyrate and iso-valerate. Regards to the five microbial genes in Model C, K00638 and K06409 have not been reported in any pathway, K00401 is directly involved in the methane metabolism pathway, K03750 relate to folate biosynthesis, and K02991 is small subunit ribosomal protein S6e.

**Acknowledgements** This research is jointly supported by Ulster University and Scotland's Rural College, U.K. and partially supported by the MetaPlat project, ([www.metaplat.eu](http://www.metaplat.eu)), funded by H2020-MSCA-RISE-2015 and the Scottish Government.

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# Relationship between dry matter intake, milk yield and energy balance, and milk fat-to-protein ratio scores in Holstein cows during the first month of lactation

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**Application** The high incidence of high milk fat-to-protein ratios (FPR: (>1.5) during the first month of lactation reflects the large negative energy balance (EB) at this time, driven mainly by low feed consumption and high milk yield.

**Introduction** While milk FPR is known to be negatively correlated with EB, there is evidence that extreme FPR values (both high and low) are associated with a greater risk of metabolic disturbances. For example, in Holstein cows high FPR values (> 1.5) have been linked with an increased risk of ketosis, while low FPR values (< 1.0) reflect an increased risk of acidosis. However, the relationship between these extreme FPR values and EB is less well understood. Thus the aim of the present study was to examine the relationship between FPR score, and dry matter intake (DMI), energy corrected milk (ECM) and estimated EB (EBest), during the first 4 weeks of lactation in Holstein cows offered predominantly grass silage based diets.

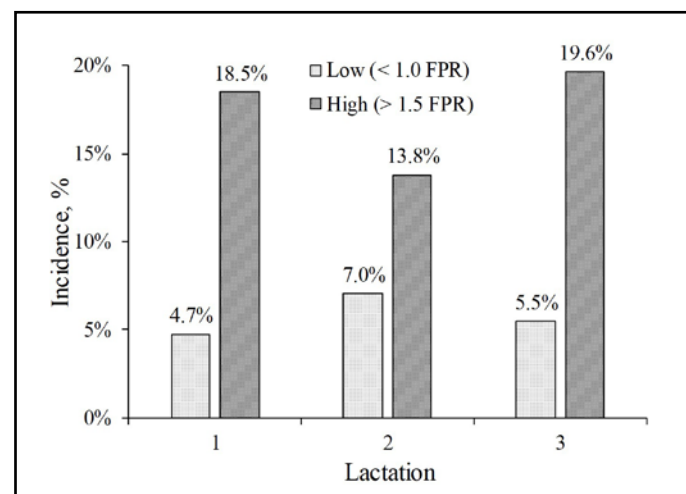
**Material and methods** This study involved a meta-analysis of individual cow data collected from 27 experiments (79 treatments, 1124 part lactations, 774 individual cows) conducted at AFBI Hillsborough in Northern Ireland. Data from all cows encompassed the first 90 to 140 days in milk. Perennial ryegrass silage was the predominant forage source in all experiments (mean forage-to-concentrate ratio on a DM basis was 48:52). Concentrate metabolisable energy (ME) contents were determined from 'book values' (FeedByte® - SRUC Consulting) for individual ingredients, while the ME content of forages offered were mostly determined via NIRS predictions. Both DMI and milk yields were recorded daily, whereas live weight and milk composition data were normally collected on a weekly basis. The data collected allowed the EB of each individual cow to be estimated daily (EBest, MJ of ME/d) using equations within 'Feed into Milk'. Mean weekly EBest values (MJ/cow/day) were subsequently calculated (n = 3926). Individual cow milk composition data was used to determine FPR on a weekly basis, from 4 until 28 days in milk, and values were then classified according to one of three FPR scores: 'Low' FPR, < 1.0; 'Normal' FPR, 1.0 to 1.5, and; 'High' FPR, > 1.5, for each of lactations 1 to 3. Mean weekly DMI, ECM, and daily EBest for animals within each FPR score category were then compared using a Linear Mixed Model, with FPR score as a fixed effect, and both study and cow included as random crossed effects in the model. Differences between least square means were tested by pairwise comparisons with the Tukey-Kramer test when relevant. For all models, statistical significance was declared at P<0.05. Statistical analyses were performed using SAS® (9.3 version).

**Results** During the first four weeks post calving (across lactations 1 to 3) there was a numerically greater mean incidence of 'High' FPR scores (17.3%) than 'Low' FPR (5.7%) scores (Figure 1). The relationships between DMI, ECM, and EBest with FPR scores are presented in Table 1. Irrespective of lactation number, milk yield increased with increasing FPR scores, while EB followed the reverse trend (P<0.001). Although DMI differed between scores in each of lactations 1 (P<0.001) and 3 (P<0.018) no consistent trend was observed.

**Conclusion** The results from this study demonstrate a large incidence of high FPR scores during the first 4 weeks of lactation, with these associated with higher milk yields and an associated large negative EB. Lower DMI's during lactations 1 and 3 also contributed to higher FPR scores. This data confirms that cows with a FPR >1.5 in early lactation are likely to have a greater risk of negative energy balance.

**Acknowledgements** This work was funded by DAERA and DAFM as part of the DAFM NutriGen project.

**Figure 1** Incidence of both 'Low' and 'High' FPR scores across lactations 1 to 3 during the first month post calving.



**Table 1** Relationships between adjusted least squares means DMI, ECM and EBest, and FPR across lactations 1 to 3 during the first month post calving.

Lactation/ Item	FPR Score			s.e.m	P-value
	< 1.0	1.0 to 1.5	> 1.5		
Lact. 1 (n= 1639)					
DMI, kg/d	13.4b <sup>a</sup>	13.7 <sup>a</sup>	13.0 <sup>b</sup>	0.38	<0.001
ECM, kg/d	22.0 <sup>c</sup>	25.6 <sup>b</sup>	27.4 <sup>a</sup>	0.55	<0.001
EBest, MJ of ME/d	-6.89 <sup>a</sup>	-19.3 <sup>b</sup>	-39.4 <sup>c</sup>	4.71	<0.001
Lact. 2 (n= 1417)					
DMI, kg/d	17.2	17.2	16.9	0.46	0.450
ECM, kg/d	30.7 <sup>c</sup>	34.7 <sup>b</sup>	37.0 <sup>a</sup>	0.79	<0.001
EBest, MJ of ME/d	-11.5 <sup>a</sup>	-33.4 <sup>b</sup>	-51.2 <sup>c</sup>	4.63	<0.001
Lact. 3 (n= 870)					
DMI, kg/d	17.8b <sup>a</sup>	18.3 <sup>a</sup>	17.5 <sup>b</sup>	0.49	0.018
ECM, kg/d	35.1 <sup>c</sup>	38.7 <sup>b</sup>	40.8 <sup>a</sup>	0.87	<0.001
EBest, MJ of ME/d	-29.9 <sup>a</sup>	-45.4 <sup>b</sup>	-66.3 <sup>c</sup>	5.90	<0.001

a,b,c Means within a row with different superscripts differ (P<0.05).



# Modelling energy balance curves during early lactation in Holstein dairy cows offered grass silage based diets

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**Application** Developing a better understanding of energy balance (EB) curves may help improve both nutritional and reproductive management of dairy cows during early lactation.

**Introduction** Modern dairy cows are often unable to consume sufficient nutrients to meet their energy requirements during early lactation, and as a consequence, experience negative EB. While EB can be measured directly for short periods of time using respiration calorimeters, longer term estimates are normally obtained as the difference between energy intake and energy required for production. While this information may be obtained from individual studies, robust estimates of EB require larger data sets, with information particularly lacking for cows offered grass silage based diets. The objectives of this study were to: 1) compare the goodness of fit of 15 polynomial random regressions to model EB trends, and, 2) using the best model identified in terms of curve fitting, to parametrise the EB performance for Holstein dairy cows during lactations 1-3.

**Material and methods** Data (dry matter intakes, milk production and composition, live weights and feed composition) for the first 90 – 154 days in milk from 27 experiments (79 treatments, 1122 part lactations, 768 individual cows) conducted at AFBI Hillsborough in Northern Ireland were used to estimate the EB of each individual cow on a weekly basis (EBest, MJ of ME/d) using equations within 'Feed into Milk'. Perennial ryegrass silage was the predominant forage source in all experiments (mean forage-to-concentrate DM ratio of 48:52). Energy balance curves for each lactation (1-3) were modelled using a total of 15 polynomial random regression models (11 models from de Vries et al. (1999), plus 4 additional models, the latter involving different model constants). The ability of each model to predict EBest for each of lactations 1-3 in terms of goodness of fit was tested using root means square error (RMSE), and Akaike's information criterion (AIC). Days in milk (DIM) were set as a fixed effect, whilst both study and cow were included as random crossed effects in the models.

**Results** Of the 15 models evaluated over lactations (1-3), the Ali and Schaeffer model described by de Vries et al. (1999) (but modified with an extra quadratic linear term, f, and 305 DIM as a constant) displayed the best goodness of fit (lower RMSE and AIC; Table 1). As parity increased, cows tended to experience larger negative EBest (Figure 2) but faster recovery rates from NADIR until achieving positive EBest.

**Conclusion** The modified Ali and Schaeffer function, (as described above), provided the best fit for the weekly EBest data. The parameters of the fitted EB curves clearly differed between lactations 1, 2, and 3.

**Acknowledgements** This work was funded by DAERA as part of the DAFM NutriGen project.

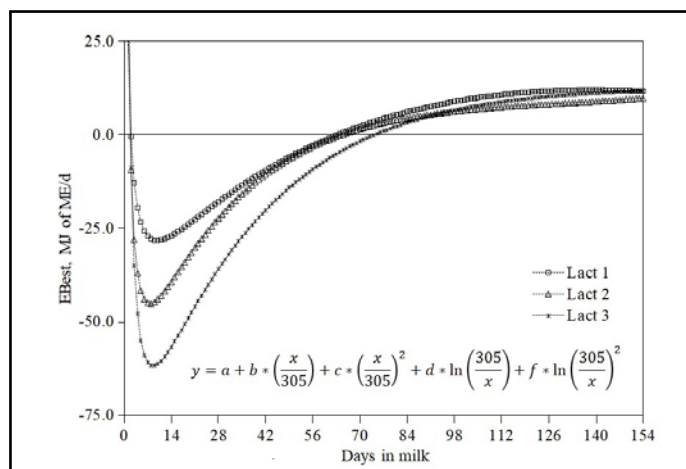
**References** de Vries et al. 1999. Journal of Dairy Science. 82, 1927-1934.

**Table 1** Prediction equations and model parametrisation for each lactation (1-3) to describe changes in EBest in Holstein dairy cows during early lactation according to a modified Ali and Schaeffer model.

Item	Lactation		
	1	2	3
Model terms1			
a	126.7±35.89	353.0±46.06	403.8±68.55
b	-96.2±78.76	-581.5±101.31	-604.7±151.01
c	-54.7±61.73	280.6±79.65	232.0±118.94
d	-85.3±17.37	-194.2±22.31	-234.5±33.3
f	12.0±2.20	24.4±2.82	30.5±4.22
RMSE2	22.2	26.6	30.4
AIC3	85432	75854	46886
Model parametrisation			
NADIR EBest, MJ of ME	-28.3	-45.2	-61.8
Days to NADIR	9.84	7.69	8.55
Days to positive EBest	63.6	65.0	74.6
Total E. deficit, MJ of ME	898	1247	1968
Recovery rate, MJ of ME/d	0.53	0.79	0.94

<sup>1</sup>Regression coefficients: a, b, c, d, f; <sup>2</sup>RMSE= Root mean square error; <sup>3</sup>AIC= Akaike's information criterion.

**Figure 1** Energy balance curves (lactation 1-3) as estimated by the modified Ali and Schaeffer model. Model terms: a, b, c, d, f; x= days in milk.





# Effects of season on nutrient composition, milk yield and estimated feed intake from grazed grass in dairy cows

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**Application** Modelling the effects of pasture quality on dry matter intake (DMI) and milk yield provides dairy farmers greater opportunities to make clearer decisions on supplementation strategies when pasture quality is poor to improve feed utilisation and the economics of global milk supply.

**Introduction** Grazing systems have been associated with greater sustainability and product quality (Dillon et al, 2006). However, although grazed grass is one of the cheapest feeds on dairy farms in Great Britain, it is often poorly utilised. Limited research has focused on pasture quality and efficiency of feed use and milk production. A greater understanding of seasonal variation in pasture quality could improve feed efficiency (AHDB, 2019) by shaping appropriate supplementation strategies that increase dairy margins. This study aimed to assess seasonal fluctuations in pasture quality and to model effects of pasture quality on dry matter intake and milk yield.

**Materials and methods** Pasture samples ( $n = 80$ ) were collected from 17 pasture-based dairy farms across the UK in 2017, directly prior to grazing. Pasture height was measured and pasture subsamples ( $n=20$ ) were collected, by traversing each paddock in a 'W' pattern, comingled and analysed for nutritional composition of each composite sample using Near Infra-Red (NIR) technology (Trouw Nutrition GB, Ashbourne, UK). Pasture samples were blocked according to season: Early (April to June) ( $n = 31$ ); Mid (July to September) ( $n = 35$ ); and Late (October to December) ( $n = 14$ ) and pasture composition was used to model the potential DMI and milk yield using Ultramix Professional (AGM Systems, Romsey, UK) with Feed into Milk (Thomas, 2004) equations. Data were normally distributed and season was applied as a fixed effect in an ANOVA General Linear Model (Minitab 17) to assess its effect on pasture nutrient composition and potential DMI and milk yield, reporting differences tested by Tukey's at  $P < 0.05$  and the results were validated using power analysis.

**Results** Pasture dry matter content was greater in Early and Mid-season compared with Late season (Table 1). ME content was not affected by season, while the crude protein content of pasture was greater in Late compared with Early season. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) of pasture was lower in Early compared with Mid and Late season. Oil content was lower in Early and Mid-season compared with Late season.

**Table 1** Seasonal variation in nutrient composition, estimated feed intake and milk yield from perennial rye grass and white clover pasture swards on 17 dairy farms across the UK.

Season	Early-	Mid-	Late-	P value
Samples, No.	31	35	14	-
Dry matter, g/kg	173 (5.9) a	176 (5.5) a	145 (8.7) b	0.011
Crude protein, g/kg DM	212 (6.0) b	223 (5.5) a, b	244 (8.9) a	0.013
Metabolisable energy, MJ/kg DM	11.8 (0.09)	11.7 (0.08)	11.6 (0.13)	0.331
Sugars, g/kg DM	86 (4.3) a	59 (4.0) b	45 (6.4) b	<0.001
Neutral-detergent fibre, g/kg DM	414 (8.5) b	485 (8.0) a	503 (12.7) a	<0.001
Acid-detergent fibre, g/kg DM	215 (5.4) b	237 (5.1) a	247 (8.4) a	0.001
Oil (Method A), g/kg DM	43 (0.9) b	44 (0.8) b	49 (1.4) a	0.001
Estimated potential:				
DMI, kg/d	11.3 (0.60) a	9.5 (0.60) a	6.0 (0.96) b	<0.001
Milk yield, kg/d	10.6 (1.38) a	6.3 (1.30) a	-1.93 (2.21) b	<0.001

a, b – Means in rows followed by differing superscript letters differ significantly at  $P < 0.05$

**Conclusion** Dry matter intake (DMI) and milk yield potential decreased as the grazing season progressed. Potential DMI of Late-season pasture was insufficient to support lactation. Thus, pasture quality data could be used to develop decision making tools that inform pasture management and animal feeding strategies to improve pasture quality and milk production per hectare.

**Acknowledgements** C.G. was funded by a University of Nottingham scholarship, while the research was funded by AHDB Dairy - Nottingham Partnership on Health, Welfare and Nutrition.

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# Effect of herbage height and density on pasture nutrients

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**Application** Mobile near-infrared spectroscopy now allows monitoring of grassland and pastures in real-time. This information can enhance forage management by improved awareness of changes in nutrient concentrations.

**Introduction** New tools and sources of information for biomass and nutrient composition measurements are being developed to help quantify temporal and spatial changes in herbage in real-time to provide more timely information for enhanced land management. Bell et al. (2018) used real-time near-infrared spectroscopy combined with a measure of herbage production to show that grazing pastures to a mean height of below 7 cm results in significantly reduced concentration of crude protein and digestibility, which may be detrimental to animal productivity. The aim of this study was to assess the effect of changes in herbage height and density on pasture nutrients among permanent and temporary swards.

**Material and methods** Grass measurements were obtained from permanent (>5 years old) and temporary ley (1 or 2 years old) pastures at 7 farms during the spring and autumn months during 2019. The same fields were sampled within each farm during the study. A total of 12 fields were studied, which were used for strip or mob grazing cattle and sheep. Across fields studied there was a diverse range of plant species including perennial ryegrass (*Lolium perenne*), timothy (*Phleum pratense*), Yorkshire fog (*Holcus lanatus*), cocksfoot (*Dactylis glomerata*), common bent (*Agrostis capillaris*), oat-grass (*Arrhenatherum elatius*), chichory (*Cichorium intybus*), plantain (*Plantago lanceolata*), red clover (*Trifolium pratense*), alsike clover (*Trifolium hybridum*) and white clover (*Trifolium repens*). In each field 5 grass samples were cut to ground level and within a 36 cm diameter wire ring (0.1 m<sup>2</sup>) randomly placed on the ground. A total of 620 herbage samples were collected for nutrient analysis during the study. A mobile near-infrared spectroscopy device (NIR4; Aunir, Towcester, UK) was used to scan cut samples for their nutrient concentrations. Nutrients measured were: dry matter, crude protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), sugars, oil, ash, digestible organic matter (DOMD), nitrate nitrogen (all expressed as grams per kilogram of dry matter) and metabolisable energy (ME, megajoules per kilogram of dry matter). A rising plate meter (F400; Farmworks Precision Farming Systems Ltd, Feilding, NZ) was used to measure herbage height. To ensure representative coverage of each field, the herbage samples and height measurements (about 30 'spot' measurements) were taken in a W-pattern across the field. Data were analysed using a linear mixed model in Genstat (version 19.1) to assess the fixed effects of month of year, if grazed (yes or no), age of sward (temporary or permanent), herbage density (kg DM/ha/cm) and height (cm) on

nutrient concentrations across fields studied. Farm was added as a random effect. Significance is attributed at  $P < 0.05$ .

**Results** The month of the year affected most nutrients ( $P < 0.001$ ) except oil content ( $P > 0.05$ ). Permanent pastures had higher dry matter and lower ME compared to temporary swards (both  $P < 0.05$ ). Grazed pastures were associated with higher nitrate ( $P < 0.001$ ) and ADF, but lower crude protein (both  $P < 0.05$ ) compared to non-grazed swards. Increasing herbage density reduced sugar, DOMD and ME (Table 1) and increasing herbage height reduced dry matter and increased oil, ME, ADF and sugars. Increasing both density and height increased dry matter, NDF and ADF but reduced crude protein, ash, DOMD and ME.

**Table 1.** Significant effects of herbage density and height on pasture nutrients after adjusting for fixed and random effects.

**Conclusion** Changes in herbage density, herbage height and time of year affect pasture nutrients, and reflect changes in botanical composition, establishment and tillering, as well as plant maturity. While the presence of grazing animals may reduce crude protein and increase less digestible plant cell wall nutrients (ADF) in the sward compared to ungrazed pastures, the animal appears to enhance plant nitrate concentrations and the potential for plant growth presumably through deposition of dung and urine. The nutrient content of pastures are typically not monitored but doing so may help farmers improve how effectively they utilise forage and improve grazing management.

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Variable	Effect (s.e) d.f. = 1			P value		
	Density	Height	Density × Height	Density	Height	Density × Height
Dry matter g/kg	0.13 (0.04)	-1.3 (1.8)	0.02 (0.008)	NS	<0.01	<0.001
Crude protein g/kg DM	-0.05 (0.01)	-1.5 (0.6)	-0.01 (0.003)	NS	NS	<0.001
NDF g/kg DM	0.16 (0.04)	2.4 (1.4)	0.03 (0.006)	NS	NS	<0.001
ADF g/kg DM	0.02 (0.01)	1.3 (0.4)	0.005 (0.002)	NS	<0.05	<0.05
Ash g/kg DM	-0.02 (0.007)	-0.1 (0.3)	-0.004 (0.001)	NS	NS	<0.001
Oil g/kg DM	-0.001 (0.002)	0.13 (0.08)	-0.0005 (0.00004)	NS	<0.01	NS
Sugars g/kg DM	-0.04 (0.01)	0.9 (0.4)	-0.003 (0.002)	<0.001	<0.05	NS
DOMD g/kg DM	-0.06 (0.01)	-0.4 (0.5)	-0.008 (0.002)	<0.01	NS	<0.001
ME g/kg DM	-0.01 (0.002)	0.05 (0.08)	-0.001 (0.0003)	<0.01	<0.01	<0.001
Nitrate %	-0.000003 (0.000004)	0.003 (0.001)	0.000006 (0.000006)	NS	NS	NS

# Using herd information and milk production data to predict dry matter intake within feed-to-yield concentrate allocation strategies

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**Application** It is possible to predict total dry matter intakes within feed-to-yield systems with a good degree of accuracy, and this could be used to improve the accuracy of concentrate allocation within these systems.

**Introduction** There is considerable interest in the adoption of 'precision feeding' approaches within dairy systems. A widely adopted approach, involves allocating concentrates on a feed-to-yield basis. This normally involves offering a 'basal diet' (often a mixture of forage and concentrate ingredients, in the form of a mixed ration) which is designed to supply the maintenance energy requirements, plus the production of a certain volume of milk, of the average cow in the herd. This is often known as the 'Maintenance Plus' or M+ value. Additional supplements are then offered to each individual cow, normally through an in-parlour or out-of-parlour feeding system, at a level designed to support milk yields in excess of those assumed to be supported by the basal diet. However, a potential limitation of this approach is the assumption that the basal diet supports a single assumed M+ value for all cows. It is postulated that if individual M+ values could be calculated for each individual cow, then concentrates could be offered with an increased level of precision. The first step in achieving this, and the objective of the current study, is to use readily available farm data to develop dry matter intake (DMI) prediction equations for individual cows. The intake of the basal diet for each cow can then be determined by deducting the known quantity of concentrate (DM basis) offered on a 'feed-to-yield' basis from the total predicted DMI. The M+ supported by the basal diet can then be determined based on the ingredient composition of the basal diet.

**Table 1.** Prediction equations for dry matter intake (DMI) within feed-to-yield dairy systems

Model	Equation	Correlation <sup>1</sup>	Adjusted R-squared	RMSE
Model 1	DMI=11.032+(0.554×Lactation number)+(0.343×ECM)+(-3.194×Fat:Protein)+(0.107×week in milk) (Equation 1)	0.84*	0.71	2.03
Model 2	DMI=3.745+(0.015×Live weight)+(0.155×Lactation number)+(0.311×ECM)+(-2.829×Fat:Protein)+(0.068×week in milk) (Equation 2)	0.86*	0.73	2.04

<sup>1</sup> Pearson's product moment correlation between predicted and actual DMI

\* P < 0.05

**Material and methods** Data were obtained from five studies conducted at the Agri-Food and Biosciences Institute, Hillsborough, UK from 2013 to 2019, which involved feed-to-yield concentrate allocation strategies. In all studies a basal diet, comprising a mixture of grass silage and concentrates, was offered, with additional concentrates then offered to each individual cow through an out-of-parlour feeding system. All studies commenced at calving, and normally involved a 3 - 4 week concentrate build up strategy, before cows moved onto the feed-to-yield approach. Studies were conducted over the first 140 - 180 days of lactation. Studies differed in a number of ways, including, concentrate feed rate through the out-of-parlour feeding, silage types offered, and assumptions used to determine concentrate allocations. The following data was available from each study for each individual cow: lactation number (1, 2, 3 and >4), week in milk, and weekly data for DMI, milk production, milk composition, and live-weight. Energy corrected milk (ECM) yield (kg/day) and milk fat : protein ratio (Fat:Protein) were subsequently determined for each week. Two multivariate linear regression models were constructed to predict daily DMI, using weekly data from experiments 1 - 4. The Model 1 included lactation number, ECM, Fat:Protein and week in milk, while the Model 2 included live weight also. These models were developed based on backward selection, and all the variables retained in the equations had a P value lower than 0.05. For diagnosing collinearity in the multivariable regression model, Variance Inflation Factor (VIF) values were estimated. Data from experiment 5 were then used to validate the two equations. The Pearson's product-moment correlation, R-squared and root mean square error (RMSE) between predicted DMI and actual DMI were obtained. All analysis was undertaken using R v3.5.3.

**Results** The two DMI prediction models are presented in Table 1. Model 1 uses data that is readily available for individual cows on many farms, while Model 2 includes live weight, which will becoming more available. The collinearity tests demonstrated that VIFs were less than 4, meaning that collinearity between predictors was not observed. These models can be used to predict DMI within dairy systems where a feed-to-yield concentrate allocation approach has been adopted.

**Conclusion** The ability to predict intakes of individual dairy cows creates opportunities to increase the precision with which concentrates are allocated to individual cows within feed-to-yield systems.

## Acknowledgements

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# Digital images can objectively measure body condition of dairy cows

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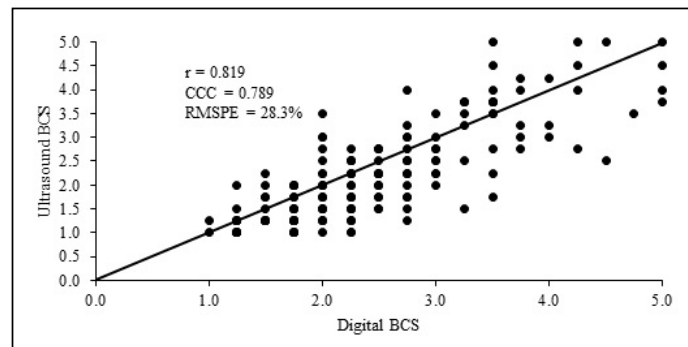
**Application** Digital images can be used to provide an objective prediction of cow body fat and the condition of a cow from tailhead dimensions.

**Introduction** Modern high milk yielding dairy cows, have a high genetic potential for mobilising body fat for milk production. However, low or high body fat can be detrimental to cow health, fertility, and longevity. The aim of this study was to assess whether cow body fat and body condition score (BCS) can be estimated from tailhead body measurements and digital images to create an objective measure.

**Material and methods** Ultrasound body fat measurements and digital photos of the tailhead were obtained from a total of 209 cows from two Holstein dairy herds, with 87 cows from Farm A and 122 cows from Farm B. A visual observation and physical examination of the cow's body fat around its tail head (manual score) was also carried out following the condition scoring method of Edmonson et al. (1989). The cows used in this study represented a range of cows at different stages of production from early, mid and late lactation and prior to calving. Body fat depth in millimetres was measured in the caudal area of the tailhead for each cow with an Easy-scan 4 ultrasound scanner (BCF, Livingstone, UK) and a digital image was also taken (5 megapixel camera; Vodafone Smart Tab 4G, Newbury, UK) of the same area. The image photo was taken from directly behind the cow at a 10° angle above the tail head and from 2 metres behind the cow. Digital software (Inkscape 0.91, Boston, US) was then used to measure the tail head width distance, distance between the hook bones and distance between the pin bones of each cow. The distances were measured in pixels and the width of the tail head was expressed as a percentage of the distance between the pin bones or the distance between the hook bones. The ultrasound fat depth measurement and digital image measurements were converted to a linear BCS from 1 to 5 with quarters by attributing values to one of 17 categories over the range of scores. The ultrasound body fat measurement was used to test the accuracy of BCS methods. Pearson correlation coefficient ( $r$ ) was multiplied by Lin's bias correction factor ( $C_b$ ) to derive the concordance correlation coefficient (CCC). The CCC was used to test the association between BCS methods. Prediction error was assessed by the square root of the mean square prediction error (RMSPE) expressed as a percentage of the observed mean ultrasound BCS.

**Results** The average BCS across cows was 2.10 for ultrasound, 2.41 for digital images and 2.76 for the manual score. The study found that BCS estimated from digital images had a high association with ultrasound BCS ( $r = 0.819$  and  $CCC = 0.789$ ; Figure 1). The prediction error for digital was moderately lower for cows with a BCS of 2.5 or more (RMSPE = 20.5%) compared to cows of less than 2.5 BCS (RMSPE = 35.5%). In comparison to digital BCS, the manual BCS had a similarly high association with ultrasound BCS ( $r = 0.790$  and  $CCC = 0.592$ ) and prediction error for cows of 2.5 BCS or more (RMSPE = 19.0%), but higher prediction error for cows of less than 2.5 BCS (RMSPE = 63.8%).

**Figure 1** Relationship between digital and ultrasound body condition score (BCS).



**Conclusion** While subjective manual scoring of cow body condition is widely used, but often not routinely done, the development of digital imaging provides an accurate, objective and non-invasive alternative to routinely monitor body condition of cows on commercial farms. This study showed that an objective measure of BCS from digital images would help identify thin cows of BCS less than 2.5.

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# Molecular Characterisation of Equine Herpesvirus 1 Isolates from Cases of Abortion, Respiratory and Neurological Disease in Ireland between 1990 and 2017

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**Application** The manifestations of EHV-1 infection are thought to be associated with the strain of virus. Genetic analysis of EHV-1 utilising multi-locus sequence typing (MLST) can be exploited to investigate EHV-1 outbreaks.

**Introduction** Equine alphaherpesvirus 1 (EHV-1) is a highly prevalent pathogen of horses in Ireland and worldwide. The virus has different clinical outcomes including respiratory disease, abortion, neonatal death and neurological disorders. The factors determining different disease outcomes after EHV-1 infection are poorly understood. A single nucleotide polymorphism of adenine (A2254/N752) to guanine (G2254/D752) within the ORF30 DNA polymerase is strongly associated with neuropathogenicity and viral shedding. A recent UK genotyping study which analysed 101 EHV-1 genomes proposed that EHV-1 has diverged into at least 13 viral clades (Bryant et al. 2018). The aim of this study was to investigate strain diversity among EHV-1 isolates from outbreaks of abortion, respiratory and neurological disease isolated in Ireland over a 28-year period using MLST and ORF68 analysis.

**Material and methods** Two hundred and seventy-two isolates from 238 EHV-1 outbreaks on 220 premises were genetically characterised by MLST. Viruses were recovered from clinical samples: nasal secretions from respiratory/neurological disease and tissues from cases of abortion/neonatal foal death (n = 247). Samples with a low concentration of virus (n = 25) were amplified by passage in cell culture. Primers were designed to amplify the loci of sequence variation between EHV-1 strains Ab4 (neuropathogenic) and V592 (non-neuropathogenic). PCR products were sequenced using Sanger technology. A concatenated amino acid sequence based on 38 amino acid differences between Ab4 and V592 and additional clade specific

substitutions was constructed for each isolate and EHV-1 genome sequences. The 38aa artificial peptide sequences were aligned using ClustalW. Phylogenetic analysis of 126 representative sequences was inferred by the Maximum Likelihood method. Tree topology was examined for UL clade resolution based on the study of Bryant et al. (2018). A chi-squared test was used to test the null hypothesis that there was no difference in the relative proportions of the G2254/D752 genotype between: isolates originating from neurological and non-neurological outbreaks, isolates from single cases of neurological disease and outbreaks with multiple neurological cases, and isolates from hypervirulent disease expression (multiple cases of abortion or neurological disease). Viruses from 222 of 238 outbreaks were characterised by ORF68 sequencing. Alignment of 441 ORF68 sequences (464bp) was used to construct an international haplotype network using PopART.

**Results** MLST grouped the 272 viruses into at least 10 of 13 UL clades. Viruses from the same outbreak had identical MLST profiles. ORF68 sequencing of EHV-1 isolates from 222 outbreaks established a divergence into seven groups. Network analysis demonstrated that Irish genotypes were not geographically restricted. Viruses from 31 of 238 outbreaks analysed had the proposed neuropathogenic marker. The association of neurological disease in those with the G2254/D752 genotype was estimated as 27 times greater than in those with the A2254/N752 genotype. Sequence analysis of isolates identified equid alphaherpesvirus 8 (EHV-8) associated abortion in horses originally attributed to EHV-1 infection. This is the first report of EHV-8, whose natural host is the donkey, causing abortion in horses. Complete genome sequences of four EHV-8 strains from two host species (horse and donkey) were determined by Illumina sequencing. Phylogenetic analysis indicated that the Irish EHV-8 strains exhibit minimal diversity. Conclusion MLST analysis in combination with epidemiological data has a potential role in tracking virus between premises and therefore in the implementation of targeted control measures. Complete genome sequences of EHV-8 strains will serve as a key reference for the development of specific assays for diagnosis and epidemiological research.

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# The Effect of Different Herbage Allowances on Dry Matter Intake and Nutrient Digestibility in Grazing Horses

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**Application** Grass is a valuable forage for horses and well-managed can provide an excellent source of nutrients and assist the horse's overall health and well-being.

**Introduction** This study investigated the impact of three different daily herbage allowances on grass dry matter intake (GDMI) and grass dry matter digestibility (GDMD) in grazing horses using naturally occurring indigestible markers.

**Materials and methods** Six Irish sport horses were assigned to three daily herbage allowances using a double Latin-square experimental design, T1 (Low): 2% BW/d, T2 (Medium): 3% BW/d and T3 (High): 4% BW/d. Each experimental period consisted of ten days of adaptation to the herbage allowance and six days of measurement. Daily herbage allowances were achieved by adjusting the grazing area offered to the horses every two days. Herbage mass disappearance was calculated using pre-grazing herbage mass relative to post-grazing herbage mass. Apparent digestibility of DM and CP was estimated using total faecal collection method. Faecal output was individualised for each horse by feeding indigestible synthetic markers daily. Three naturally occurring indigestible markers acid insoluble ash (AIA), acid detergent insoluble ash (ADIA) and acid detergent lignin (ADL) were used to estimate DM digestibility.

**Results** GDMI on T1 ( $13.0 \pm 0.75$  kg DM/d) was significantly higher compared to T2 ( $17.5 \pm 0.82$  kg DM/d) and T3 ( $20.6 \pm 0.93$  kg DM/d) ( $P < 0.05$ ). Both DMD (T1:  $40.6 \pm 0.01$ , T2:  $53.9 \pm 0.09$  and T3:  $62.10 \pm 0.07\%$ ) and apparent digestibility of protein (T1  $40.2 \pm 0.03$ , T2  $59.8 \pm 0.2$  and T3  $72.3 \pm 0.01\%$ ) were significantly different between the treatments ( $P < 0.05$ ). Daily herbage allowances presented significantly different digestible DM and CP intakes. Estimated digestibility coefficients using AIA (T1  $65.6 \pm 3.57$ , T2  $65.8 \pm 3.38$  and T3  $61.2 \pm 2.56$  %) and ADIA (T1  $69.2 \pm 6.08$ , T2  $68.2 \pm 4.67$  and T3  $64.9 \pm 6.53$  %) were not significantly affected by daily herbage allowances ( $P > 0.05$ ).

**Conclusion** This study describes nutrient availability on a grass-based diet for horses using three different digestibility methods at different grass allowances. All methods highlight the potential source of digestible nutrients available from grass for horses.

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# Equine (*Equus caballus*) social network construction within small domestic groups

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**Application** The ability to assess social structure in small domestic horse (*E. caballus*) groups would be of value to appraise management practices and optimise welfare through better informed companion selection.

**Introduction** Evidence suggests that keeping horses in groups is beneficial as increased opportunities for social interaction have been linked to improved welfare and trainability. The consideration of social structure within these groups is also recommended, so that attempts may be made to minimise inter-horse aggression and subsequent injury risk. Manual observation of interactions is often considered the most reliable way to determine group structure, yet is time consuming and prone to observer bias. The use of inter-individual proximity may be a more practical alternative for researchers and possibly those responsible for equine management, but first requires validation in the species of interest to ensure reliability (Castles et al., 2014; Davis et al., 2018; Farine, 2015). Consequently, this study aims to determine if equine social networks created using different techniques are comparable.

**Materials and Methods** Three independent established groups of mature horses; 'Group A' (n=4; mean age 20±4.55[SD] years), 'Group B' (n=3; mean age 17.67±7.09[SD] years) and 'Group C' (n=4; mean age 16±7.17[SD] years), were manually observed for a total of 20 hours per group during May/June 2019. Horses remained in their usual mixed-grass field throughout the study, with no supplementary provision of forage or resources, other than water. All social interactions that occurred were recorded with the use of a study-specific ethogram, and used to construct four social networks for each group, which considered (1) 'all observed', (2) 'affiliative', (3) 'grooming' and (4) 'agonistic' interactions. A proximity-based network was also created, based on inter-individual distances between group members obtained every 10-minutes from global positioning system (GPS) units attached to the 'field safe' head collar on each horse. Mantel tests were run with 1000 permutations in Socprog2.9 to determine if networks based on observed interactions are structurally similar to those based on inter-individual proximity. For half of the observation time (10/20hrs) horses also wore fly rugs with the neck removed. Attached to each rug was a non-commercial version of the Orscana® sensor, located below the left hip of each horse. This sensor contained an internal accelerometer set to log horse movement every minute. These movement data were used to investigate the effect of filtering

proximity data by activity level, so that all interactions occurring at times of high activity were removed, has on its agreement with interaction-based methods. A simple form of cost-benefit analysis was also undertaken across the methods to further inform future methodologies.

**Results** Mantel tests identified that proximity networks were similar to networks based on affiliative interactions between horses, with strong significant agreement seen in 'Group A' (Z=0.85438, P=0.05) and 'Group C' (Z=0.88925, P=0.05), and a moderate but non-significant relationship in 'Group B' (Z=0.61582, P=0.475). Proximity was not seen to be significantly associated with any other methods and filtering interactions by activity level did not influence any agreement seen when the chosen threshold was used. When the practicality of each method was considered, the use of GPS units was advantageous in the fact that it enabled data to be collected objectively and remotely, decreasing researcher time (and subsequent staffing costs) which would likely justify the initial cost of purchasing these devices. However, it is also worth considering that the use of GPS-derived data alone does not enable directed networks to be created, and could therefore be considered a less informative method, potentially making it inappropriate to meet some research aims.

**Conclusion** These findings suggest that GPS-derived proximity may be a viable alternative to manual observation when affiliative interactions are of interest. Although, more work is warranted to establish how generalisable these results are in larger groups, and how variables, such as field size, group composition and resource provision influence method agreement, the results of which will be of use to inform both equine management, companion selection and future study methodologies.

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# Characterising the pepsin concentration and activity from full, part-full and empty equid stomachs

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**Application** In vitro models are useful for equid gut health and nutrition studies without the need for using animals in research. Understanding pepsin concentrations in the horse will help inform the design of an equid in vitro stomach model.

**Introduction** In vitro models have previously been reported as an alternative to using live animals which is more cost effective and can be more ethically favourable than conducting research trials with animals. Previously reported in vitro gut models of horses have focussed upon hindgut fermentation. Foregut studies in animals have been predominantly based on pigs, for horses porcine values are often extrapolated and applied to the horse. In monogastrics the pepsin concentrations in the stomach are reported to be between 0.5-1 mg/ml. The aim of this study was to characterise the pepsin concentration in full, part-full and empty stomachs of horses to identify pepsin activity under these conditions.

**Material and methods** This study was granted ethical approval by the RAU ethics committee. Cadaver stomachs (n=13) were collected from horses that were slaughtered for human consumption. Post slaughter stomachs were collected and chilled to 4°C to transport to the laboratory. Incisions were made into the cardiac, fundic and pyloric regions to record pH. Stomach content was then removed and strained through a muslin bag, the liquid was then centrifuged at 15000 x g at 4°C and then frozen at -80°C. A further 11 ex-clinical samples were provided by a local veterinary practice from horses that had undergone diagnostic gastroscopy. The ex-clinical samples were already frozen at -80°C and were shipped to the university on dry ice. In total there were 24 samples of gastric juice, breeds collected from comprised ISH, Thoroughbred and New Forrest ponies, mean age 12 ( $\pm 8.86$ ) years. Samples were categorised into three groups, full stomachs, half full stomachs and fasted stomachs (ex-clinical samples).

Pepsin concentration and activity were analysed using a modification of the azocoll method (Will et al. 1984) whereby endogenous proteases in the sample digest azo impregnated collagen which leads to a colour change reaction. The assay was measured kinetically for activity and at end point for concentration over 30 minutes at 37°C. This was measured against a commercial pepsin (Fisher 10264440) standard curve.

Pepsin concentration was calculated using linear regression and activity via regression factoring in time and substrate loss. Calculated concentrations and activity were analysed by unbalanced ANOVA and least significant difference in Genstat 18th edition.

## Results

	Diet				
	Full	Part Full	Empty	s.e.d	P
Pepsin Activity (mg/ml/min)	0.031	0.031	0.031	0.00179	n.s.
Pepsin Concentration (mg/ml)	0.541	0.581	0.446	0.06127	n.s.
Relationship between concentration and activity R <sup>2</sup>	0.18	0.00	0.81a		<0.05

**Table 1** Pepsin activity and concentrations from full, part-full and empty stomachs of horses

**Conclusion** Our findings suggest that the pepsin concentration of equids is similar to that of other monogastrics (0.56  $\pm$  0.06 mg/ml). Concentration of pepsin and pepsin activity did not differ between the full, part-full and empty stomachs. Concentration was slightly lower in the empty, ex-clinical samples where horses had been fasted prior to gastroscopy, but not significant. These data provide a useful insight into the required pepsin concentrations when designing an in vitro stomach model for horses.

**Acknowledgements** The authors gratefully acknowledge B&W Equine Vets and L J Potter Ltd.

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# Contemplating the Five Domains model of animal welfare assessment: UK horse owner perceptions of equine wellbeing

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**Application** Owners assess their horse's welfare, and this influences management choices. This suggests that equine welfare knowledge may impact owner perceptions thus potentially influencing management decisions.

**Introduction** The Five Domains model (Green & Mellor, 2011) introduced the concept of all aspects of an animal's life influencing mental state and welfare. In equestrianism there is a lack of awareness of this notion (McGreevy et al, 2018) and this may compromise the welfare of domestic horses (*Equus caballus*) by failing to recognise them as sentient beings with species specific needs. The present study aimed to explore how evidence-based information can be effectively communicated to equestrians and whether increased awareness of equine welfare needs has any impact on assessment of their own horses' quality of life.

**Material and methods** A four-part survey was distributed to horse owners via Google Forms publicised on equestrian social media feeds in July 2019. Section one gathered demographic details from participants. Section two used Likert scales graded between 0-10, with 0 being 'not at all' and 10 being 'all the time', to determine how well participants felt they met their horses' needs, according to each of the Five Domains: Nutrition, Environment, Health, Behaviour and Emotional State, with a separate question about overall welfare. Section three used a short intervention in the form of an info-graphic of the Five Domains, originally distributed in Horses and People Magazine Australia. Section four gathered information on post-intervention welfare perceptions. Further open questions assessed views on which situations evoke positive or negative emotions in horses and identified barriers to improving welfare. Scores and responses as codes were subject to basic counts. Non-parametric tests were used, generated using Minitab 19 software and

analysed using Wilcoxon signed-ranks tests. Differences between scores for multiple categories were identified using Friedman and Kruskal-Wallis tests. The free-text responses were categorised using thematic analysis post-hoc and independently assessed by two researchers, with a third assessor consulted in the case of any disagreement.

**Results** A total of 259 usable surveys were returned from participants within the United Kingdom. A significant difference between scoring for different categories of the Five Domains ( $p=0.009$ ) pre-intervention was found, with scoring in the emotional category lower than other categories. There was a significant difference pre-intervention between scoring for emotions and overall welfare ( $p<0.001$ ) but no significant difference post-intervention ( $p=0.369$ ), with emotional score significantly higher post intervention ( $p<0.001$ ). There was also a significant difference in assessment of behaviour ( $p=0.017$ ) and in health assessment ( $p=0.018$ ) with both significantly lower post intervention but no significant difference in assessment of environment ( $p=0.998$ ) or nutrition ( $p=0.889$ ). There was a significantly lower welfare score post intervention ( $p<0.001$ ). Pre-intervention, there was a significant difference in scores for how often respondents felt their horses felt positive emotions and overall welfare score ( $p<0.001$ ) with scores for emotional wellbeing lower than for overall welfare. Post-intervention there was no significant difference between these scores. Within free-text responses, four higher order themes were identified for each question (Table 1).

**Conclusion** This study provided a valuable insight of how horse owners currently assess welfare and their perceptions of equine emotional state. Knowledge of the Five Domains model, imparted through a simple, accessible infographic, did impact these views by encouraging consideration of the factors influencing horses' ability to experience positive and negative emotions, significantly affecting equine welfare assessment. Free-text responses showed that lasting behavioural change may be hindered by factors outside the control of many equestrians, such as livery restrictions and resistance to new ideas, and this should be addressed through further research to identify individual motivations and how opportunities for welfare improvement can be more readily available. Additionally, development of an objective method of assessing equine emotional state is vital if horses whose lives might presently be dominated by negative emotions are to be given the chance for more positive experiences and enjoy a meaningful existence.

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**Table 1** Higher order themes informing participants beliefs (n=259)

Question	Higher order theme	Respondents mentioned
What situations do you believe would evoke the most positive mental state in horses?	Natural Environment	25%
	Positive human interactions	22%
	Company of conspecifics	53%
	Food	22%
What situations do you believe would evoke the most negative mental state in horses?	Confinement	52%
	Isolation	56%
	Negative human interactions	38%
	Hunger	33%
What barriers or limitations do you see to avoiding a negative mental state and promoting a positive mental state in horses?	Facilities	55%
	Attitudes	43%
	Knowledge	22%
	Financial	15%

# Practical challenges and scientific opportunities in rearing young livestock

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Young livestock are one of the most expensive, but important components of livestock production. They can, however, be one of the components of the herd that is more difficult to monitor and as such become less closely and well managed, which leads to inefficiency and unnecessary climate change. This paper aims to clarify the importance of their management in terms of financial cost and evaluates the potential for investment. It will explore how to optimise the impact of health and nutrition on the development and the future functionality of livestock. It will look closely at young livestock in terms of survival, the effect of health on future functionality, impact of growth on productivity and contribution to farm income, along with the effect on potential farm financial and environmental costs. It will explore the challenges and opportunities faced by the agricultural sector, including issues raised by consumer perception, animal welfare, climate change and global food security. It will examine some of the scientific opportunities and developments in terms of the application of molecular and scanning technology, microbiome evaluation, cell function and signalling, animal function and welfare, animal interactions and animal-human interactions. It will look at technological developments in animal monitoring and the application of big data sets in the assessment of animal disease, immunity, activity and nutrient use. The aim will be to assess opportunities for improvements in the development and longevity of farmed livestock and how such development can be applied to lower the impact on the environment and climate change, while maintaining food security and enhancing farm incomes and the welfare of animals.

**Key words:** Youngstock, Income, Environment, Microbiome, Technology.



# Effect of calfhood respiratory disease on rearing targets in Holstein-Friesian dairy heifers

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**Application** Better understanding of the relationship between calfhood disease and age at first calving would be invaluable for the evaluation of the economic value of disease prevention in reducing rearing costs for farmers.

**Introduction** A target of 24 mo. of age at first calving (AFC) is a long-standing recommendation that many dairy heifers do not achieve. At an estimated £2.87 (Boulton et. al., 2017) for every additional day over the target AFC, it is essential to regularly assess heifer live weight gain (LWG) and evaluate factors that prevent heifers achieving adequate LWGs to reach rearing targets of 55% mature weight (MW) for service at 13 to 15 mo. of age and 85% MW at FC at 24 mo. of age (Margerison and Downey, 2005). However, there is little data regarding the effect of calfhood disease on heifer LWG, MW and AFC. This study aimed to assess the effect of bovine respiratory disease (BRD) treatments on dairy heifer LWG, time to reach 55% and 85% MW, and AFC.

**Material and methods** Animal health records were collected from 318 Holstein-Friesian heifers born into an all-year-round calving system at the Nottingham University Dairy Centre (Sutton Bonington, Leicestershire, UK). The data set included both healthy animals and those treated for BRD, which had calved for the first time between 2016 and 2019. The number of treatments each calf received was categorised as: none, one, two, three and four or more. Live weight at first calving was measured post calving using load cells fitted to the milking robots (Lely Astronaut A3; Lely UK Ltd). Daily gain from birth to calving was calculated by dividing LWFC - 45 kg (assumed birth weight) by AFC. MW for the herd was calculated as mean live weight (LW) of animals in third lactation and above, which was then used to calculate 55% and 85% MW. Age at 55% and 85% MW were calculated by dividing LW at 55 and 85% MW by daily gain. Data were analysed using the GenStat 19th statistical package (VSN International Ltd., Oxford), with number of treatments as the fixed effect. While LW and daily gain data were analysed using a Generalised Linear Model (GLM) with normal distribution of errors and age-related data were analysed using a GLM with a log-linear model assuming Poisson distribution of errors.

**Results** Heifers that received  $\geq 4$  treatments calved 18 d later than animals that received no treatment (Table 1). Heifers receiving  $\geq 4$  treatments had lower LWGs than those receiving no treatment. Age at 55% and 85% MW were greater for heifers treated 3 and  $\geq 4$  times. There was no difference in LWFC between treatment groups.

**Table 1** Mean (SE) age and live weight at first calving, daily gain, and age at 55% and 85% mature weight (MW) according to the number of treatments received, 0 to  $\geq 4$ , between 11/04/14 to 11/12/17

	Treatments administered, No.					P Value
	0	1	2	3	$\geq 4$	
No. animals	108	61	48	46	55	
First calving						
Age, days	772 (7.1) <sup>b</sup>	773 (8.8) <sup>b</sup>	786 (10.3) <sup>ab</sup>	780 (10.7) <sup>ab</sup>	790 (9.1) <sup>a</sup>	< 0.001
Live weight, kg	677 (8.7)	676 (8.5)	684 (12.7)	656 (9.4)	664 (9.3)	0.344
Daily gain, kg/d	0.831 (0.01) <sup>a</sup>	0.823 (0.01) <sup>b</sup>	0.818 (0.02) <sup>c</sup>	0.787 (0.01) <sup>d</sup>	0.787 (0.05) <sup>d</sup>	0.026
Age at:						
55% MW, <sup>d</sup>	504 (7.6) <sup>b</sup>	508 (8.8) <sup>b</sup>	517 (12.3) <sup>ab</sup>	531 (8.4) <sup>a</sup>	532 (8.7) <sup>a</sup>	< 0.001
85% MW, <sup>d</sup>	735 (11.6) <sup>b</sup>	735 (11.7) <sup>b</sup>	746 (16.4) <sup>a</sup>	765 (10.8) <sup>a</sup>	766 (11.6) <sup>a</sup>	< 0.001

**Conclusion** Heifers repeatedly (3 to  $\geq 4$ ) treated for BRD had lower LWG and reached maturity and calving targets later ( $\geq 4$ ). At £2.87/d this would increase heifer rearing costs by £51.66 per head, excluding treatment costs.

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# Microbiological quality of water offered to pre-weaned calves on Northern Irish dairy farms

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**Application** The source of water supply and location of water drinkers for neonatal calves impacts on the microbiological quality of the water and potentially on calf health.

**Introduction** Water is an essential nutrient in the diet of young calves with previous research indicating that lack of water consumption can reduce starter concentrate intake in the first month of life (Kertz et al, 1984; NRC, 2001). Due to an underdeveloped immune system, provision of a contaminated water source to neonatal calves may have a negative effect on calf health and development. However, there is a dearth of knowledge relating to the quality of water being offered to calves during early life. The aim of the present study was to determine microbiological levels of water offered to pre-weaned calves on dairy farms across Northern Ireland.

**Material and methods** Assessments of the water facilities of 66 dairy farms across Northern Ireland were conducted as part of a larger calf housing and rearing environment survey. The source of the water, type of calf pen, type and location of drinker were ascertained. For determination of total viable count (TVC), Coliforms and E.coli, a total of 4 water samples were collected from each farm on two occasions. Water sampling was achieved by submerging a disposable, sterile container into the water bucket/ drinker before transferring to a sterile bottle containing 20g/ml of Sodium Thiosulfate (Deltalab 500ml bottle, Deltalab, Barcelona, Spain). Samples were refrigerated until preparation and analysis within 24 hours of collection. Determination of TVC was completed by a series of decimal dilutions mixed with Yeast Extract Agar medium in petri dishes. Plates were incubated at 22oC and 37oC and upon completion the number of

colonies growing at each temperature were counted and the count per ml was calculated. The detection and enumeration of coliforms and E.coli was completed by membrane filtration followed by incubation with Membrane Lauryl Sulphate Broth (MLSB). Due to the nature of the microbiological counts, the values were log transformed and analysed using REML variance components analysis (Genstat) by applying the water source, the pen type, drinker type and drinker location by including them as fixed effects while the farm was included as a random in the models.

**Results** The mean TVC at 22oC, TVC at 37oC, coliform count and E.coli count were 1,810,955 cfu/ml, 316,449 cfu/ml, 14,507 cfu/100ml and 6,538 cfu/100ml, respectively. The predicted means for water source (Figure 1), drinker type (Figure 2), drinker location (Figure 3), and pen type (Figure 4) are shown. Water samples that were sourced from bore wells had significantly higher TVC, coliform and E.coli counts than samples sourced from a mains supply across all counts, indicating the use of bore well water as a risk to pre-weaned calf health. Additionally, water samples taken from drinkers located outside the pen had significantly higher TVC at 22oC and a trend indicated they had higher coliform counts. No other significant effects were seen.

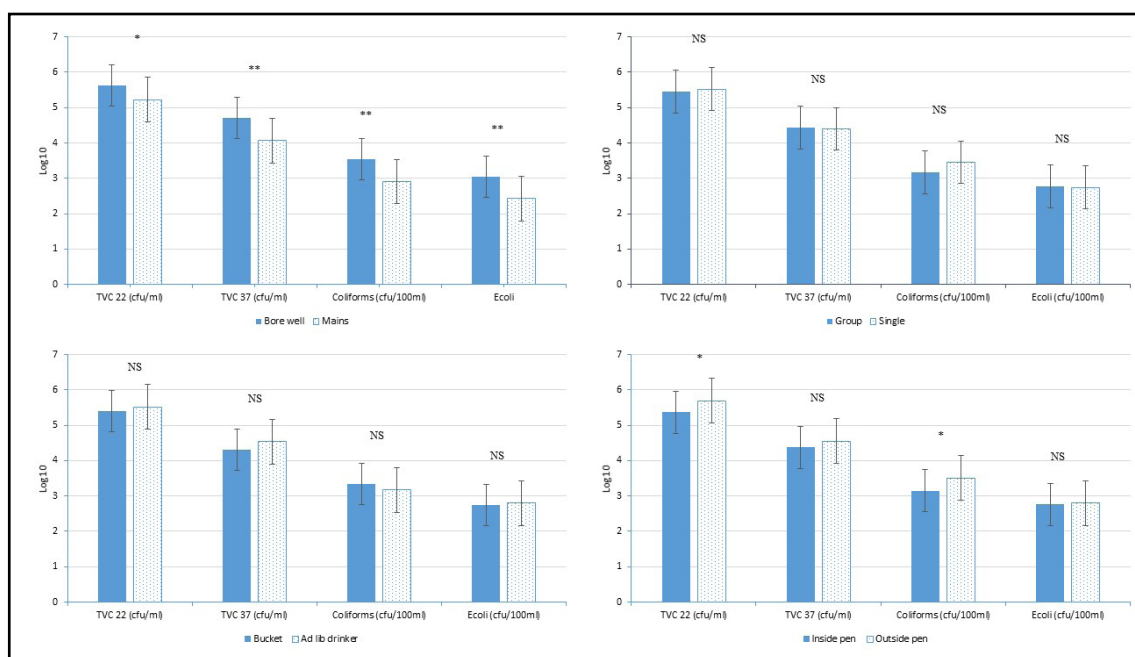
Effect of water source (Figure 1), pen type (Figure 2), drinker type (Figure 3) and drinker location (Figure 4) on water quality in calf pens

**Conclusion** Results showed a large variation in the microbiological quality of water samples collected from calf drinkers, with counts being at levels potentially damaging to calf health and development. Further investigation into the management surrounding water hygiene is needed to further explain the variation in microbiological counts.

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# Effect of early life nutrition on the transcriptional profile of visceral adipose tissue in heifer calves

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**Application** This study highlights the underlying biological response in visceral adipose tissue as a consequence of an enhanced dietary intake for the first 21 weeks of life in heifer calves. Results from this study may be further utilised for the potential optimisation of enhanced early-life nutrition programs for heifer calves.

**Introduction** Enhanced dietary intake during the critical early life period has been shown to have a positive effect on subsequent lifelong growth and production potential of cattle. However the effect of enhanced nutrition during the early life period on the biochemical regulation of many metabolically important tissues including adipose tissue is unknown. Adipose tissue, in particular visceral adipose represents an endocrine organ with important functions affecting feed intake, immune function, growth and reproductive development (Coelho et al., 2013). The objective of this study was to evaluate the effect of an enhanced dietary intake during early life on the visceral adipose tissue in Holstein-Friesian cross Angus heifer calves.

**Material and methods** Holstein-Friesian X Angus heifer calves with a mean ( $\pm$ S.D.) age and bodyweight of 19 ( $\pm$ 5) days and 51.2 ( $\pm$ 7.8) kg, respectively, were assigned to either a high-energy diet (HE; n = 15) or a control diet (CONT; n = 15). During the pre-weaning period, HE heifers received 1500g of milk replacer (MR) and ad libitum access to concentrate while CONT received 500g of MR and a maximum of 1 kg concentrate. Post weaning (9 weeks of age) HE continued to receive concentrate ad libitum while CONT received a maximum of 1 kg. Expected growth rates were 1.2 kg/day and 0.5 kg/day for the HE and CONT treatment groups respectively. At 21 weeks of age all calves were euthanized and tissue samples were harvested from the visceral adipose fraction. Total RNA was isolated from all samples and then verified for yield and quantity. cDNA libraries were prepared from total RNA samples using the Illumina TruSeq mRNA kit and subsequently sequenced on an Illumina NovaSeq sequencer. Resultant sequencing reads were first checked for quality, following which sequencing reads were then aligned to the bovine reference genome (UMD3.1) using STAR, with the number of reads mapping to each gene also determined using STAR software. Differentially expressed genes were then determined using EdgeR, resultant differentially expressed

genes (adj. P-value <0.1; fold change >1.5) were then input into pathway analysis programs to determine biochemical pathways over-represented from the list of genes that were differentially expressed using Ingenuity Pathway Analysis.

**Results** Heifer calves on the HE and CONT grew, on average at 1.18 and 0.50 kg/day, respectively, during the experimental period. This led to a bodyweight difference at euthanasia of 78.4 kg (HE: 189.6 kg; CONT: 110.2 kg). Differential feeding between 3-21 weeks of life affected the visceral adipose transcriptome. This was manifested as 1214 genes identified as differentially expressed between HE and CONT treatment groups. Specifically 720 genes were up and 494 genes were down-regulated in the HE calves compared to the CONT calves. Pathway analysis identified a number of biological pathways that were significantly enriched (P-value <0.05 including those involved in metabolic processes, energy production and adipogenesis (Table 1).

**Table 1.** Statistically significantly enriched pathways following differential feeding between 3-21 weeks of life in heifer calves.

Biological pathway	P-value
Oxidative Phosphorylation	<0.001
Sirtuin	<0.001
Gluconeogenesis I	<0.001
Glycolysis I	<0.001
Adipogenesis	<0.01

**Conclusion** Results of our biological pathway analysis indicate greater metabolic processing and energy production in the HE treatment group, consistent with their higher plane of nutrition and performance. There was also evidence for upregulation of adipogenic processes in the HE compared to the CONT calves. This study provides an insight into the underlying molecular response of enhanced early life nutrition on visceral adipose in heifer calves.

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# The effect of inclusion of oregano essential oil in milk replacer and of concentrate pellet size on growth performance of dairy calves to 70 days of age

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**Application** The inclusion of 2 ml/head/day of oregano oil (Orego-Stim Liquid) added to the milk replacer can improve live weight at weaning in dairy calves.

**Introduction** Pre-weaning growth rates of calves have an impact on first lactation milk yield (Chester-Jones et al., 2017). The use of oregano essential oil (OEO) has been shown to improve calf health and growth rates (Liu et al. 2017). Physical form of calf starter concentrates also affects calf growth rates and feed conversion efficiency (Bach et al., 2007); however, there is little information on the effect of pellet diameter. This project evaluated the effect of OEO offered in milk replacer and of pellet size on the performance of dairy-bred calves up to 70 days of age.

**Material and methods** At 5 days of age, 70 Holstein-Friesian calves were assigned to one of four treatments in a 2 x 2 factorial arrangement comparing milk replacer (with or without 2 ml OEO per day (Orego-Stim Liquid, Anpario plc)) and concentrate pellet size (3 or 5 mm diameter), allocated according to birth weight and gender. All calves received their mothers' fresh (>1 hour) colostrum for 4 days and were offered milk replacer (150 g powder/L) and concentrates ad libitum via automatic feeders from 5 days of age. Milk replacer was offered at 6 L per day from day 5 to 33. At day 33 it was reduced to 2 L until day 56 (weaning). Calves were housed in straw bedded pens, with 15 calves per pen. Milk replacer intake was recorded daily from 5 to 56 days and concentrate intakes recorded daily from 5 to 70 days. Scales were placed at the concentrate

feeders to record full bodyweight at each feeder visit. Forage was offered as straw pre-weaning and silage post-weaning. A blood sample was taken between 24 to 36 hours of birth and analysed using the zinc sulphate turbidity (ZST) test. The direct and interactive effects of essential oil and pellet size were measured by REML analysis with variance components, using a linear mixed model (GenStat 16.2) with pen as the random effect. Where significant treatment effects were identified ( $P < 0.05$ ), differences were tested using Fisher's protected-adjusted multiple comparisons.

**Results** Mean ZST scores (35.2 units) did not differ between treatments. There were no interactions between the inclusion of OEO and pellet diameter on any parameter; therefore, only treatment effects are presented. Calves receiving OEO had greater live weight at 56 d ( $P = 0.026$ ) and tended to be heavier at 70 d ( $P = 0.052$ ). Pellet size had no significant effect on weaning weight, 3 mm diameter pellet tended to improve 70 d live weight ( $P = 0.073$ ). Mean milk replacer intake was 368 g lower ( $P = 0.038$ ) in calves offered the 5 mm diameter pellet. The calves receiving the 5 mm diameter pellets ate 168 g/d ( $P = 0.028$ ) more starter concentrate. Addition of OEO had no effect on total milk replacer or concentrate intake.

**Conclusion** The inclusion of 2ml/head/day of OEO in in milk replacer resulting in a greater weaning weight, at 56 d of age, in Holstein-Friesian calves. Offering calves a starter concentrate with a 3 mm diameter pellet reduced pre-weaning milk replacer intake, but tended to result in a greater live weight at 70 d of age.

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	Milk Replacer Additive		Pellet Diameter		SED	P Value	
	OEO +	OEO -	3mm	5mm		OEO	Pellet
Weight d56 (kg)	72.9	70.9	72.3	71.4	1.09	0.026	0.275
Weight d70 (kg)	87.0	84.1	86.7	84.5	1.52	0.052	0.073
Mean concentrate intake (g/d)	1,060	1,011	999	1073	66.6	0.165	0.163
Mean milk intake (l/d)	4.8	4.8	4.8	4.9	0.256	0.402	0.038

**Table 1** The effect of inclusion of oregano essential oil (0 v. 2 ml/d) and 3 or 5 mm diameter pellet on live weight and average daily milk and concentrate intake of dairy calves.



# Effect of housing calves individually or pairs, along with environmental enrichment, on the behaviour, feed intake and growth rate of dairy calves

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**Application** Individually housing calves lowered cross sucking but not non-nutritive behaviour. Cross sucking between pair housed calves was lowered by bedding down with straw directly following milk consumption.

**Introduction** Individual housing of calves lowers cross sucking and disease risk, but creates challenges in terms of calf cognition and welfare and thus consumer concerns. Conversely pair housing of calves enables cross sucking to occur. Cross sucking occurs most commonly within the first twenty minutes following milk consumption (Margerison et al., 2003) and is indicative of poor welfare (Boissy et al., 2007; Held and Špinká, 2011). This study aimed to assess the effect of calf housing and environmental enrichment (EE) on feed intake, growth rate, cross sucking and play behaviour of dairy calves.

**Material and methods** Calves (n=60) were randomly selected and housed in pairs (n=48; n=24) and individually (n=12). At 5 weeks of age each pen of calves were offered one of four

EE's: No EE (No); cereal straw am and pm (CSAP), 2 buckets (BK) or manila rope with a chain (MRC) for one hour directly following milk feeding. Calf behaviour was measured for three consecutive days, on the day enrichment was added and 7 days after for 5 minutes before and 30 minutes following milk consumption. Calf weight, feed and water intake were measured weekly. Data was assessed using Minitab (17.0) and normally distributed feed intake data was assessed using AVONA GLM. Behaviour data was not normally distributed and assessed using Kruskal Wallis, applying animal as a random, and housing and EE as fixed effects in the models. Means and medians were presented with relevant P values (Table 1).

**Results** Housing calves in pairs reduced non-nutritive, but increased cross sucking behaviour (Table 1). Offering cereal straw directly following milk consumption, reduced non-nutritive and cross sucking (P<0.001), whilst increasing play, nutritive behaviour and oral engagement with straw, but did not affect starter intake or growth rate.

**Conclusion** Feed intake and growth rate were not affected by individual, pair housing, or EE. Individually housing calves lowered cross sucking, but not non-nutritive sucking. Pair housing calves increased cross sucking. Offering small amounts of cereal straw as bedding, directly following milk consumption, reduced cross sucking, increased nutritive and play behaviour whilst MRC or buckets did not.

**Acknowledgements** Suputo (formerly Dairy Crest) and DenkaVit for funding this research.

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**Table 1** Mean feed consumption and median behaviour of calves housed either as an individual or in a pair and offered either: No, manila rope with a chain (MRC), 2 buckets (BK) or cereal straw am and pm (CSAP) as an enrichment directly following milk replacer meals

	Individual	Pair housed			SEM	P Value	
	No	No	MRC	BK			CSAP
Starter intake, g/d	0.69	0.70	0.73	0.77	0.76	0.041	0.704
Milk intake, L/d	9.5	9.5	9.5	9.5	9.5	0.07	0.989
Growth rate, g/d	0.91	0.90	0.91	0.91	0.92	0.031	0.795
Milk consumption, s/d	460	433	504	459.0	384	0.38	0.086
Cross sucking, s 1	0.0 <sup>b</sup>	21.0 <sup>a</sup>	20.5 <sup>a</sup>	10.0 <sup>b</sup>	0.0 <sup>c</sup>	-	<0.001
Non-nutritive behaviour, s 1	72.0 <sup>a</sup>	49.5 <sup>b</sup>	48.5 <sup>b</sup>	52.5 <sup>b</sup>	1.5 <sup>c</sup>	-	<0.001
Playing behaviour, s 1	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	28.0 <sup>a</sup>	-	<0.001
Nutritive behaviour, s 1	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	259.5 <sup>a</sup>	-	<0.001

a, b, c Means in the same row that do not share a letter are significantly different at P<0.05

1 Observed over 40 mins daily, consisting of two 20 mins periods directly following two milk meals /d



# Evaluation of milk replacer protein composition on calf growth and performance

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**Application** Whey protein concentrate and skim milk powder are comparable sources of dairy protein in milk replacer diets for calves to 8 weeks of age.

**Introduction** Dairy ingredients are the main source of protein in milk replacers (MR); these include skim and whey produced as co-products from the processing of dairy products destined for the human food industry. Skim milk powder (SMP) is whole milk with the fat removed and contains 80% casein and 20% whey protein. Liquid whey is the fraction remaining following the precipitation of caseins. Ultrafiltration of whey produces whey protein concentrate (WPC). In the acid conditions of the abomasum, whole milk forms a clot due to the enzymes renin and pepsin acting upon the casein protein in digesta. MR with a high casein (SMP) content results in curd formation, whereas when whey is the primary source of protein, no curd forms. Previous research has shown no effect of curd formation on digestibility or performance in calves fed at low levels (ca. 600 g DM / d) (Terosky et al., 1997; Lammers et al., 1998). The aim of this experiment was to determine the effects of four dietary ratios of SMP and WPC as the primary protein source in MR offered at high levels of up to 1050 g DM / d.

**Material and methods** Holstein Friesian calves (n=80) were randomly assigned, according to birth date, gender and birth weight (range 40.8 to 41.7 kg) to one of four dietary treatments. All MR (26% CP, 16% fat, reconstituted at 150 g / L) diets contained 25.35% dairy protein that was contributed by different ratios of SMP to WPC: T1 0:98; T2 29:69; T3 58:40; and T4 86:11, with amino acids contributing 0.65% of the protein. All calves received 3.5 L colostrum within 2 h of birth and a further 2 L within 12 to 16 h of birth and continued to be offered twice daily

(2.5 L per feed) until 5 d of age. The MR was offered at a rate of 5 L / d from 5 to 10 d, 7 L / d from 11 to 34 d, 5 L / d from 35 to 49 d and 2 L / d from 49 to 55 d of age. Calf starter (CS) (18% CP) and fresh water was available ad libitum from birth, while chopped straw was offered from 56 d of age. Individual intake of MR, CS and water were recorded daily and body weight (BW) was measured weekly. Health status was assessed daily (faecal score, 1=normal consistency; 2=slightly liquid; 3=moderately liquid; and 4=primarily liquid). Pneumonia was recorded when calves displayed visual indicators as described in the Wisconsin Scoring System. Data was analysed using GenStat (version 18.1, VSN International Ltd). Statistical models included the fixed effects of time and MR treatment along with additional fixed effects of birth weight and gender and housing block fitted as a random term. Data was analysed using a linear mixed model (feed intake and growth data) and a generalised linear mixed model (health data). Significance was determined by comparing the F-statistic against the F-distribution or Wald test compared against a Chi-squared distribution, as appropriate.

**Results** There were no treatment differences in total intake of; MR (range 40.9 to 41.1 kg DM; P=0.08) between 5 and 55 d of age, CS (range 52.4 to 58.1 kg DM; P=0.42) and water (range 144 to 159 L; P=0.59) between 5 and 70 d of age. Mean CS intake across treatments was >1.5 kg DM / d per calf and mean BW across treatments ranged from 71.9 to 74.5 kg at weaning at 56 d of age and between 86.3 and 89.4 kg at 70 d of age. The ADG from 0 to 14 d and 14 to 56 d of age were not related to protein source (SMP:WPC ratio; P>0.10). In the post weaning period ADG ranged from 0.97 to 1.14 kg / d between 56 and 70 d of age across treatments. There were no significant differences in number of episodes of scour and / or respiratory illness between treatments. Mean faecal score was below 2 across the experimental period and was not affected by dietary treatment.

**Conclusion** Growth rate and health status was similar across treatments in both the pre and post-weaning periods. In this experiment, WPC and SMP were comparable sources of dairy protein in MR diets offered to calves at high feeding rates.

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	Skim milk powder: Whey protein concentrate				SED	P-value
	T1 0:98	T2 29:69	T3 58:40	T4 86:11		
ADG 0 to 14 d of age, kg/d	0.40	0.31	0.34	0.38	0.065	0.544
ADG 14 to 56 d of age, kg/d	0.63	0.61	0.66	0.64	0.035	0.478
Scouring episodes, No.	0.39	0.54	0.63	0.75	-	0.485
	(0.15-0.98)	(0.23-1.13)	(0.27-1.44)	(0.34-1.66)		
Respiratory episodes, No.	0.57	0.67	0.71	0.44	-	0.679
	(0.26-1.26)	(0.31-1.44)	(0.33-1.50)	(0.19-1.03)		

**Table 1** Effect of dietary treatment (ratio of SMP to WPC) on calf growth and health

# The effect of weaning method and post weaning management on calf behaviour and performance.

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**Application** Quietweans (QW) reduced abnormal behaviour around weaning compared with abrupt weaning (AW), but had no effect on post weaning calf growth.

**Introduction** The use of QW's, a nose piece device that prevents calves suckling their dams, reduce the behaviours, such as vocalisation and pacing that are associated with weaning (Haley et al., 2005). However, there is conflicting results showing reduced performance from QW insertion to weaning (Enriquez et al., 2010) indicating that behavioural differences may occur earlier with the QW, in this two-step weaning process. Moreover housing indoor or outdoor following weaning can effect calf performance and behaviour equally (Lynch et al., 2011), with deferred housing will after weaning lowering biochemical stress responses. The aim of this study was to assess the effects of post weaning environment and the use of QW on calf performance and behaviour.

**Material and Methods** This experiment allocated 48 suckler calves, which were allocated to a 2 (weaning method) x 2 (post weaning environment) factorial design study. The weaning treatment included either an AW or using QW's. The post weaning environment was either being housed on slatted pens in groups of 6 or grazed in groups of 12. The calves were grazed along with their dams prior to the commencement of the study. Treatments were balanced for calf age, sex and weight. Concentrates were offered post weaning at 2 kg/head. Behaviour was monitored using IceQube pedometers which were attached to the calves' legs on day 0 to record lying time, lying bouts and standing time. This data was recorded every 5 minutes using the CowAlert system. Behaviour was also monitored via visual

assessment which started on day -3 and finished on day 22. QW's were inserted, weaning undertaken, pedometers removed, housing of outdoor calves and turnout respectively happened at days 4, 11, 14, 97 and 133. Calves were weighed at each of these time points and regularly after weaning to get a liveweight and calculate daily liveweight gains (DLWG) (kg/day). All data was analysed using GenStat. Performance data was analysed using REML mixed model analysis with date of birth, calf weight day 0, weaning allocation and post weaning environment and weaning environment allocations interactions as fixed effects. Behaviour was analysed using REML mixed model analysis and generalized linear mixed model analysis with day, weaning allocation, post weaning environment and interactions as fixed periods.

**Results** There were no significant interactions between weaning and environment treatments for animal performance ( $P>0.05$ ). Weaning method did not influence liveweight (LW) around weaning but there was a tendency for QW animals to be lighter at day 97 & 133 (Table 1). The calves kept outdoors were significantly lighter 7 post weaning but by 14 days post then were significantly heavier which may be due to weather conditions post weaning. There was a significant effect on behaviour on days 6 & 7 ( $P=0.02$  &  $P=0.003$ ). There was significant interactions for lying and standing behaviours on days 12 & 13 ( $P<0.001$  &  $P=0.004$ ). On day 14 the significant are from post weaning environment only ( $P>0.001$ ).

**Conclusion** The use QW's reduced abnormal behaviour post weaning by reducing standing time, which could be indicative of pacing. QW's had no significant effects on performance. However, trends were seen at housing and turnout for QW reducing LW. Housing environment (indoors or outdoors) after weaning had an effect on liveweight on day 18 & 32 due to weather conditions post weaning.

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**Table 1:** The effect of treatment on calf performance (kg)

	Day	Weaning		s.e.d	Environment		s.e.d	Significance	
		QW (kg)	AW (kg)		Inside (kg)	Outdoors (kg)		Weaning	Environment
Initial LW	0	239	236	9.1	-	-	-	NS	-
Insert QW	4	235	234	1.9	-	-	-	NS	-
Weaning	11	229	227	2.0	228	228	2.0	NS	NS
	18	234	235	2.7	241	228	2.7	NS	***
	32	249	251	3.4	245	255	3.4	NS	**
Housing	97	292	301	4.6	300	293	4.6	0.063	NS
Turnout	133	333	343	5.4	339	338	5.4	0.072	NS

# The genetics of ewe maternal characteristics and lamb survival

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Improving lamb survival is a goal worldwide, with several countries now including it in genetic evaluation schemes. However, genetic gain is limited by low heritability and the difficulty of recording the required phenotypes, particularly under extensive grazing conditions. Can genetic gain be improved and is it possible to achieve current generation gains as well?

## Can trait definitions be improved?

Lamb survival is generally defined over a specific period, such as from birth to weaning (LSW), irrespective of when losses occur. Australian researchers have reported higher heritability for rearing ability in twin and multiple-bearing Merino ewes than those bearing single lambs (Hatcher et al. 2014, Bunter et al. 2018, Greeff et al. 2019), with Hebart and Brien (2018) finding that additive genetic and maternal variances for LSW of twin and multiple-born lambs were 3 times and 6 to 10 times higher than that for singletons, respectively. Higher genetic gains could therefore be possible by evaluating lamb survival within litter size classes, at least where there are a significant proportion of single-bearing ewes.

## Is indirect selection useful?

Based on a larger dataset than that initially reported by Brien et al. (2010), the most correlated indirect criteria with LSW were lamb rectal temperature at birth (RT), the time taken for the lamb to bleat (BLT) and to follow the ewe (FOLL) after release by a shepherd, lambing ease (LE) and a score of lamb birth vigour (OBV), with genetic correlations of  $0.74 \pm 0.11$ ,  $-0.43 \pm 0.18$  and  $-0.52 \pm 0.23$ ,  $-0.37 \pm 0.14$  and  $-0.35 \pm 0.13$  respectively (Brien et al. unpublished). LE is included in genetic evaluation in Australia, Ireland (named lambing difficulty) and the UK. Maternal behaviour score (MBS), which is relatively easy to score, is under trial in Australia (Bunter et al. 2019). A similar trait, 'ewe mothering ability', scored on ewes lambing indoors, is recorded in Ireland, as are scores of 'ewe milk supply' and lamb vigour (Sheep Ireland 2020). Adding OBV to a selection index could boost genetic gain in LSW by 36% and just adding RT could, in theory, boost genetic gain for survival by a considerably larger 60% (Brien et al. unpublished). The recording of BLT and FOLL is onerous and inclusion in breeding programs is not recommended.

## Can genomic information improve genetic gains?

Sheep Improvement Limited in New Zealand have recently included genomic information in single step procedures for calculating breeding values, with genetic trends for direct and maternal lamb survival combined appropriately doubling in dual purpose flocks as a result (S. McIntyre, 2019, personal communication).

## Can gains be obtained in the current generation?

Total flock improvements of 2-4% in reproductive rate (NRR) by culling twice-dry ewes and keeping the best 50% of ewes based on NRR for a year longer have been reported by Hatcher et al. (2018), however more work is needed to fully appreciate the contributions that could be made from improving ewe rearing ability.

**In conclusion**, there is evidence to suggest that genetic gains in lamb survival can be improved in breeding programs via better trait definitions, use of indicator traits and genomic information, and that current generation gains are also possible.

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# Dissection of the direct and maternal responses to selection for piglet survival in outdoor sows produced by sires selected based on postnatal survival recorded indoors

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**Application** Sires with high breeding values for postnatal piglet survival based on observations recorded indoors will also improve survival of their piglets kept outdoors.

**Introduction** Piglet survivability is an important trait from economic and welfare perspectives. The trait is complex, as it is influenced by genes of the piglet to survive (direct genetic effect) and genes of the dam associated to mothering abilities (maternal genetic effect). The significant heritabilities and genetic correlation between the direct and maternal effects estimated by Nguyen et al. (2019) suggested that genetic selection is expected to improve piglet survival. The use of boar breeding values for piglet survival based on observations recorded indoors to improve survival of piglets raised outdoors is still a concern, due to possible genotype-environment interactions. Therefore, the aim of this study was to evaluate whether the selection of sires for maternal or direct piglet survival breeding values based on indoor observations resulted in genetic responses under outdoor conditions.

**Material and methods** Data were obtained from 22,481 piglets raised on an outdoor farm. In the first generation, 26 Landrace boars were classified into high and average (control) maternal breeding values (EBVs) for piglet survival during the nursing period, referred to as postnatal survival (HM and CM, respectively). These boars were mated with randomly selected commercial sows. From the progeny of each group, 285 and 283 daughters (from the HM and CM groups, respectively) were selected for mating with 42 Large White boars (previously selected for high and average (control) direct EBVs for the same trait, HD and CD, producing the 3rd generation piglets. The EBVs of all sires were estimated based on records of piglets raised indoors. The piglets were divided into four groups based on their maternal and direct selection background: CDCM, CDHM, HDCM and HDHM. The differences between high and average breeding values of the 3rd generation piglets were multiplied by 4 and 2 to obtain the selection response for maternal and direct effects, respectively, because only the sires had been selected for

maternal and direct effects (in the first and second generations, respectively; Smith 1977). Although a Bayesian multivariate threshold model was fitted for peri- and postnatal survival and birthweight, only the results of the latter survival trait, on that selection took place, are presented.

**Results** Average EBVs of piglets of each selection scenario i.e. no selection for maternal and direct effects (CDCM), selection for maternal (CDHM) and direct (HDCM) and their combination (HDHM), and the differences ( $\Delta$ ) of these groups to predict the responses are shown in Table 1. The selection only for maternal effects resulted in the largest maternal and direct response of  $\Delta Ma=1.28\%$  and  $\Delta Da=2.60\%$  in postnatal survival, whereas selection for direct effects showed a negative and positive response in maternal ( $\Delta Mb=-0.96\%$ ) and direct ( $\Delta Db=1.10\%$ ) effects of piglet survival so that the overall response was small (0.14%). This indicates the strong antagonism between direct and maternal effects if selection is only for direct effects and that maternal effects are the most limited factor to achieve a high overall selection response in piglet survival. This interpretation is consistent with the result that the overall response increased to 1.46% when selection was for both direct and maternal effects.

**Table 1** Group average breeding values (EBV), selection responses and standard errors (SE)

Maternal selection response (%)		Direct selection response (%)	
Group	Maternal EBV	Group	Direct EBV
CDCM	88.42	CDCM	87.81
CDHM	88.74	CDHM	89.11
HDCM	88.17	HDCM	88.36
HDHM	88.38	HDHM	88.62
Group difference	$\Delta M$ (SE)	Group difference	$\Delta D$ (SE)
$\Delta Ma = CDHM - CDCM$	1.28 (0.20)*	$\Delta Da = CDHM - CDCM$	2.60 (0.17)*
$\Delta Mb = HDCM - CDCM$	-0.96 (0.20)*	$\Delta Db = HDCM - CDCM$	1.10 (0.17)*
$\Delta Mc = HDHM - CDCM$	-0.16 (0.16)ns	$\Delta Dc = HDHM - CDCM$	1.62 (0.17)*

\*: significant, ns: not significant,  $\Delta M$  and  $\Delta D$ : maternal and direct selection response

**Conclusion** This study showed that substantial selection responses in maternal and direct effects for postnatal piglet survival were achieved when selection is emphasised only on maternal genetic effects. Because the selection of boars was based on EBVs obtained using data recorded from indoor raised piglets and the selection experiment was carried out in an outdoor farm, the achieved selection responses suggest that genotype by environmental interactions are of negligible importance in comparison to the influence of change in selection pressure on direct and maternal genetic effects.

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# Inbreeding in Charollais sheep

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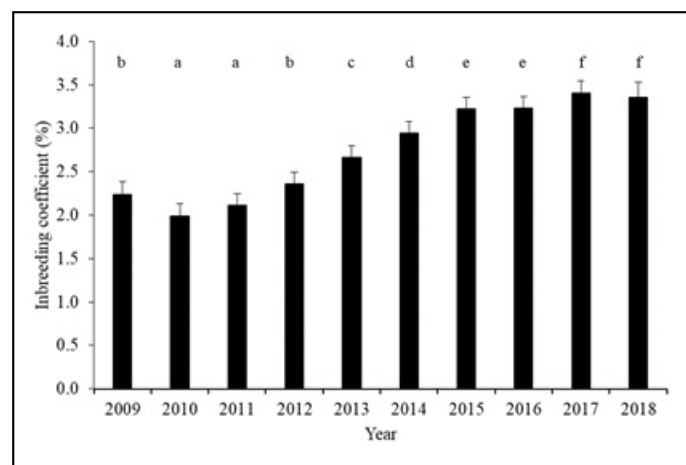
**Application** The level of inbreeding in the Charollais sheep population has increased in recent years. Charollais lambs that were heavier at eight-weeks of age and/or born via embryo transfer were less inbred.

**Introduction** The population of Charollais breeding ewes in the UK is relatively small at about 0.4% of the total sheep population of 16 million ewes, however, as a terminal sire the Charollais breed represents about 8.4% of sires used on commercial farms (Charollais Society, 2019). The intensive use of a few related animals (i.e. inbreeding) can result in deleterious effects on animal performance. In a finite population, inbreeding is inevitable. The objective of this study was to assess differences in the mean inbreeding coefficient among flocks, study years, lamb eight-week body weight categories and lambs born via embryo transfer.

**Material and methods** Data were obtained from 35,220 individual Charollais lamb records between the years of 2000 to 2018. The dataset consisted of 146 commercial flocks that were using performance recording in the UK. The records collected included the identification of individual lambs and their dam, sire, date of birth, eight-week body weight, if the lamb was born following embryo transfer, and the lamb's calculated inbreeding coefficient. The data represented a subset of the current total UK Charollais sheep population at 2% of breeding ewes and 0.5% of sires (of UK origin). The inbreeding coefficient was determined using methodology of Meuwissen and Luo (1992). Data from the most recent 10 years of the study period (from 2009 to 2018) were analysed using a linear mixed model in Genstat (version 19.1) to assess differences in the mean inbreeding coefficient among flocks, study years, lamb eight-week body weight categories and embryo transfer lambs. This subset of data consisted of 102 commercial flocks and 20,103 individual Charollais lambs. Significance was attributed at  $P < 0.05$ .

**Results** The mean inbreeding coefficient of lambs has increased in recent years from about 2% in 2010 and 2011 to 3.4% in 2017 and 2018 ( $P < 0.001$ ; Figure 1). Lambs with an eight-week body weight  $> 32$ kg had a lower mean inbreeding coefficient ( $P < 0.001$ ). Lambs born via embryo transfer had a lower mean inbreeding coefficient ( $P < 0.05$ ).

**Figure 1.** Predicted mean inbreeding coefficients for lambs during each year from 2009 to 2018 after adjusting for effects of year of birth, eight-week body weight, embryo transfer, flock and lamb sire. Columns with different letters are significantly different at  $P < 0.05$ . Standard error bars are shown.



**Conclusion** The mean inbreeding coefficient of the Charollais sheep studied showed an increase in recent years from 2% to 3.4%. Heavier lambs at eight-weeks of age were found to have lower inbreeding coefficients and the use of embryo transfer reduced the level of inbreeding in the population studied.

**Acknowledgements** The authors thank Samuel Boon at Signet for providing the dataset, and the UK Charollais Sheep society for allowing the use of the data.

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# Genetic benefits of foreign sire contributions to a domestic sheep industry; including a New Zealand-Ireland case study

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**Application** The strategic use of progressive sheep genetics sourced and used within a domestic environment will contribute to an increase in genetic gain and cumulative profit for the domestic sheep industry.

**Introduction** Santos et al. (2015) demonstrated that the sheep industry in New Zealand has a threefold higher rate of genetic gain in maternal traits than Ireland's. Results from a recently established flock in Teagasc Athenry (INZAC) confirm the superiority of New Zealand sheep genetics in comparison to Irish genetics. Gene flow models are a useful way of quantifying the effect of breeding strategies on the industry. Certain subpopulations will contribute differently towards improvement in genetic gain in comparison to others, due to placing different emphasis on various aspects of their breeding strategies. The objective was to analyse whether a domestic sheep population, specifically; Ireland, would benefit from the use of foreign genetics; from New Zealand and if so, how best to incorporate those genetics into the industry.

**Material and Methods** A recursive model with multiple flows of predicted genetic merit across subpopulations of sheep was created in Microsoft Excel. The model predicts the genetic improvement in overall economic merit of future generations of commercial sheep as a consequence of the incorporation or exclusion of foreign genetics. Subpopulations generated included conservative breeders (CON), progressive breeders that source domestic genetics only (PRO), progressive breeders that use a combination of foreign and domestic genetics (PROFOR), foreign flocks who supply rams to the domestic market (FOR) and also, commercial farmers (COM) who source rams or semen from the aforementioned subpopulations. Each subpopulation has annual merit predicted for rams born, rams mated, ewes mated and lambs born. Base genetic merit values and genetic trends were derived from the Sheep Ireland Replacement Index and the New Zealand Maternal Worth in 2019. Aspects of the model that can be varied include genetic trends, selection proportions, market share of different subpopulations and level of superiority of foreign sires over domestic sires. Cumulative and annualised benefits, discounted to present values, were generated from the predicted index

values of commercial ewes over a twenty year horizon. These benefits were expressed relative to a base situation whereby commercial flocks sourced 87% and 13% of rams from conservative and progressive breeders respectively, and never used foreign genetics (Table 1).

Scenario	Description	Additional industry benefit (€m)	
		Cumulative	Annualised
Base		49.18	3.95
1A	Shift market share only	+88.86	+7.13
1B	Shift market share + reduce selection proportions	+132.94	+10.67
2	Direct foreign genetics for five years	+97.30	+7.81
3	Static foreign trend	+76.89	+6.17

**Results** Scenario 1A shifted market share away from CON towards PRO, at a rate of 5% per annum, resulting in a cumulative and annualised benefit of €88.96m and €7.13m above the base, respectively (Table 1). Scenario 1B shifted the market share but reduced selection proportions to model farmers retaining only the top 20% of animals. This generated the greatest cumulative and annualised benefits of €132.94m and €10.67m above the base, respectively. Scenario 2 modelled an optimised PROFOR scenario, e.g. five years of upgrading to foreign sires directly into commercial flocks, followed by shifting half of the market share of PRO to PROFOR. This increased the cumulative and annualised industry benefits up to €97.30m and €7.81m, an increase above shifting market share alone but it does not take the implementation and importation costs into account. Scenario 3 modelled COM flocks sourcing foreign genetics directly from abroad through a single shipment with a 5% per annum shift in market share away from CON with a transfer of half of the market share from PRO breeders to FOR sourced rams. This scenario accumulated the lowest long term benefit out of all the scenarios described; i.e. €76.89m and €6.17m for cumulative and annualised benefits, respectively because of a failure to accumulate incremental gains over time.

**Conclusion** Large gains of €132.94m accumulated over 20 years could be achieved by the Irish sheep industry without the use of foreign genetics, but through shifting the market share away from conservative breeders towards progressive domestic breeders and through careful selection of replacements. However, short term tactical use of progressive foreign genetics may increase the proportion and rate at which this shift occurs, highlighting the potential benefit of their use.

**Acknowledgements** Kindly supported by AbacusBio and the Teagasc International Training Awards.

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# Preference elicitation methods for more appropriate breeding objectives: a systematic review of applications to date

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**Application** The use of methods for the elicitation of preference towards appropriate genetic improvement programs is growing in popularity, with numerous methods applied over the past three decades. However, there is no comprehensive, systematic review of methods, meaning inconsistencies in the framework of analysis persist; the current paper addresses this substantial knowledge gap, together with presenting aggregate ranks of cattle traits as an indication of uncertainty in preference estimation across multiple studies.

**Introduction** In agricultural systems, breeding objectives are usually profit maximising; however, increasing attention is being paid to the more abstracted values of users (Byrne et al., 2016). Attempts to elicit user-preference towards the formulation of breeding objectives not only seek to increase user-ship, and so increase the rate of genetic gain, but also to understand what traits and trait-complexes may become more important in future production circumstances, towards more resilient, future-proof agricultural systems. The absence of key stakeholders in the derivation of breeding objectives risks genetic improvement programs becoming unsuitable or inappropriate (Nielsen et al., 2014). Thus, in the context of this issue, the current paper seeks to present and discuss the sum of methodological applications in the derivation of more appropriate breeding objectives to date. This review represents the first comprehensive, systematic review of this rapidly developing, important field.

**Material and methods** A systematic review was undertaken to compile literature using various methods to elicit preference for traits in respective breeding objectives, following a Rapid Evidence Assessment protocol. The review captured literature from both animal and plant breeding, as the primary interest was in methods. Data was then extracted from the papers, which included details of the method and analytic approach, objective, sample size and sample population, location of study, and numerous additional data points. Furthermore, using a minimum violations and branch-and-bound approach, we present the first demonstration of an aggregate rank for the most common study subject, cattle, across two broad production systems, based on multiple partial ranks of included studies (Cook et al., 2007).

**Results** We discuss 84 peer-reviewed articles. Some studies contained more than one rank of traits, giving approximately 200 ranks of traits. The subjects of study included cattle, pigs, sheep, chickens, and fish amongst some less familiar study subjects. Livestock were the focus of more than 80% of the studies, with the most common subject being cattle (~40%). Sample size of studies ranges from 9 to 1371, with a median of 166. Although the majority of studies were based in low income countries, studies from high income countries were responsible for most of the more sophisticated and scientifically robust methodological approaches. Discussion of relative merit of methodological approaches is accompanied by the illustration of clear differences and high uncertainty in preference estimates for cattle traits in two broad production systems.

**Conclusion** Rank aggregation can reveal trends in preferable trait-types, for example highlighting increasing recognition of the importance of adaptation traits in a changing production system. The inclusion of stakeholder preference in breeding objective design may be a key aspect of sustainable agricultural systems in future; we present a catalogue of methodological applications to date as a resource to research and industry in identifying, selecting and modifying the most appropriate preference-elicitation tools for their requirements. Future research must adopt a consistent framework in order that opportunities to characterise respondent preference towards the derivation of appropriate genetic improvement programs are not underexploited with insufficient and inefficient methodological approaches.

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# Effect of early life nutrition on transcriptional regulation of the hypothalamic-pituitary-testes axis in bull calves

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**Application** This study highlights the effect of enhanced early life nutrition on the transcriptional regulation of the hypothalamic-pituitary-testes (HPT) signalling axis. miRNA results from this study as well their mRNA targets may hold potential for use as molecular biomarkers for the selection of bull calves that will undergo puberty earlier in life.

**Introduction** Improved plane of nutrition and metabolic status are known to advance sexual development in the bull calf. This is mediated through neuroendocrine signalling at the level of the HPT biochemical signalling axis, whereby enhanced nutrition may lead to early release of hypothalamic gonadotropin releasing hormone (GnRH). The pulse generation of GnRH may subsequently result in production of follicle stimulating hormone and luteinizing hormone in the anterior pituitary and ultimately development of the testes tissue. However the precise molecular mechanisms leading to this effect are currently unknown. The objective of this study was to evaluate differentially expressed miRNA, which contribute to transcriptional repression, and thus may act as mediators to the underlying molecular control regulating the effect of enhanced plane of nutrition on the early development of the HPT signalling axis and ultimately to advanced sexual development in the bull calf.

**Material and methods** Holstein-Friesian bull calves with a mean (+SD) bodyweight and age of 48.8 (5.3) kg and 17.5 (2.8) days, respectively, were assigned to either a high energy (H; n=15) of moderate energy (M; n=15) plane of nutrition. Calves assigned to the H diet were offered 10L of milk replacer, ad libitum concentrate and 500 g/day of hay, with those within the M group offered 4L of milk replacer, 500 g of concentrate and 500 g hay per day. Diets were designed to allow for overall growth rates of 1.0 and 0.5 kg/day for each H and M treatment groups, respectively. At 12 weeks of age all calves were euthanised and at the same time tissue samples of the arcuate nucleus region of the hypothalamus, the anterior pituitary and the parachyma of the testes were sampled for subsequent RNAsequencing. Total RNA containing miRNA was isolated from all tissue samples collected using the Qiagen RNeasy Plus Universal

kit. cDNA libraries were then prepared from the small-RNA fraction of each sample using the Illumina TruSeq Small RNA kit and subsequently on an Illumina HiSeq2500 sequencer. Resultant sequencing reads were first checked for quality and then trimmed to retain sequencing reads of appropriate miRNA length (15-30 nucleotides in length). Retained reads were then aligned to miRNA regions of the bovine reference genome using MiRDeep software. Differentially expressed (DE) miRNA were determined using EdgeR. mRNA targets of DE miRNA were then determined using TargetScan.

**Results** As expected based on the differential feeding program, calves in the H treatment group were heavier at the time of euthanasia (112 kg and 88 kg for H and M groups, respectively,  $P < 0.001$ ). Small RNA sequencing of calves fed varying planes of nutrition during the first 12 weeks of life resulted in the identification of differentially expressed miRNA between H and M treatment groups in each tissue of the HPT axis. Specifically within the arcuate nucleus one miRNA was up-regulated in H calves. Similarly in the anterior pituitary four miRNA were up-regulated in H treatment group, with a further one additional miRNA down-regulated in H calves. Finally seven miRNA were differentially expressed within the testes tissue. Specific miRNA differentially expressed in each tissue are outlined in Table 1. In particular one miRNA bta-miR-2419-3p, was up-regulated in H calves compared to M calves across both arcuate nucleus and anterior pituitary tissues.

**Table 1.** miRNA differentially expressed in the HPT axis following differential feeding in early life in bull calves

Tissue	miRNA up-regulated in H v M groups	miRNA down-regulated in H v M groups
Arcuate nucleus	bta-miR-2419-3p	
Anterior pituitary	bta-miR-451, bta-miR-144, bta-miR-10172-3p, bta-miR-2419-3p	bta-miR-205
Testes parenchyma	bta-miR-34b, bta-miR-34c, bta-miR-2419-5p, bta-miR-11995, bta-miR-146b	bta-miR-223, bta-miR-10a

**Conclusion** Results from this study show that early life plane of nutrition affects the transcriptional regulation of the HPT axis in the young bull calf. Further analyses are warranted at the mRNA to identify if miRNA targets were also differentially expressed as a consequence of early life plane of nutrition.

## Acknowledgements

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# Effect of plane of nutrition on testicular growth and sexual development in Holstein-Friesian bull calves

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**Application** This study highlights key genes contributing to early testicular growth and development in dairy bull calves. Furthermore, following appropriate validation, these data may have utility as potential molecular biomarkers for early puberty onset in dairy bulls.

**Introduction** Semen from young genetically elite AI sires often exceeds supply, within seasonal dairy production systems (Byrne et al, 2018). Consequently there is a requirement to identify appropriate rearing management strategies for bull calves destined for AI, to ensure (i) early onset of puberty and sexual maturity and (ii) the timely availability of high quality semen.

Improved early life (prior to six months of age) nutrition has been shown to advance testicular and sexual development in bull calves, most likely mediated through complex interplay between metabolic signals and neuroendocrine cells within the hypothalamic-pituitary-testicular (HPT) axis (Kenny et al., 2018). However the precise timing and underlying biochemical mechanisms of this effect are currently unknown. The objective of this study was to examine the effect of plane of nutrition during the first three months on the molecular control of testicular development in the dairy bull calf.

**Material and methods** Thirty Holstein Friesian bull calves with a mean (SEM) age and body weight of 17.5 (2.8) days and 48.8 (5.3) kg, respectively, were assigned to either a high (H; n=15) or moderate (M; n=15) plane of nutrition, to achieve an average target growth rate of 1.0 and 0.5 kg/day, respectively. Calves on H and M received 1.5 and 0.5 kg of milk replacer

(MR) per day, reconstituted at 15 and 12.5% (g/L), respectively. Calves on H were offered concentrate ad libitum, while those on M received, 500g day<sup>-1</sup>. Both groups were offered 500 g of hay daily. Throughout the trial calves were weighed and blood sampled regularly for the evaluation of hormone (AMH) and metabolite (IGF-1, insulin, BHB, NEFA, cholesterol, leptin and glucose) concentrations. At 87 days ( $\pm 2.141$ ) of age, all calves were euthanized and testes recovered for subsequent molecular analyses. RNA was isolated from the testes of all calves and the expression of candidate genes involved in spermatogenesis (PCNA, THY1 and UCHL), steroid hormone biosynthesis/gonad development (FSHR, AMH, LHR and AR), testicular/Sertoli cell development (CLDN11, GATA4 and INSL3) and cholesterol biosynthesis (MVK, StAR and EBP) was assessed using qPCR.

**Results** Calves offered a higher plane of nutrition were heavier at slaughter (112 v 88 kg,  $P < 0.001$ ), reflective of their higher ADG (0.88 v 0.58 kg,  $P < 0.001$ ). Similarly, H also had heavier testes (29.2 v 20.1 g,  $P < 0.05$ ) compared to M calves. Systemic concentrations of metabolites and metabolic hormones, in general, reflected the improved metabolic status of the H compared with M calves. However neither NEFA nor cholesterol concentrations were affected by diet. Similarly, serum concentrations of AMH, indicative of testicular maturity, was also unaffected by diet. In contrast, candidate gene expression analysis showed that plane of nutrition during the first 12 weeks of life affected the relative transcript abundance for genes involved in cholesterol biosynthesis (EBP) spermatogenesis (INSL3) and Sertoli cell development (CLDN11). In each case, gene expression was greater in the H compared to the M calves ( $P < 0.05$ ; Figure 1).

**Conclusion** Results from this study indicate that offering bull calves a high plane of nutrition during the first three months of life advances and sexual development, consistent with previous finding from our group where bull calves were offered a high plane of nutrition for up to 20 weeks of age. Differentially expressed genes identified in this study could be harnessed, following appropriate validation, as indicators of precocious sexual development, within the context of genomically assisted selection programs for cattle.

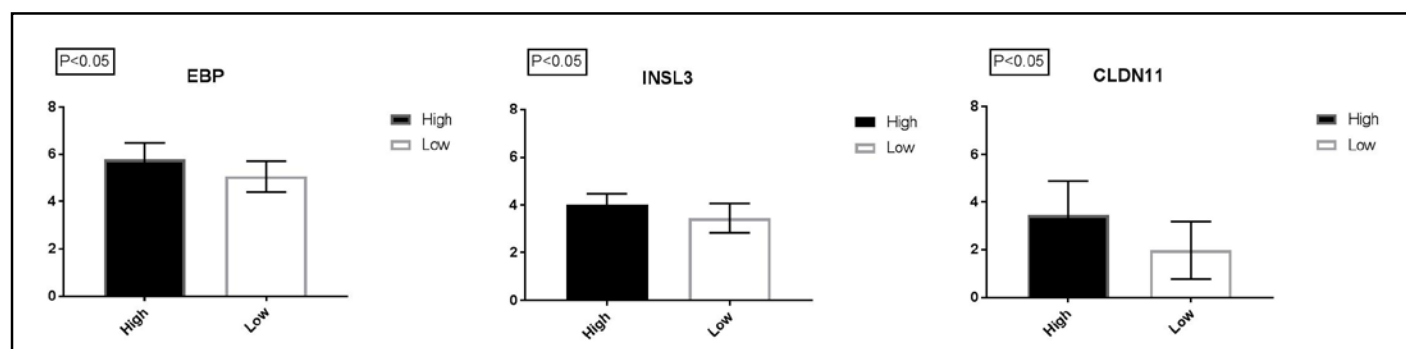
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Figure 1





# Effects of dietary protein level on feed intake, milk production and nutrient digestibility by lactating Holstein-Friesian cows

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**Application** Increasing dietary protein level in excess of the optimum does not result in a linear increase in milk yield, instead a linear increase in milk urea N ensues, a sign of increased urinary N excretion.

**Introduction** Protein overfeeding is evident on many commercial dairy farms, with the belief that high dietary protein results in increased milk production. Although protein underfeeding may result in sub-optimal milk production, protein overfeeding can also result in low milk productivity due to the high cost of protein and milk loss owing to the energy cost associated with the excess nitrogen excretion. Besides, protein overfeeding exacerbates the environmental impact of dairy production. Consequently, this study assessed the effects of increasing dietary protein level on feed intake, milk yield, composition and nutrient digestibility by lactating Holstein-Friesian cows.

**Material and Methods** The study used 24 lactating Holstein-Friesian cows (17 multiparous and 7 primiparous). The cows were between 149 and 202 days in milk (DIM). Four TMR diets were formulated with a target to contain 130, 150, 170 or 190 g CP/kg of DM from 50% ryegrass silage and 50% concentrate mix (DM basis). The cows were assigned to 6 groups of 4 cows based on DIM, parity, milk yield, live weight (LW) and body condition score. One cow from each group was randomly allocated to 1 of the 4 TMR diets. Thereafter, the 24 cows were randomly

grouped into 12 pairs (ensuring that no pair consisted of cows on the same diet), and then enrolled in the study (in that order) for a 28-day period. The first 20 days were for adaptation to the diets, followed by a 5-day period of individual feeding, and finally 3 days in 2 respiration chambers for measuring gas emissions. Details of the gas emissions objective are not included here. To ensure that all the 12 pairs were on the study for the same number of days, the enrolment of cows in the study was arranged in such a way that each pair was enrolled 3 days after the preceding pair. During the 5-day individual feeding period, day 1 was for adaptation and the final 4 days for data collection (feed intake, milk yield, faeces and urine). Data were analysed using the PROC MIXED procedure of SAS (2003). Diet was the only fixed effect in the model. The effect of pre-treatment milk yield and parity were considered covariates, while the effect of cow within order of enrolment was considered random. Linear and quadratic effects of diet were also estimated.

**Results** Diet affected ( $P < 0.05$ ) total DMI (Table 1). The average DMI for the 170 diet was 1.9 kg/day higher than the average of the other three diets. The average DMI (% LW) ranged from 2.8 to 3.1. Diet did not affect ( $P > 0.05$ ) DM digestibility. However, CP digestibility was affected ( $P < 0.05$ ) by diet, exhibiting a linear ( $P < 0.05$ ) increase with increasing protein level. There was a trend ( $P = 0.073$ ) for diet differences in milk yield. The average yield ranged from 23.2 to 28.1 kg/cow/day. A maximum yield of 28.5 kg/cow/day at a dietary protein level of 171 g CP/kg of DM was derived from the quadratic relationship ( $R^2 = 0.865$ ) between milk yield and dietary protein level. Diet did affect ( $P < 0.05$ ) milk urea N, exhibiting a linear increase with increasing dietary protein level.

**Conclusion** A maximum milk yield of 28.1 kg/cow/day at an optimum dietary protein level of 171 g CP/kg DM was derived from the quadratic relationship ( $R^2 = 0.865$ ) between milk yield and dietary protein level. Increased protein level resulted in a linear increase in milk urea N. These results demonstrate that offering protein levels in excess of the optimum is not a corrective strategy for milk production.

## Acknowledgement

Department of Agriculture, Environment and Rural Affairs

**Table 1.** Effect of dietary protein level on intake, apparent digestibility and milk yield and urea N composition

	Actual	Dietary protein (g CP/kg of DM)				SE	P-value		
		142	153	174	191		Diet	Linear	Quadratic
DM Intake, kg/day									
Silage	8.5	8.8	9.5	8.9	0.385	0.351	0.216	0.270	
Concentrate	10.0 <sup>b</sup>	10.3 <sup>ab</sup>	11.4 <sup>a</sup>	10.5 <sup>ab</sup>	0.436	0.022	0.480	0.039	
Total	18.5 <sup>b</sup>	19.1 <sup>ab</sup>	20.9 <sup>a</sup>	19.4 <sup>ab</sup>	0.798	0.0001	0.228	0.206	
Digestibility									
DM, g/kg	691	711	689	705	13.4	0.573	0.746	0.857	
CP, g/kg	613 <sup>b</sup>	650 <sup>b</sup>	644 <sup>b</sup>	679 <sup>a</sup>	15.7	0.0003	0.0007	0.457	
Milk yield and urea N composition									
Milk, kg/day	23.2	25.5	28.1	25.6	1.55	0.073	0.156	0.120	
Urea N, mg/kg	128 <sup>b</sup>	129 <sup>b</sup>	199 <sup>a</sup>	207 <sup>a</sup>	10.6	<0.0001	<0.0001	0.7538	

<sup>ab</sup>Least square means in the same row with different superscripts differ ( $P < 0.05$ )



# Relationship between the protein molecular structure and in vitro protein digestibility of two insect species intended for inclusion as a feed ingredient

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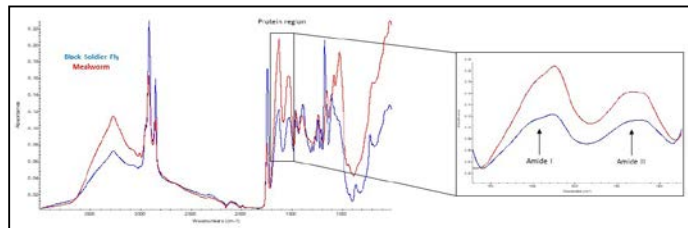
**Application** FTIR spectroscopy represents a rapid, direct, non-destructive technique to predict the in vitro protein digestibility of Black soldier fly and Mealworms.

**Introduction** Insects are a potential source of alternative feed proteins, being Black soldier fly (BSF) and Mealworms (MW) the most popular insect species considered to use in animal diets. However, protein quality relies not only on total protein and amino acid content but also on protein inherent structures (i.e.  $\alpha$ -helix and the  $\beta$ -sheet), which may influence protein quality and nutrient utilisation, availability, or digestive behaviour (Yu, 2005). Fourier-transform infrared (FTIR) spectroscopy is a non-destructive technology to determine the protein molecular structure and the secondary structural composition (Lei et al., 2018). This is the first work assessing the relationship between the protein molecular structure of insects and the in vitro protein digestibility in a non-ruminant model.

**Material and methods** Three different batches of dried BSF larvae and MW were obtained from Chubby Mealworms (West Yorkshire, UK). Samples were milled and sieved (<200 $\mu$ m) prior to analysing. Nitrogen content was analysed by the Kjeldahl method, and crude protein (CP) was calculated using  $N \times 4.76$  (Janssen et al., 2017). The in vitro CP digestibility (CPdig) was calculated according to Boisen & Fernandez (1995). The molecular spectra data were collected and corrected with the background spectrum using Attenuated Total Reflectance (ATR)-FTIR spectrometer. The spectra were generated using the mid-infrared region (400–4000  $\text{cm}^{-1}$ ) with 32 co-added scans and a spectral resolution of 4  $\text{cm}^{-1}$ , in a transmission mode. The IR spectra were performed by the OMNIC 7.2 software. The targeted functional groups in this project are the band amide I (ca. 1655  $\text{cm}^{-1}$ ) and amide II (ca. 1550  $\text{cm}^{-1}$ ), both peaks detectable between the region of ca. 1700  $\text{cm}^{-1}$  to 1500  $\text{cm}^{-1}$ . Absorption peak heights for secondary protein structure  $\alpha$ -helices (ca. 1660  $\text{cm}^{-1}$ ) and  $\beta$ -sheets (ca. 1630  $\text{cm}^{-1}$ ) are detectable in the amide I area using the 2nd derivative function of OMNIC 7.2. The ratios of amide I to amide II (Aml/AmlI) and  $\alpha$ -helix to  $\beta$ -sheet ( $\alpha$ H/ $\beta$ S) spectral intensities were calculated. All samples were analysed in triplicate. Data were analysed by a one-way ANOVA with species as the main treatment, and Pearson's correlations between protein structure profiles and nitrogen content and digestibility were obtained (SPSS 25th edition).

**Results** Previous studies showed a relationship between the protein molecular structure and digestibility of different vegetal and animal feedstuffs (Bai et al., 2016). However, this is the first work assessing the relationship between the protein molecular structure of insects and their protein digestibility in vitro. Figure 1 shows the example FTIR spectra of BSF (blue) and MW (red), with an amplification of the protein region. Table 1 shows data related to the CP, CPdig, Aml/AmlI ratio and  $\alpha$ H/ $\beta$ S ratio, and their correlations. MW showed a higher ( $P < 0.001$ ) CP and in vitro CPdig than BSF, which were positively ( $> 0.80$ ) and negatively ( $< -0.80$ ) correlated with the Aml/AmlI ratio and  $\alpha$ H/ $\beta$ S ratio, respectively (Table 1). The insect-killing method, drying process and gut loading might have had an effect on the molecular structure and digestibility.

**Figure 1** FTIR spectra (enlargement of protein region) of Black Soldier Fly and Mealworm



**Conclusions** FTIR spectroscopy allows assessing differences in the molecular structure of insect proteins, which may influence their digestibility. The higher Aml/AmlI ratio found in MW in comparison with BSF seems to be correlated with a higher crude protein content and in vitro CP digestibility. These findings suggest better suitability of TM than BSF as a protein feed ingredient.

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**Table 1**

Species	CP	CPdig	Aml/AmlI Ratio	$\alpha$ H/ $\beta$ S Ratio	Correlations	Aml/AmlI Ratio	$\alpha$ H/ $\beta$ S Ratio
BSF	37.6	0.85	1.68	0.94	CP	0.94***	-0.82***
MW	44.1	0.91	1.78	0.85			
SEM	1.37	0.012	0.023	0.019			
P	***	***	***	***	CPdig	0.84***	-0.83***

BSF = Black Soldier Fly; MW = Mealworm;  
CP = crude protein;  
CPdig = crude protein digestibility;  
Aml/AmlI = Amide I/Amide II;  
 $\alpha$ H/ $\beta$ S =  $\alpha$ -helix/ $\beta$ -sheet;  
SEM = standard error of the mean  
Significance level =  
\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

# Reducing dietary protein content in dairy cows fed red-clover and grass silage based rations decreases nutrient digestibility but not performance

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**Application** Dry matter intake and diet digestibility were reduced by low protein diet but not milk performance. The protein levels should be taken into consideration when formulating red-clover and grass silage-based rations for dairy cows.

**Introduction** Reducing the dietary crude protein (CP) content in dairy cow diets can decrease purchased feed costs and increase the efficiency of nitrogen use, but may reduce performance if animals do not receive sufficient metabolisable protein (MP; Sinclair et al., 2014). The majority of research to date has focused on the effect of feeding low protein diets based on lucerne and maize silages (Sinclair et al., 2015). Red clover is the second most popular forage legume globally and is high in CP (Boller et al., 2010). However, there is limited information on the effect of feeding low protein diets based on red-clover silage. The aim of the current study was to investigate the effect of feeding low CP diets, based on red-clover and grass silage, on animal performance and diet apparent digestibility in dairy cows.

**Material and methods** Eighteen multiparous Holstein-Friesian dairy cows that were  $71 \pm 14.3$  days in milk, producing  $45.3 \pm 5.72$  kg milk per day, and with an average live weight of  $690 \pm 48.2$  kg at the beginning of the study were used in a  $3 \times 3$  Latin square design with 3 periods, each of 28 days duration. The first 21 days of each period were for adaptation to the diets, with sample and data collection undertaken during the final 7 days. The cows were randomly allocated to 1 of 3 dietary CP concentrations: Control (C, 175 g CP/kg dry matter (DM); supplying 100% of MP requirements), Medium (M, 165 g CP/kg DM; supplying 100% of MP requirements) and low CP (L, 150 g CP/kg DM; supplying 95% of MP requirements). The diets were formulated to be isoenergetic and have a concentrate:forage ratio of 0.47:0.53 (DM basis), with a 50:50 ratio of red-clover to grass silage (DM basis), and were fed as a total mixed ration through roughage intake feeders (Insentec, Marknesse, Netherlands) once daily. Milk samples were taken during the sampling week of each period at two consecutive morning and evening milking. Faecal samples were collected from all cows at approximately 1000 and 1600 h for 5 days during the sampling period and subsequently analysed for acid insoluble ash as an indigestible marker. Intake, performance and digestibility

parameters were analysed as a Latin square design in Genstat (18th edition, VSN International Ltd, UK), where treatment and period were considered as fixed effects and cow as a random effect.

**Results** Mean DM intake was 1.6 kg/day lower ( $P < 0.001$ ) when cows were fed L compared to C or M, but there was no effect ( $P > 0.05$ ) on milk yield, milk composition, or live weight change. Apparent N use efficiency (calculated as milk N output divided by dietary N intake) was highest ( $P < 0.001$ ) in cows when fed L compared to C or M. In contrast, the apparent total tract digestibility of N and fibre were lower ( $P < 0.05$ ) in cows when fed L compared to C or M.

**Table 1.** Milk performance, live weight and whole tract diet digestibility in dairy cows offered high (C), medium (M), or low crude protein (L) diets based on red-clover and grass silage.

	Diets			SEM	P value
	C	M	L		
Dry matter intake, kg/day	25.0 <sup>a</sup>	25.2 <sup>a</sup>	23.5 <sup>b</sup>	0.33	0.001
Milk yield, kg/day	35.0	34.7	34.6	0.51	0.810
Milk fat, g/kg	41.4	42.9	42.6	0.68	0.252
Milk protein, g/kg	32.0	31.6	31.6	0.23	0.422
Live weight change, kg/d	0.14	-0.10	-0.01	0.246	0.792
Apparent N use efficiency (%)	24.7 <sup>b</sup>	25.6 <sup>b</sup>	30.4 <sup>a</sup>	0.40	<.001
N digestibility (kg/kg)	0.68 <sup>a</sup>	0.64 <sup>a</sup>	0.55 <sup>b</sup>	0.022	0.001
Neutral detergent fibre digestibility (kg/kg)	0.68 <sup>a</sup>	0.67 <sup>a</sup>	0.58 <sup>b</sup>	0.021	0.003

Means within a row with a different superscript differ significantly ( $P < 0.05$ )

**Conclusions** Reducing dietary CP concentration from 175 to 150 g/kg DM in red-clover and grass silage-based rations had no effect on performance, but reduced DM intake and apparent total tract nutrient digestibility, which may have a long-term effect on body energy reserves. Apparent N use efficiency was improved in cows receiving the lowest protein diet.

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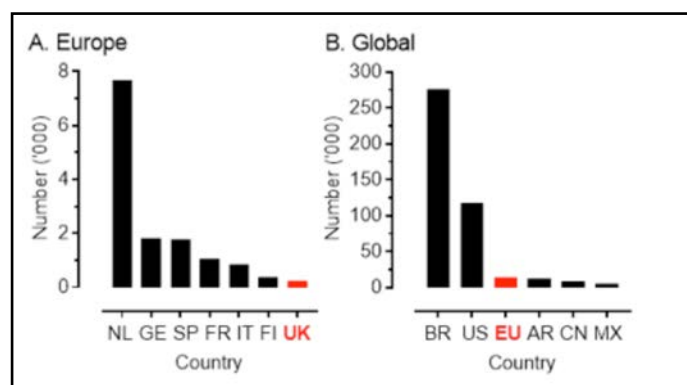
# Advanced reproductive technologies (ART) in animal breeding: Opportunities and issues related to efficiency and safe use

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**Application** Use of such technologies in farm-animals can greatly enhance genetic improvement with existing approaches and establishes a robust platform for use of advanced biotechnologies such as gene editing.

**Context and opportunities** Pigs and cattle account for ~60% of meat and ~85% of milk consumption globally and a sustainable supply of these commodities both to developed and developing markets requires increased productivity through rapid selection for greater feed conversion efficiency and improved disease resistance. Producers have, in recent years, turned to high-throughput genomic platforms (SNP chips) to determine genetic merit for these traits in newborns. Genomic screening can be realised more efficiently via reductions in generation interval when evaluations are made on biopsied cells from in vitro produced (IVP) embryos. Use of such embryos (especially with sexed semen) can also increase selection intensity. That is, making the first selection step before the embryo is implanted removes lower genetic merit animals immediately. The development of robust platforms for embryo production and transfer has the following additional advantages: (i) it facilitates the national and international transport of genetically superior stock in a high animal-welfare, low-cost and bio-secure manner, (ii) provides the means of establishing bio-banks of genomically evaluated stock and rare/endangered breeds, and (iii) lays the foundation for the application of advanced biotechnologies such as zygotic gene editing and the generation and use of stem cells (discussed elsewhere at this meeting). However, the commercial use of these platforms in UK cattle breeding currently lags behind that of our European partners, and use in Europe as a whole languishes behind that in both North and South America (Fig 1).

**Figure 1.** In vitro produced bovine embryo transfer (ET) in major countries within (A) Europe and (B) the world during 2017.



**Recent technological advancements** In contrast to cattle (Black and Sinclair, 2017), the uptake of ART in commercial pig breeding is hindered by a series of species-specific biological constraints that relate to oocyte recovery, IVP, gamete/embryo cryopreservation and ET. These constraints are the subject of ongoing research endeavours where there is cause to be optimistic, with recent improvements in in vitro oocyte maturation and non-surgical deep uterine embryo transfer leading to significant improvements in litter size and farrowing rates (Martinez et al., 2015; Yuan et al., 2017).

**Issues related to efficiency and safe use** Aneuploidy is a major factor associated with pregnancy failure in human ART and is observed in IVP cattle embryos (Turner et al., 2019); although its contribution to pregnancy failure is uncertain. We are currently assessing the merits of aneuploidy screening in IVP cattle embryos using SNP data derived from embryo biopsies used for genomic evaluations. This allows simultaneous identification of genetically desirable and developmentally competent Day 7 embryos for transfer. Chromosomal mosaicism, however, could limit the predictive ability of this test, and is the subject of ongoing investigations. Issues also surround fetal development, obstetrical complications and neonatal morbidity, all of which are determined by the epigenetic regulation of specific genes (particularly imprinted genes) during the periconceptional period (Sinclair et al., 2016). This too is the subject of ongoing investigations which seek to refine existing IVP methodologies, and will be discussed at this meeting.

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# Opportunities for higher value meats, specificity of chicken

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The meat sector is not spared the change in consumption trend. The "one-size-fits-all" offer has to evolve towards a massive range of alternatives to satisfy growing individual(istic) expectations. The number of labels on meat packaging claiming better welfare, better taste, more natural or more social attributes and so on is increasing. Yet, most consumers are lacking the knowledge to grasp the true meaning of these claims. Furthermore, consumers' perception of chicken is different from that of beef, leading to different expectations. Looking at successful meat labels, recent labelling changes and peer-reviewed research, we will draw conclusions on key factors for success and future opportunities for higher value meat, especially chicken.

# Opportunities and challenges for added-value chicken meat

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Throughout the history, the poultry industry has undergone profound modifications mainly driven by the global population growth as well as by the changes in consumers' lifestyle and needs. The same driving forces will be responsible for defining the role of the poultry production in feeding the world in the coming years. In the future, everybody involved in the poultry meat production chain will face different challenges including those related to the changes in consumers' perception in relation to the sustainability of the production chain, to food safety and animal welfare concerns.

The concept of "meat quality" is extremely complex and is commonly thought to be the result of genetic, nutrition and environmental conditions. Indeed, meat quality includes different aspects related to the intrinsic traits of the meat itself (such as its sensorial, sanitary and nutritional traits as well as the genetic background of the animals) but it also embraces extrinsic factors including those related to the consumers' perception of the meat itself primarily related to the production system from which it belongs to, which is currently gaining more and more importance.

Nowadays, poultry meat is considered as a commodity and, as this term implies, aside from the place and from the producer it belongs to, all its traits are highly standardized and uniform all over the world. This is a direct consequence of the poultry meat production system which is currently based on the intensive farming of few commercial hybrids selected for meat production purposes. Indeed, being selected for their fast growth rate, the commercial hybrids intended for meat production are slaughtered at young age (around 35 days-old) and, therefore, the sensory profile of the resulting meat is extremely mild. This peculiar trait of chicken meat makes it particularly suitable for further processing (as any flavor enhancer/spice can be easily added) but may also represent a constraint when differentiation needs to be achieved. Undeniably, the selection practices carried out in the past fifty years permitted to improve the production performances and allowed to reduce the price of chicken meat which is now cheap and affordable for people having different income levels. However, from the other side, the intensive farming of few commercial hybrids is resulting in the loss of most of the autochthons and local breeds that can be found worldwide. Also, the increasing incidence of myopathies that are currently affecting the pectoral muscles of the fast-growing genotype should be kept in mind. Thus, different aspects should be considered when thinking about the opportunities and challenges related to the production of high-values meats. Indeed, differentiation can be achieved throughout different strategies.

As previously mentioned, meat quality is the result of genetic, nutrition and environmental conditions. Thus, it might be easily understood that the first strategy to achieve differentiation is

through the choice of the genotype to be reared according to the production system (intensive vs. extensive) that will be implemented. In this specific framework, rearing medium- or slow-growing genotype instead of the commercial fast-growing and high breast-yield hybrids, would result in meat having a different nutritional profile and stronger flavor, which can partly be ascribed to the slaughter age of the birds and to their feeding. However, it should be mentioned that this choice is associated to longer production cycles and to an overall reduction in the sustainability of the production chain.

Aside from changing the genetic of the birds, nutritional strategies can be developed with the aim to differentiate the quality of the resulting meat. Indeed, as chickens have a monogastric digestive system, the lipid composition of their feed can strongly affect the fatty acid profile of the meat. Aside from this well-known aspect, nowadays, researchers coming from different countries in the World are investigating the impact of including alternative protein sources (including green peas, seaweeds and insects) in feed formulation on the sensorial traits and technological properties of meat. Thus, promoting the use of protein sources that commonly do not find any application and that are not intended for human consumption, this strategy would allow to confer to the meat peculiar traits that can be related to the protein source included in the feed as well as to improve the sustainability of the production chain. In addition, also the inclusion of molecules with potential beneficial effects on the productive performances as well as on meat quality traits and on its stability during storage (i.e. antioxidants, minerals, etc.) might represent a profitable differentiation strategy, especially in the case in which the added compounds can be mentioned as a claim on the product's label.

Aside from the genetic and nutritional aspects, the implementation of processing procedures aiming at producing high-value products (i.e. ready to cook and ready to eat) might be considered effective strategies to differentiate chicken meat. Indeed, the current consumers' lifestyle results in an increasing demand of meat products requiring only minimum efforts for their preparation. Thus, the implementation of processing steps, such as among the others the pre-cutting of the fillets and the addition of marinade solutions might represent effective strategies that the companies can carry out with the aim to differentiate and produce an added-value product from chicken meat.

However, to be perceived and have positive implications on consumers' preferences and attitude to buy, all these genetic, nutritional and processing strategies need to be supported by proper information and transmitted knowledge to the consumers. It should be mentioned that, in general, consumers are getting more sensitive about food quality and animal welfare concerns and they tend to prefer products associated to the concept of "naturalness" and belonging to a non-industrial system as well. Food products containing additives are perceived as not natural and will not be longer desired.

For this reason, the terms "natural" frequently mentioned on chicken meat's label, all the claims related to its organic or extensive production system as well as the development of clean labels can be considered as useful tools to differentiate these productions from the conventional one.



# Predicting nitrogen excretion from beef cattle on diets with contrasting protein content

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**Application** Prediction equations developed in this study could be used for the prediction of manure nitrogen (N) output (MNO) from beef cattle fed diets with diverse protein contents, using N intake (NI) and feed metabolisable energy (ME) content as predictors.

**Introduction** Mitigating MNO from beef systems improves farm profitability and reduces groundwater and atmospheric pollution (Hristov et al. 2011). Reliable prediction equations for MNO can inform management decisions related to the mitigation of N losses but literature equations tend to under-predict nitrogen outputs in manure, urine and faeces, especially in animals with high N excretion (Angelidis et al. 2019). This study aimed to develop diet-specific prediction equations for MNO from beef cattle which were fed diets with different protein contents, using NI as a sole predictor, or in combination with ME.

**Materials and methods** A database was constructed using individual beef cattle digestion data from the Agri-Food and Biosciences Institute (Hillsborough, UK), Centre for Dairy Research (University of Reading, UK) and Beltsville Agricultural Research Center (US Department of Agriculture). Data represented a large variation in animal traits and production characteristics, such as animal breed (purebred and crossbreds including Holstein, Hereford, Angus and others), bodyweight (BW; 153–631 kg), production stage (growing, finishing), diet forage proportion (20–100% of dry matter (DM)) and diet ingredients and chemical composition. Four data sub-sets were created based on diet crude protein (CP) content: (i) all data (84–217 g/kg DM, n=570), (ii) low-medium-CP (LMP) (84–143 g/kg DM, n=190), (iii) high-CP (HP)

(144–162 g/kg DM, n=190), and (iv) very high-CP (VHP) (163–217 g/kg DM, n=190). Prediction equations for MNO were developed using each of the datasets and residual maximum likelihood analysis in GenStat (17th edition), so that the potential random effects of the experiment and individual animal, were accounted for. An external database (n=289) based on a literature review and treatments means from 69 beef digestibility studies, was used to validate the equations; the external database was also split into the same four diet CP groupings as the original database. Equations developed from all data were validated across all four external datasets, but equations developed for specific diet CP ranges were validated against the corresponding external dataset.

**Results** MNO was positively correlated to NI and negatively correlated to ME in all equations. The equation with NI as a sole predictor, produced from the whole database (Eq. 1a), showed similar MPE across the different validation databases. Equations produced from the protein sub-datasets (Eq. 1b, LMP; Eq. 1c, HP; Eq. 1d, VHP) showed lower MPE (i.e. improved prediction accuracy) than Eq. 1a in all corresponding external validation datasets. When ME was used as an additional predictor, the prediction accuracy was increased in the whole, LMP and HP validation datasets (Eq. 2a, 2b and 2c, respectively), while it was markedly improved in the VHP case (Eq. 2d). This highlights the importance of dietary ME supply for the improvement of N use efficiency and its usage as a predictor of MNO in beef cattle fed rich in CP diets.

**Conclusion** This study presents for the first time equations for the prediction of MNO from beef cattle within specific ranges of dietary CP. The results demonstrate that the prediction accuracy in MNO from beef cattle is improved when the equations used have been developed from datasets of similar diet CP. Using equations that have been developed from generic datasets that include a great range of diet CP may reduce the prediction accuracy of MNO. In addition, including ME in the prediction equation may marginally increase the prediction accuracy in LMP and HP diets but highly improves accuracy in VHP diets.

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**Table 1** Single and multiple linear prediction of manure nitrogen output (MNO)

	Equations <sup>a</sup>	n	R <sup>2</sup>	MPE <sup>a</sup>	MPE <sub>LMP</sub>	MPE <sub>HP</sub>	MPE <sub>VHP</sub>	Eq
MNO =	17.34 (6.208) + 0.673 (0.0167) NI	570	0.94	0.242	0.237	0.239	0.226	(1a)
	13.87 (4.703) + 0.699 (0.0316) NI	190	0.94	-	0.232	-	-	(1b)
	5.472 (5.3840) + 0.753 (0.0304) NI	190	0.94	-	-	0.229	-	(1c)
	0.970 (5.5646) + 0.772 (0.0303) NI	190	0.91	-	-	-	0.201	(1d)
	82.79 (8.538) + 0.691 (0.0148) NI - 5.922 (0.5297) ME	564	0.94	0.317	0.429	0.322	0.129	(2a)
	72.34 (10.128) + 0.691 (0.0278) NI - 5.004 (0.8378) ME	188	0.94	-	0.404	-	-	(2b)
	51.28 (15.045) + 0.739 (0.0280) NI - 3.662 (1.1648) ME	189	0.95	-	-	0.287	-	(2c)
	103.6 (13.85) + 0.754 (0.0263) NI - 8.698 (1.0859) ME	187	0.94	-	-	-	0.113	(2d)

a Units: g/d for NI; MJ/kg DM for ME <sup>b</sup> MPE derived from an external validation process

# Intake and performance of finishing beef steers accommodated indoors on concrete slats or rubber covered slats

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**Application** Under the conditions of this experiment finishing cattle accommodated on rubber-covered concrete slats had superior performance to those on concrete slats.

**Introduction** Concern for the welfare of finishing cattle in relation to housing conditions, such as floor type, has been expressed at National, EU and OIE level. There is the view that conventional slatted floors without access to lying-areas should be replaced by more 'animal-friendly' floor types. A recent meta-analysis study (Keane et al., 2018a) concluded that using concrete slatted floors overlaid with rubber (RM) instead of concrete slatted floors (CS) had no effect on performance of beef cattle; however, studies conducted at Teagasc, Grange (Earley et al., 2017; Keane et al., 2015; 2017) ; showed differences in average daily live weight gain (ADG) in favour of RM although the numerical increases in carcass weight obtained were not statistically significant. Furthermore, there was evidence that improvement in animal ADG was product-specific. The objective of this study was to determine effect of CS with or without RM on performance of beef cattle finished indoors.

**Material and Methods** One hundred and forty late-maturing 'continental' breed steers [initial weight 597 (SD 3.5) kg] were used. Following a dietary acclimatisation period of 21 days animals were weighed on two consecutive days, blocked by age and weight and, from within block, randomly assigned to one of 2 treatments; i) Concrete slats (CS) and ii) Rubber-covered slats (RM) [Durapak Agri Ltd., Co. Cork], for a 120 d 'finishing' period. Animals were penned in groups of four at a space allowance of 3.0 m<sup>2</sup> per animal; there were 17 CS and 18 RM pens. Animals had free access to a total mixed ration (TMR) diet comprised of 60:40 grass silage: concentrate on a dry matter basis. Feed was weighed into each pen daily and refusals were measured twice weekly. Representative samples of the TMR offered were obtained bi-weekly for dry matter determination. Animals were weighed at 14 day intervals; ADG was determined by linear regression. At the end of the finishing period animals

were slaughtered in a commercial abattoir; carcass weight, conformation score and fat score were determined. Pen was the experimental unit. Data were statistically analysed using the PROC MIXED procedure of SAS (SAS Institute, Inc.); the model included the fixed effects of treatment.

Results Animal ADG, feed conversion ratio, slaughter weight and carcass weight were superior ( $P < 0.001$ ) for RM compared to CS (Table 1). However, there was no difference ( $P > 0.05$ ) in daily feed dry matter intake, kill-out proportion, carcass conformation score and fat scores among treatments.

**Table 1** Effect of concrete slats versus rubber mats on dry matter intake, daily live weight gain, feed conversion ratio and carcass traits of finishing steers

	Concrete Slats	Rubber Mats	s.e.	P-value
Dry matter (DM) intake (kg/day)	12.1	12.3	0.15	0.32
Average daily live weight gain (ADG) (kg)	0.98	1.15	0.031	<0.001
Feed conversion ratio (kg DM/ kg ADG)	12.4	10.6	0.28	<0.001
Slaughter weight (kg)	717	735	3.5	<0.001
Carcass weight (kg)	403	414	2.5	0.001
Kill-out proportion (g/kg)	563	564	3.4	0.89
Carcass conformation score <sup>1</sup>	8.9	9.2	0.19	0.10
Carcass fat score <sup>2</sup>	8.7	8.5	0.25	0.47

<sup>1</sup> Scale 1 (Poorest) to 15 (best) conformation; <sup>2</sup> Scale 1 (Thinnest) to 15 (fattest)

**Conclusion** Under the conditions of this experiment, performance and feed efficiency of finishing steers were enhanced on RM compared to CS.

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# Does overlaying concrete slats with rubber affect performance of dairy bred bulls?

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**Application** The effect of overlaying concrete slatted floors with rubber on animal performance has been unclear. A meta-analysis of data from three similar studies has provided evidence that attaching rubber to concrete slats resulted in improved performance in dairy bred bulls.

**Introduction** Concern has been raised about the welfare of beef cattle accommodated on fully slatted concrete floors. However there has been conflicting evidence on the effect of overlaying concrete slats with rubber on performance, with some authors reporting an improved performance in cattle on rubber compared with concrete slats (Keane et al 2015; Brscic et al 2015), while others have reported no effect of floor type on performance (Earley et al 2015, 2017). There are many variables, such as animal gender; breed and slaughter weight; pen space allowance; group size; pre-experiment accommodation; rubber product type and diet noted in these studies, which could potentially have an impact on the effect of floor type on animal performance. Keane et al (2018) previously carried out a meta-analysis of the effect of floor type on performance of beef cattle and concluded that using rubber covered slats instead of concrete slats had no effect on beef cattle performance. However the eight studies were used in this meta-analysis had many variables including using a range of rubber products and both bulls and steers. The aim of this study was to carry out a meta-analysis of three similar studies carried out recently at AFBI to assess the effect of attaching rubber to concrete slatted floors on the performance of dairy bred bulls.

**Material and methods** Data for the performance of dairy bred bulls were collated from three studies which examined performance of cattle on fully slatted concrete floors and fully slatted concrete floors overlaid with rubber strips (Comfort Slat Mat, Irish Custom Extruders Ltd, Dublin, Ireland). Individual animal performance data was used from two studies (Murphy et al, 2018 and Lowe et al, 2019) and from a further unpublished study carried out at this research Institute. A total of 148 dairy bred bulls were used which had a mean starting live weight of 241 (s.d. 44.2.0) kg. The results were analysed using linear mixed model methodology (REML estimation method). Experiment and pen within experiment were fitted as random effects, while floor type was fitted as a fixed effect.

**Results** Bulls accommodated on rubber-covered slats had greater live weight gain and estimated carcass gain compared with bulls finished on concrete slats (Table 1). There was also a tendency ( $P < 0.10$ ) for bulls accommodated on rubber to have a higher slaughter weight and carcass weight than those accommodated on concrete slats.

**Table 1** Animal performance of dairy bred bulls accommodated on concrete slats or rubber covered slats

	Concrete slats	Concrete slats overlaid with rubber	sem	P value
Days to slaughter	196.6	196.8	6.32	0.982
Slaughter weight (kg)	524	539	5.6	0.059
Live weight gain (LWG) (kg/d)	1.48	1.54	0.018	0.013
Carcass weight (kg)	266	275	3.5	0.094
Estimated carcass gain (kg/d)	0.75	0.78	0.009	0.006

**Conclusion** Dairy bred bulls finished on slats overlaid with rubber had higher live weight gain and estimated carcass gain than those finished on concrete slats.

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# Using machine learning techniques to estimating feed intake from feeding behaviour and liveweight in finishing beef steers

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**Application** Currently available techniques for measuring intake in housed cattle are expensive and time consuming. This study shows the potential of using liveweight and feeding behaviour variables (which can be obtained from neck mounted accelerometers/collars) to estimate individual animal intake.

**Introduction** The global human population is expected to exceed 9 billion by 2050 with meat consumption also projected to increase. Increasing the efficiency of which feed is converted into beef allows for improved sustainability to achieve these increased levels of production. There also exists large variation in feed efficiency within groups of animals offered the same feed. In order to measure feed efficiency, it is important to accurately record the input (dry matter intake) and output (weight gain) on an individual animal basis. Current techniques for measuring feed intake on an individual animal basis are labour intensive, prone to error and ultimately too expensive for commercial use. The aim of this study was to assess the suitability and efficacy of using feeding behaviour as a proxy to estimate feed intake.

**Material and Methods** In total, 80 steers were used (n=40 per diet) and two diets were allocated to two pens (four pens in total; 20 steers per pen). The two diets (fed as total mixed rations) were generated using a diet mixing wagon and consisted of (g/kg dry matter) forage to concentrate ratios of either 494:506 (MIXED) or 80:920 (CONC). Fresh water was provided ad libitum using a water trough, and diets were offered at approximately 1.05 times average daily intake to all steers using 32 electronic feeders (HOKO, Insentec, Marknesse, The Netherlands). Individual daily fresh weight intakes (kg/day) were recorded for each animal using the electronic feeding equipment, and dry matter intakes (DMI, kg/day) and feeding behaviour variables subsequently calculated. Feeding behaviour variables included: total number of visits to the feeder (TOTVISIT), total time spent at the feeder (TOTFEEDTIME), total time where intake was consumed (TIMEWITHFEED) and average length of time during each visit to the feeder (AVVISITLENGTH). These feeding variables were chosen due to ease of obtaining these measurement from neck mounted accelerometers (e.g. collars, however not used in this study). In total 3 techniques for creating prediction models for

estimation of intake were based on (i) an animal's proportion of TOTFEEDTIME relative to the group (GRP), (ii) random forests (RF) and (iii) support vector machine (SVM). The GRP model was used to estimate fresh weight intake as it is a simplistic model using quantity of feed consumed on a group basis (feed delivered-feed remaining) and TOTFEEDTIME. RF and SVM models used to predict DMI using feeding behaviour and weight of individual animals. Each model was used to predict CONC and MIXED diets separately, giving 6 prediction models in total, (i) GRP\_CONC, (ii) GRP\_MIXED, (iii) RF\_CONC, (iv) RF\_MIXED, (v) SVM\_CONC and (vi) SVM\_MIXED. The coefficient of determination (R<sup>2</sup>), root mean squared errors (RMSE) and mean absolute errors (MAE) were calculated for each model.

**Results** The GRP models predicted fresh weight intake with an R<sup>2</sup> = 0.65 and 0.70, RMSE = 3.13 and 4.32 within CONC and MIXED diets respectively. The RF models predicted DMI with an R<sup>2</sup> = 0.44 and 0.11, RMSE = 1.44 and 1.32 within CONC and MIXED diets respectively. The RF models predicted DMI with an R<sup>2</sup> = 0.41 and 0.40, RMSE = 1.63 and 2.54 within CONC and MIXED diets respectively. These results are summarised in

**Table 1.**

Model	R <sup>2</sup>	RMSE	MAE
GRP_CONC	0.65	3.13	2.46
GRP_MIXED	0.70	4.32	3.48
RF_CONC	0.44	1.44	1.17
RF_MIXED	0.11	1.32	1.06
SVM_CONC	0.41	1.63	1.25
SVM_MIXED	0.40	2.54	2.02

**Conclusions** Feeding behaviour and liveweight can be used to estimate DMI of finishing beef steers with moderate accuracy. Whilst the more simplistic GRP models (fresh weight intake) showed highest R<sup>2</sup>, these models had highest errors.

**Acknowledgements** This work was carried out with funding from the BBSRC and Innovate UK.



# Replacement of *ad-libitum* concentrates with fermented whole crop wheat silage in intensive finishing diets for continental cross dairy-bred steers

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**Application** Similar levels of performance and profitability can be achieved with finishing steers fed *ad-libitum* concentrates or diets containing 500-750 g/kg DM fermented whole-crop wheat silage.

**Introduction** Beef cattle are often intensively finished on *ad-libitum* concentrates based on cereals and protein supplements, with feed cost representing 75% of the variable costs (AHDB, 2016). Recently the high price of cereals and protein supplements, together with market volatility has threatened the long-term sustainability of beef production. In addition, the UK is only 30% self-sufficient in vegetable protein sources, with the rest imported from overseas. This represents a serious strategic risk. High energy and protein forages have the potential to replace concentrates in intensive beef systems with limited effects on animal performance (Kim, et al., 2000). Fermented whole crop wheat (WCW) is a high-energy forage, which can be widely grown in the UK. The objective of the experiment was to investigate the effect of replacing *ad-lib* concentrates with fermented WCW in intensive finishing systems for continental cross dairy-bred steers.

**Material and Methods** Whole crop wheat (Var. Shabras Beret Gold) was harvested in July 2018 at growth stage 85 (Zadocks et al., 1974), with a dry matter (DM) content of 430 g/kg and a cutting height of 16.0 cm, using a self-propelled forage harvester fitted with a Kemper header. The WCW was treated with an additive (Biotal, Wholecrop Gold) at 2.0 l/tonne prior to ensiling in an Ag-bag. Sixty British-Blue X Holstein steers (406 kg) were allocated by live-weight (LW) to one of four treatments consisting of *ad-libitum* concentrates (Control, C), or 250 (25WCW), 500 (50WCW) and 750 (75WCW) g/kg DM WCW, plus concentrates. The four concentrates were based on barley, sugar beet pulp, rapeseed meal and wheat distillers and formulated to supply 140 g/kg DM CP in the overall diet. Animals were housed in groups of five in straw-bedded pens, with three pens per treatment. All diets were fed *ad-libitum* as total mixed rations, with animals on treatment C being offered straw in racks. Feed intake was monitored by recording the quantity offered and weighing back refusals twice weekly.

Animal LW was recorded at the beginning of the experiment and then at 30 day intervals until slaughter. Cattle were selected for slaughter at approximately 600-650 kg LW fat class 3, prior to processing by ABP Shrewsbury. The data were analysed by ANOVA as a randomised block design using Genstat 18.

**Results** The WCW silage was well fermented (pH 4.4; NH<sub>3</sub>-N 30 g/kg TN), contained 94 g/kg DM CP and 288 g/kg DM starch, with a predicted ME of 10.4 MJ/kg DM. The DMI was higher and feed conversion ratio (FCR) poorer for steers fed the WCW diets than those fed diet C. However, the other performance parameters were not significantly different (Table 1). Numerically, days to slaughter increased with increasing WCW proportion and daily LW gain decreased.

**Table 1:** Effects of dietary whole crop wheat proportion on animal performance and diet costs.

	Control	25WCW	50WCW	75WCW	SED	P
Initial LW (kg)	406	406	406	406	1.4	0.940
Final LW (kg)	641	640	633	640	11.5	0.865
Days to slaughter	152	153	157	166	7.8	0.320
Daily LW gain (kg/d)	1.59	1.53	1.45	1.42	0.126	0.553
Dry matter intake (kg/day DM)	9.40	12.25a	12.50a	12.21a	0.287	<0.001
Feed conversion ratio (kg/kg)	6.00	8.03a	8.62a	8.64a	0.438	0.003
Killing out (%)	54.6	54.2	53.8	53.6	0.42	0.166
Diet costs (£/tonne DM)	216	188	163	139	---	---
Margin (£/kg LW gain)	0.99b	0.78a	0.84ab	0.98b	0.080	0.084

**Conclusion** Replacing concentrates with fermented WCW increased intake, which helped to maintain performance, but resulted in a poorer FCR. Diet cost decreased with increasing WCW proportion, such that margins were similar for diets based on *ad-libitum* cereals or containing 500-750 g/kg DM WCW (Table 1).

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# Effect of grazing management system on live weight performance of dairy x beef steers

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**Application** Cell Grazing (CG) management can extend the grazing season and produce equivalent or higher live weight (LW) performance compared to Set-Stocking (SS).

**Introduction** The traditional approach to grazing livestock management has been to use SS or continuous stocking methods, where animals have access to a relatively large grazing area for prolonged period(s) of time at low to medium stocking rates. In contrast, CG is a planned, rotational, time-controlled grazing method which builds upon some fundamental ideas proposed by Voisin (1959) and Savory (1969). It encourages the use of high stocking densities and frequent movements of animals to mimic the natural grazing behaviours of large wild herds of grazing ungulates. Harry Weir, director of KiwiTech International, further developed this concept in NZ during the late 1980's and commercialised it as TechnoGrazing (Charlton and Weir, 2001), which is now beginning to gain popularity in the UK. There is much anecdotal evidence regarding the benefits of management planned, rotational grazing versus other more extensive grazing management approaches, yet the scientific evidence in support of CG is lacking and inconsistent. Thus, a study has been established at Rothamsted Research, North Wyke, Devon to quantify and compare the perceived production and environmental benefits of CG versus SS.

**Material and methods** The study was carried out during 2019 using a ley re-seeded in 2013 with a perennial ryegrass and white clover mix. Eighteen-month-old dairy x beef steers were assigned to either SS (n=15) or CG (n=9), based on LW and breed type. Paddocks were setup in triplicate with a fixed size of 1.75 ha for SS and 1.0 ha for CG, the latter of which was sub-divided into 42 cells. Mobs of 5 steers were randomly allocated to SS paddocks and 3 steers to CG paddocks, giving similar stocking rates of ca. 1.9 LU/ha and total grazing area per head of ca. 0.3 ha. SS paddocks were continually grazed for the duration of the grazing season. CG paddocks were rotationally grazed with a daily area allocation determined by average pasture cover (APC) and ad-lib feed demand (2.5% of animal LW). SS animals were turned out on 3 April, however lower than expected grass growth rates necessitated the temporary removal of animals on 18 April as these paddocks had been grazed to below the minimum target APC to maintain target LW gain. SS

animals were kept on spare pasture while APC in SS paddocks recovered, the LW gain during this period was not used in the data analysis. Animals returned to SS paddocks on 15 May and were housed on 22 October. CG animals were turned out on 26 March and housed on 6 November. LWs were recorded at turnout, at ca. three-weekly intervals during grazing and at housing. Data were analysed in GenStat 20 (VSNI, Hemel Hempstead, UK) using analysis of variance (ANOVA).

**Results** The total duration of the grazing season was 175 days for SS and 225 days for CG. Turnout LW was the same for both treatments (P>0.05) averaging 513kg. Average LW at housing was 686 kg for SS and 692 kg for CG (P>0.05). No difference was seen for average daily gain (P>0.05), which was 0.74 and 0.80 kg/day for SS and CG, respectively. CG was significantly higher for total LW gain per animal (P=0.002) and per ha (P=0.051) compared to SS.

**Table 1** Effect of grazing management on mean live weight (LW) performance of dairy x beef steers at pasture

	Set-stocking (n=15)	Cell-grazing (n=9)	s.e.d	p
Total LW gain per animal (kg)	130	181	14.1	0.002
Average daily LW gain (kg/day)	0.74	0.80	0.072	NS
Total LW gain per ha (kg/ha)	371	542	40.3	0.051

**Conclusion** CG allowed for a longer grazing season compared to SS grazing management which has the potential to reduce the cost of production. CG animals achieved equivalent daily gain and significantly better LW per animal and per ha compared to SS. This indicates that LW output per animal and per ha can be increased by adopting CG and implementing planned grazing management.

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# The effect of pattern of concentrate allocation and Yea-Sacc<sup>®</sup> supplementation on the performance and rumen pH in high yielding dairy cows

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**Application** Supplementing the diet of dairy cows with an active yeast may increase rumen pH and may therefore reduce the risk of subacute ruminal acidosis, but had no effect on performance.

**Introduction** It has recently been reported that 58% of dairy herds in the UK had significant diet selection of the total mixed ration (TMR; Tayyab et al., 2018). When cattle sort through the TMR they change both their level and pattern of concentrate intake (Leonardi and Armento, 2003), which may alter rumen pH, increase the risk of subacute ruminal acidosis and compromise performance. The use of active dry yeasts can stabilise rumen pH by scavenging excess oxygen and reduce the lactic acid concentration in the rumen, which has been shown to increase DMI and milk yield (Desnoyers et al., 2009). The objective of the study was to determine the effect of pattern of concentrate allocation when fed without, or in combination with, a live yeast on animal performance and rumen pH.

**Material and methods** Four multiparous dairy cows that were fitted with permanent rumen cannulas and were 69.3 (SE ±12.1) days post-calving were used in a 2 x 2 factorial Latin square design study. There were four treatment periods of 35 days duration, with data collection and sampling undertaken during the final 11 days. All cows were fed a partial mixed ration with a concentrate to forage ratio of 30:70 (DM basis) and a grass silage to maize silage ratio of 45:55 (DM basis). The diets were either unsupplemented (-) or supplemented (+) with Yea Sacc<sup>®</sup> (Alltech UK) at the rate of 1 g/ cow/ day. An additional 4 kg concentrate/ cow/ day was fed either in an even pattern of allocation (E; 4 x 1 kg every 4 h between 0600 and 1700 h) or an uneven pattern of allocation (U; 4 kg provided in one meal at 0600 h). The four treatments were therefore: E+, U+, E-, and U-. During the final 11 days of each period dry matter intake (DMI) and milk yield were recorded daily, with milk samples collected on four occasions for subsequent analysis of fat and protein. Rumen pH was measured every 3 h from 0600 to 2100 h for three days during the sampling period using a portable pH meter (Hanna Instruments, Bedfordshire, UK). Performance parameters were analysed as a 2 x 2 factorial design by ANOVA, and the change in rumen pH was analysed as a repeated

measures ANOVA, with main effects of pattern of concentrate allocation (C), yeast supplementation (Y) and their interaction (C x Y) using Genstat (v18.1).

**Results** Pattern of concentrate allocation and yeast supplementation had no effect ( $P > 0.05$ ) on DMI, milk yield, or milk fat or protein content, with mean values of 23.1 kg/d, 39.2 kg/d, 39.9 g/kg and 29.1 g/kg, respectively (Table 1). Compared to unsupplemented animals, yeast supplementation tended ( $P=0.084$ ) to increase mean rumen pH by 0.07. The pattern of concentrate allocation and yeast supplementation had no effect ( $P > 0.05$ ) on maximum or minimum rumen pH, with mean values of pH 6.41 and 5.45, respectively, with the lowest pH recorded at 3 h post feeding in cows fed U and 12 h post initial concentrate feed when fed E.

**Table 1.** Even (E) or uneven (U) pattern of concentrate allocation when fed with (+) or without (-) Yea-Sacc<sup>®</sup> on the performance and rumen pH in dairy cows

	Diet				P-value			
	E+	E-	U+	U-	s.e.d	C	Y	C x Y
Total dry matter intake, kg/d	23.2	22.6	22.9	23.6	0.48	0.367	0.943	0.103
Milk yield, kg/d	39.0	40.0	39.1	38.8	1.30	0.560	0.717	0.512
Milk fat, g/kg	38.2	41.2	39.1	40.9	5.43	0.933	0.552	0.876
Milk protein, g/kg	27.8	30.1	27.8	30.6	2.65	0.871	0.219	0.904
Mean rumen pH	5.85	5.77	5.83	5.78	0.05	0.890	0.084	0.745
Maximum rumen pH	6.27	6.31	6.31	6.29	0.09	0.948	0.866	0.589
Minimum rumen pH	5.45	5.48	5.47	5.49	0.07	0.737	0.665	0.967

**Conclusion** Supplementation with yeast tended to increase mean rumen pH but had no effect on DMI, milk yield or milk composition. Pattern of concentrate allocation changed the diurnal pattern of rumen pH, but had no effect on animal performance.

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# Supplementation strategies for a high quality grass silage: starch-based or fibre-based concentrates, offered with or without straw

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**Application** A high starch concentrate (373g starch/kg) can be used to supplement a high quality grass silage to promote dry matter intakes and milk protein content in mid-lactation cows without the need to include straw in the diet.

**Introduction** Increasing the quality of grass silage within dairy cow diets can improve dry matter intakes (DMI) and milk yields, while offering opportunities to reduce concentrate inputs. However, high quality silages have lower fibre contents, which may have a negative impact on rumen function and digestive efficiency (Mertens, 1997). Supplementing high quality silages with starch-based concentrates has the potential to exacerbate this situation. Therefore, it is common practice to supplement high quality silages with 'fibre-based' concentrates, and/or add straw to the diet. Straw is associated with increased retention time of digesta in the rumen (Nandra et al., 1993), and this may improve digestive efficiency and stabilise rumen pH. The current study was designed to examine the effect of concentrate type and straw inclusion in the diet on cow performance, when offered alongside a high quality grass silage.

**Materials and Methods** A three-period change-over design study (period length, 4 weeks) using 24 mid-lactation multiparous Holstein-Friesian dairy cows, examined supplementation strategies for a high quality grass silage (dry matter (DM), 418 g/kg; crude protein, 170 g/kg DM; metabolisable energy, 12.1 MJ/kg DM). Four treatments, in a 2 × 2 factorial arrangement, compared the effects of concentrate type (High-starch or High-fibre) and straw inclusion (Straw or No-straw) on cow performance. The concentrates had a starch and neutral

detergent fibre content of 373 and 258 g/kg DM (High-starch), respectively, and 237 and 339 g/kg DM (High-fibre). Concentrates were formulated using NutriOpt (Nutreco, Netherlands). Silage and concentrates were offered as a total mixed ration in a 57:43 DM ratio. Straw was added at 4% of total DM, replacing part of the silage component of the diet. Effect of diet on intakes, milk yield and composition, faecal scores and body tissue reserves were measured during the final week of each period with cow as the experimental unit. Data was analysed using linear mixed model methodology with constant + treatments as fixed model and block + block × cow + block × period as random model.

**Results** There were no interactions between concentrate type and straw inclusion for any of the cow performance or digestibility parameters examined. Silage DMI and total DMI were reduced with the High-fibre concentrate, and with straw inclusion (Table 1), likely as a result of increased fibre slowing rate of passage. Neither concentrate type nor straw inclusion had an effect on milk yield or milk fat content ( $P > 0.05$ ). The High-starch concentrate increased milk protein content ( $P < 0.001$ ), while straw inclusion decreased milk protein content ( $P = 0.036$ ). Treatment had no effect on cow body weight, condition score or faecal scores ( $P > 0.05$ ).

**Conclusions** Neither concentrate type nor straw inclusion had a significant impact on milk yield or milk fat + protein yield. A carefully formulated High-starch concentrate, when offered alongside a high quality grass silage, increased DMI and milk protein content, compared to a High-fibre concentrate, with no negative effects on faecal scores. Straw inclusion reduced DMI and milk protein content, with no beneficial effect on milk fat content or faecal scores.

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	Concentrate type		Straw inclusion		SED	P-Value	
	High-starch	High-fibre	No-straw	Straw		Concentrate	Straw
Silage DMI (kg/d)	14.7	14.2	15.1	13.7	0.21	0.001	<0.001
Total DMI (kg/d)	26.1	25.3	26.0	25.4	0.37	0.006	0.014
Milk yield (kg/d)	32.9	33.3	33.5	32.7	0.82	0.562	0.161
Fat (g/kg)	44.9	45.0	45.2	44.7	0.77	0.879	0.319
Protein (g/kg)	38.1	37.3	37.9	37.5	0.29	<0.001	0.036
Fat+protein yield (kg/d)	2.71	2.73	2.77	2.66	0.064	0.692	0.403

**Table 1** Effect of concentrate type and straw inclusion on cow performance

# Effect of varying doses of ocimum gratissimum (scent leaf) leaf extract on faecal worm egg count of West African dwarf goats

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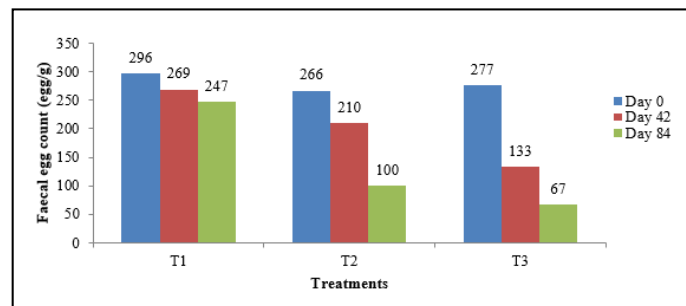
**Application** Ocimum gratissimum (scent leaf) leaf extract reduced faecal worm egg count in growing West African dwarf goats

**Introduction** A lot of phyto-genic plants have been assessed by scientists. This is necessitated by the need to find alternative to antibiotics which usage has been restricted and/or outrightly banned in some countries. Phyto-genics are added to feed to improve animal performance and this has been the focus of several studies in recent years. An example of such plant is Ocimum gratissimum. Ocimum gratissimum is a valuable medicinal plant that is wide spread in West Africa. Researchers agree that this plant has several medicinal values that depend on certain active chemical substances. These active chemical substances are believed to have physiological impact on the human and animal body. Therefore the aim of this study is to assess the effect of varying doses of Ocimum gratissimum extract on faecal worm egg count of West African dwarf goats

**Material and Methods** Ocimum gratissimum (OG) leaves were harvested from villages around the University and air-dried for 3-5days after which they were ground to pass through 1mm sieve. A portion was kept in a dry form for analysis while another portion of the milled sample was extracted. Extraction was by hot infusion, 100ml of hot water was added to 10g of plant sample. This was left to stand for 20 minutes and then filtered using WHATMANN NO1 filter paper. This is a modification of the method described by Imaga and Bamigbetan (2013). The extract (700ml) was prepared weekly and stored in the refridgerator for daily use. The dietary treatments consisted of a basal diet (Panicum maximum) and concentrate supplement which were offered to the animals at 5% of their body weight in a ratio of 60:40 respectively. Eighteen West African dwarf goats were divided into three treatment groups (with six replicates each) in a completely randomized design and were given oral doses (0, 5 and 10ml) of OG leaf extract. Faecal samples were collected directly from the rectum of each animal to determine the faecal egg count at the start of the study and this was repeated every six weeks. The experiment lasted 84days.

**Results** Ocimum gratissimum leaf extract (OGLE) significantly ( $p < 0.05$ ) influenced the faecal worm egg count by the end of the study with the highest and least values of 249 and 67 egg/g respectively obtained from goats dosed 0ml and 10ml of O. gratissimum leaf extract (figure 1).

**Figure 1:** Faecal worm egg count of West African Dwarf goats dosed Ocimum gratissimum leaf extract



**Conclusion** Faecal worm egg counts reduced with increase in the dosage of OGLE. The highest reduction was obtained in goats receiving 10ml dosage

**Acknowledgement** The authors appreciate Tertiary Education Trust Fund (TETFUND) for funding this research

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# What effect do *Sturnus vulgaris* (European Starlings) have on total mixed rations on dairy farms?

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**Application** Starlings consuming exposed total mixed rations affect the amount and physical structure of feed available for cows, which has significant financial implications for dairy farms in the South West of England

**Introduction** *Sturnus vulgaris* (European starling) create a problem for dairy farms in the South West of England in the winter months by consuming feed intended for cows. As well as loss of feed due to direct consumption, there may be changes to proportions of ingredients or particle sizes in a total mixed ration (TMR). Starlings were reported to preferentially consume energy dense portions of a TMR (Deppenbusch et al. 2012). There are other implications, including feeding on silage clamps, particularly maize and whole crop wheat, and the biosecurity risk from travel between farms (Medhanie et al. 2015). Once the location of a farm has been established, particularly where cows are fed along feed fences, starlings are hard to deter. The aim of this study was to determine whether starlings influence the amount and the physical structure of feed, specifically looking at selection of components of the TMR by starlings.

**Materials and Methods** Nine farms were recruited, utilising knowledge from Mole Valley Feed Solutions regarding locations and starling issues on farms. The study was carried out in Cornwall and North Devon in mid-December 2015. Each farm was visited twice a day, in morning and afternoon. At the morning visit, whilst TMR was being dispensed to cows, two 2 kg samples of TMR were taken directly from the mixer wagon and placed in three locations. In each location, one sample was covered to prevent starling access, and one left uncovered. Locations were selected to be beyond the reach of cows, but could be accessed by starlings. 1 kg of TMR was also taken and bagged immediately as a control. Samples were then left for 7 hours on each farm. At the afternoon visit, samples were weighed and bagged. Physical structure of TMR was measured using a Penn State Particle Separator, following the Penn State Extension Protocol; each sample was shaken 40 times, and weight of feed on each sieve was recorded. Dry matter of TMR samples was measured using a Koster tester. Data were analysed using a REML linear mixed model (Genstat 19th Edition) with farm as a random effect and sample covering as a fixed effect.

**Results** Uncovered samples of TMR, which were accessed by starlings, weighed less and had lower dry matter concentration after seven hours than covered samples that could not be accessed by starlings ( $P < 0.001$ ; Table 1). More feed was in the 19mm ( $P < 0.001$ ) sieve, and less feed was in the 8mm ( $P = 0.002$ ) and 4mm ( $P < 0.001$ ) sieves for uncovered samples compared with covered samples. No change in the bottom sieve ( $P = 0.873$ ).

**Table 1:** Effects of exposure to starlings on weight, dry matter content and physical composition of TMR samples after seven hours

	Covered	Uncovered	SEM	P Value
TMR Sample Weight (g DM)	952	660	26.3	<0.001
Dry Matter (g/kg)	481	412	9.0	<0.001
TMR 19mm Sieve (g/100g)	22.1	34.8	2.2	<0.001
TMR 8mm Sieve (g/100g)	47.7	42.5	1.9	0.002
TMR 4mm Sieve (g/100g)	28.2	20.6	1.5	<0.001
TMR Bottom Sieve (g/100g)	2.0	2.1	0.2	0.873

**Conclusion** Starlings consumed considerable quantities of TMR from exposed samples. Increase in dry matter concentration of TMR, and changes to physical feed structure suggest that starlings selected specific small and medium sized TMR elements. These elements would be concentrate components of TMR, which are more expensive and nutrient-dense than forage components. Both consumption of TMR and changes in physical feed structure by starlings are potential financial losses to farms. Understanding bird biology and movement requires research to reduce financial losses related to starlings.

**Acknowledgements** We are grateful to Mole Valley Feed Solutions and the participating farmers.

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# Prediction of manure nitrogen excretion for dairy cattle using data collated at four physiological states (calf, growing, lactating and dry period)

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**Application** Accurate prediction of nitrogen (N) excretion can provide dairy producers with useful information for better management of feed N sources at the farm level.

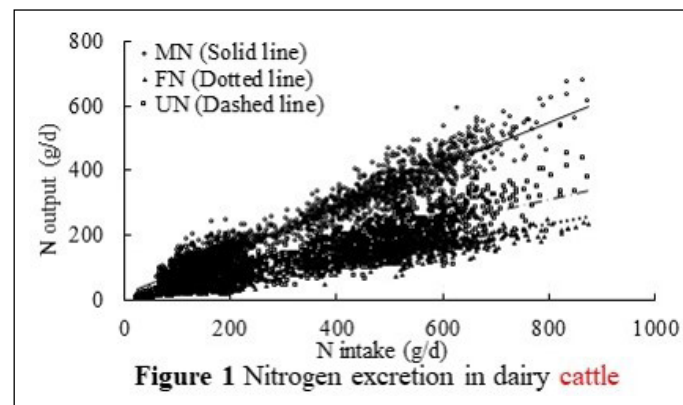
**Introduction** Nitrogen excretion from dairy production is of considerable environmental concern due to its capacity to pollute water source and atmosphere. Lots of prediction equations have been published for quantification of manure N excretion from dairy cattle at various physiological states (e.g., Yan et al., 2006; Reed et al., 2015). However, there is no information available for estimates of N excretion from dairy cattle in a completed physiological period. The objective of the present study was to develop prediction equations for N excretion in dairy cattle using data collated at four physiological states (calf, growing, lactating and dry period).

**Material and methods** Nitrogen intake and output data (n = 1916) used were collated from 63 studies undertaken at the Agri-Food and Biosciences Institute between 1984 and 2017, and comprise 54 calves, 454 growing cattle, 954 lactating cows and 454 dry cows of pure Holstein-Friesian and crossbreed, Norwegian (lactating cows only n = 40) and Swedish Red (lactating cows only n = 18). All calf data were obtained before weaning and offered milk replacer with free access to water, starter concentrates and forage. Dry cows were offered fresh grass-only diets at maintenance levels. Growing cattle and lactating cows were offered ad libitum forage-only diets or mixed diets of forage and concentrates. Before commencing digestibility trials, all animals were housed in loose cubicle accommodation and offered experimental diets for at least 20 d, and then transferred to metabolism crates/units and housed there for 8 d with total feed intake and faeces and urine outputs recorded daily. The REML procedure was used to develop relationships between N intake and N excretion in faeces, urine and total manure, with the effect of experiment being removed.

**Results** Live weight data of calves ranged from 49 to 114 kg, growing cattle from 143 to 649 kg, lactating cows from 379 to 781 kg, and dry cows from 421 to 722 kg. Milk yield for lactating

dairy cows ranged from 5.9 to 49.1 kg (mean 22.3, s.d., 6.90 kg). The whole dataset contains a large range in dietary N content (11 to 43 g/kg DM, s.d. 5.4), ME content (9.0 to 14.9 MJ/kg DM, s.d. 0.88), N intake (25 to 874 g/d, s.d. 195.8), faeces N output (4 to 261 g/d, s.d. 60.0), urine output (8 to 452 g/d, s.d. 80.1) and total manure N output (11 to 679 g/d, s.d. 132.8). These data were used to develop prediction equations for N excretion in faeces, urine and manure (Table 1 and Figure 1). Nitrogen outputs in faeces, urine and manure all are significantly related to N intake (P < 0.001), with very high R<sup>2</sup> values (0.90 to 0.97). Adding live weight, forage proportion, diet N content, diet ME content individually or together as supporting predictors reduced root mean square error by 2.8 to 6.9 units and increased the R<sup>2</sup> values by 1 to 2 units (equations not presented).

**Figure 1** Nitrogen excretion in dairy cattle



**Table 1** Prediction equations for N excretions in dairy cattle

	Equation <sup>1</sup>	R <sup>2</sup>	RMSE <sup>2</sup>	Eq. no
FN =	0.221 (0.0044) * NI + 28.7 (2.67)	0.95	24.0	1
UN =	0.417 (0.0080) * NI - 1.95 (4.258)	0.90	37.0	2
MN =	0.647 (0.0074) * NI + 23.8 (3.71)	0.97	31.1	3

<sup>1</sup>Values in subscript parentheses are SE <sup>2</sup>RMSE = Root mean square error

**Conclusion** The present study provides novel information for accurate quantification of N excretion of dairy production in a complete physiological period. Nitrogen intake can be used as a single predictor to estimate faecal N, urine N and manure N outputs in dairy cattle production.

**Acknowledgements** This project was funded by the Department of Agriculture, Environment and Rural Affairs of Northern Ireland (17-S-267).

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# Prediction of manure nitrogen output for a whole lactation period using long-term production data of dairy cows

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**Application** Manure (faeces and urine) nitrogen (N) output of dairy cows during a lactation period can be predicted from N intake or a combination of milk yield, live weight (LW) and dietary N concentration.

**Introduction** Annual manure N (MN) excretions and associated ammonia emissions from dairy production systems are often predicted from annual N intakes, with the latter estimated from feed intake using energy rationing systems. An alternative approach is to estimate annual MN output directly from cows' performance. The objective of the present study was to evaluate if there were significant relationships between N output and N intake, milk yield and LW of dairy cows in a whole lactation, and then develop prediction equations for MN output for a whole lactation using N intake or animal and dietary factors.

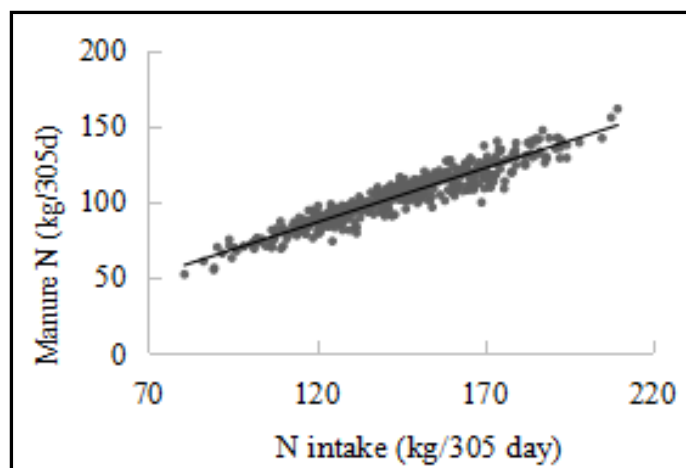
**Material and methods** Dairy cow data ( $n = 476$ ) used were collated from 8 full lactation production studies undertaken at the Agri-Food and Biosciences Institute between 2006 and 2013. Cows (286 Holstein, 161 Jersey-Holstein crossbred and 29 Swedish Red-Jersey-Holstein crossbred) were either totally housed throughout the experimental period ( $n = 89$ ), or housed in early lactation and late lactation, and grazed in mid lactation ( $n = 387$ ). Diets offered comprised predominantly grass silage, grazed grass and concentrates. Feed intake, LW and milk production were recorded throughout experimental periods, except for intake of grazed grass which was estimated using the 'Feed into Milk' models. The MN outputs (kg) for individual cows during whole experiment periods (ranging from 250 to 390) were calculated as the difference between N intake and a sum of N retention for LW gain and pregnancy (gravid uterus N and fetus N). Total N intake (NI305, kg/305d), energy corrected milk yield (ECM305, ton/305d) and MN (MN305, kg/305d) for individual cows were scaled to 305d assuming 305d lactation and 60d dry period in a yearly basis. These data were then used to develop prediction equations for MN305 using NI305 or a combination of ECM305, mean LW (kg) and dietary N concentration (Nc, g/kg DM) by the REML procedure, with the effects of experiment, cow breed/genotype, parity, management regime and dietary forage type and proportion being removed.

**Results** The current dataset contains a large range in LW (379 to 769 kg), ECM305 (3.62 to 13.22 ton), NI305 (76 to 210 kg), MN305 (52 to 161 kg) and dietary CP concentration (0.123 to 0.224 kg/kg

DM). The prediction equations for MN305 ( $P < 0.001$ ) developed are presented in Table 1 and Figure 1. The NI305 was the best predictor for MN305 with  $R^2$  of 0.96 (Eq. 1). Compared to the equation using ECM305 as a single predictor (Eq. 2), adding LW and Nc as supporting factors (Eq. 3) significantly improved the prediction accuracy for MN305 with  $R^2$  values increased from 0.64 to 0.69. An internal validation was undertaken using 2/3 present data to develop similar equations and 1/3 data to validate these new equations. The validation indicates that these equations could be used to accurately predict MN for dairy cows over a whole lactation period. The present study highlights that although the variation of MN excretion is best described by N intake, the equation using ECM305, LW and Nc may be important and practical.

**Table 1.** Prediction equations for MN output over 305d lactation of dairy cows

Equation	R <sup>2</sup>	Eq.
MN305 =		
$0.78_{(0.0112)} * NI_{305} - 11.9_{(2.92)}$	0.96	1
$5.65_{(0.4992)} * ECM_{305} + 61.5_{(7.91)}$	0.64	2
$4.54_{(0.458)} * ECM_{305} + 0.086_{(0.009)} * LW + 3.17_{(0.271)} * Nc - 65.8_{(10.17)}$	0.69	3



**Figure 1** N intake vs Manure N output

**Conclusion** Manure N outputs of dairy cows over 305 d lactation can be predicted from N intake, or a combination of annual milk yield, live weight and dietary N concentration. This provides an alternative measure to quantify annual N excretion and associated ammonia emissions in dairy production systems.

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# Factors influencing the water content of milk collected from UK dairy farms

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**Application** Milk which contained higher levels of lactose, fat, protein and urea was associated with a higher freezing point depression (FPD), indicating a reduced water content. Milk FDP was also highest in the summer.

**Introduction** Raw milk composition with the correct physicochemical properties is essential for the manufacture of dairy products (Schwendel et al., 2015). The freezing point (FP) of raw milk reflects the water content in milk. The relative level of water in milk is derived by converting the measured FP of milk to a positive integer known as the freezing point depression (FDP). The lower the FDP value, the greater the milk water content. Excessive water (more than 90%) in raw milk incurs a price penalty. Water content in milk is affected by a number of factors such as animal species, stage of production, nutritional composition and seasonal climate (Henno et al., 2008). The aim of this study was to investigate the effects of milk composition and season on the water content of bulk tank raw milk collected on commercial UK dairy farms.

**Material and methods** Ten dairy farms located in the west of the UK, from a single milk processor, were selected to be representative of UK dairy production systems. A total of 5,733 herd milk data records were collected between the years 2016 and 2017. Data was analysed for bulk tank milk volume on date of collection, bulk tank milk composition, and milk freezing point (°C). Milk samples were sent to the National Milk Recording Laboratories (NML) for analysis. Milk fat, protein, lactose, urea and casein values were determined through fourier transformed infrared spectroscopy. The freezing point of raw milk was measured using thermistor cyroscrope. Milk samples were supercooled and the freezing point measured. All samples were analysed within 24 hours of collection. The effect of season during the year and each year were also investigated. The months of the year were grouped into periods as follows: March, April, May and June (Spring), July, August, September and October (Summer) and November, December, January and February (Winter). Data were analysed using a linear mixed model in Genstat (version 19.1) to identify the factors effecting FPD across farms studied. Significance is defined as  $P < 0.05$ .

**Results** The milk contents of lactose, fat, protein and urea all had an effect on bulk tank milk FPD (Table 1). The summer months had a higher FPD compared with other months during the year (Table 1). Also, the FPD was higher in 2016 than in 2017 on farms analysed in this study.

**Table 1.** Significant effects of milk lactose, fat, protein, urea, season and year of study on the predicted mean freezing point depression values for milk after adjusting for fixed and random effects. Means with different superscript letters in the same row differ significantly  $P < 0.05$ .

Variable	Content (g/kg)				F statistic	SED	P value
Lactose	<44.4 512.3 <sup>a</sup>	44.4 – 45.2 515.1 <sup>b</sup>	45.3 – 46.0 517.6 <sup>c</sup>	>46.0 519.7 <sup>d</sup>	121.3	0.22	<0.001
Fat	<37.5 514.4 <sup>a</sup>	37.5 – 39.7 514.8 <sup>b</sup>	39.8 – 45.9 516.2 <sup>c</sup>	>45.9 519.4 <sup>d</sup>	117.1	0.24	<0.001
Protein	<31.9 514.5 <sup>a</sup>	31.9 – 32.7 515.6 <sup>b</sup>	32.8 – 35.7 516.3 <sup>c</sup>	>35.7 518.3 <sup>d</sup>	47.7	0.23	<0.001
Urea	<0.20 515.7 <sup>a</sup>	0.20 – 0.23 516.0 <sup>ab</sup>	0.24 – 0.27 516.3 <sup>b</sup>	>0.27 516.8 <sup>c</sup>	14.7	0.15	<0.001
Season	Spring 515.7 <sup>a</sup>	Summer 517.0 <sup>b</sup>	Winter 515.9 <sup>a</sup>		76.8	0.13	<0.001
Year	2016 516.8 <sup>a</sup>		2017 515.6 <sup>b</sup>		125.9	0.10	<0.001

**Conclusion** Farmers should aim to increase the content of milk solids in their milk to prevent receiving a price penalty due to excessive water content. Milk FPD was highest in the summer months, indicating less water present.

**Acknowledgements** The authors gratefully acknowledge Arla Foods for providing data for analysis.

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# Isolation and characterisation of myogenic precursor cells in pigs: towards development of an in vitro model for the study of muscle growth and development

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**Application** The projected rise in world population to 9.7 billion by 2050 will require an increase in production of meat globally to meet the protein requirements without compromising environmental and financial sustainability. Efficient increase in meat production will require an in-depth knowledge of muscle growth and development.

**Introduction** Being a multiparous species able to produce up to 40 offspring per female a year, the pig provides a potentially very efficient source of meat. A significant issue with modern high prolificacy breeds is the high variability in birth weights within litters, which in turn results in variable growth efficiency and carcass quality. Growth efficiency is closely related to lean body, i.e. the proportion of skeletal muscle relative to body fat. Therefore, understanding the biological drivers of muscle growth is essential for devising efficient strategies to maximise meat production in pigs. A technical limitation to the development of robust models to study muscle development in vitro is that efficient protocols to isolate and maintain in culture muscle stem/precursor cells from pig are lacking thus precluding meaningful studies. The objective of this study was to develop a protocol for efficient isolation of muscle progenitor cell from pig muscle.

**Material and methods** Semitendinosus muscle was obtained from new born male piglets. Immunohistochemistry was used to identify muscle precursor cells in muscle based on the expression of the surface markers, CD56 and Pax7. To isolate progenitor cells, muscle samples were digested enzymatically followed by incubation with antibodies targeting endothelial cells (CD31), haematopoietic cells (CD45) and myogenic cells (CD56). Myogenic (CD45-/CD31-/CD56+) and non-myogenic (CD45-/CD31-/CD56-) cell fractions were isolated using fluorescent activated cell sorting (FACS). Cells in both fractions were expanded in culture and characterised by qPCR analysis for muscle regulatory factors (PAX7, MYOD, and MYF5). In addition, cells were tested for their ability to form myotubes (In media supplemented with 1% insulin selenium transferrin) or adipocytes (in media supplemented with fetal bovine serum,

dexamethasone, Indomethacin, 3-isobutyl-1-methylxanthine and Insulin) and subsequently analysed using immunocytochemistry and qPCR for specific lineage markers. QPCR values below are always provided as arbitrary units relative to a standard curve.

**Results** Immunocytochemistry data confirmed that CD56 co-localises with the muscle stem cell-specific marker, PAX-7, thus providing a suitable marker for isolating myogenic cells from muscle. We found that compared to non-myogenic cells (CD56-), myogenic cells (CD56+) obtained by FACS were enriched for the muscle regulatory factors, PAX7 (27.1±5.0 vs 2.9±2.3; P<0.05), MYOD (10.1±1.7 vs 1.8±0.7; P<0.05) and MYF5 (18.5±4.8 vs 2.7±1.1; P=0.06), as determined by qPCR. In addition, CD56+ but not CD56- cells were able to fuse and form myotubes under appropriate conditions. In contrast, both cell fractions were able to generate adipocytes in vitro, although CD56- cells had a higher capacity to do so, as confirmed by the higher expression of lipid transport protein FABP4 in differentiated cultures from CD56- relative to CD56+ (48.9±8 vs 8.4±4.8; P<0.05).

**Conclusion** We showed that CD56+ marks myogenic progenitor cells in pig muscle. Moreover, we established a protocol for efficient isolation of myogenic cells from pig muscle based on selective CD56 expression. We showed that although myogenic cells are predominantly contained with the CD56+ fraction, this fraction also contains some adipogenic precursor cells. This data provide a strong foundation for the robust generation of pig muscle tissue development for meaningful interrogation of candidate genes involved in muscle growth and development.

**Acknowledgements** Financial support for this study was provided by the Biological and Biotechnology Research Council (BBSRC), UK and the Edinburgh Global Research Scholarships.

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# Relationship between glutathione peroxidase activity and the fatty acid profile of poultry meat

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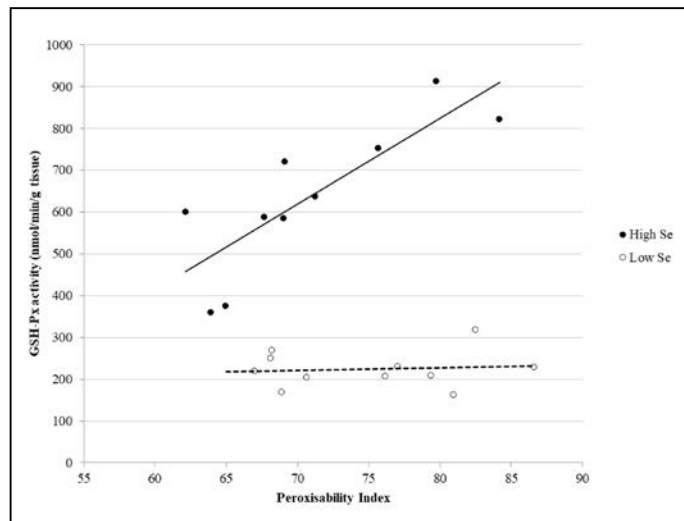
**Application** An adequate supply of dietary selenium is required to ensure tissues with high unsaturated fatty acid concentrations can protect themselves from lipid oxidation with increased GSH-Px activity.

**Introduction** Glutathione peroxidase (GSH-Px) is a family of selenium (Se)-containing enzymes which regulate the production of reactive oxidative species during normal respiration, preventing damage to biological fluids and tissues. This damage can also happen post-mortem, particularly in meat with a high concentration of unsaturated fatty acids (FA), resulting in lower shelf life and poorer meat quality. GSH-Px activity is dependent upon Se status (Colakoglu et al., 2017), whereas GSH-Px gene expression is independent of Se status, but may be upregulated during periods of oxidative pressure (Sneddon et al., 2003). The main objective of this study was to identify the relationship between oxidative pressure (measured as tissue polyunsaturated fatty acid (PUFA) content and peroxidisability index, PI), and GSH-Px activity in the breast and thigh tissue of poultry offered Se containing diets.

**Materials and methods** Twenty four male Ross 308 day-old chicks were ad libitum fed a starter diet (Target Feeds, Shropshire, UK) containing no supplemental Se until 15 days of age before being randomly allocated to one of two dietary treatments – a grower/finisher Control diet containing no supplemental Se, and the same diet but containing 0.35 mg/kg (as fed) Se in the form of hydroxy-selenomethionine (Selisseo®, Adisseo, France). Birds had ad libitum access to both diets and water, and were slaughtered at 42 d. Breast and thigh tissue was collected post-mortem, homogenised and analysed for GSH-Px activity using a colorimetric kit (Abcam, Cambridge, UK). Remaining tissue was freeze dried and stored at -20°C until analysed for FA profile using the method of Sukhija & Palmquist (1988). Effect of dietary Se on GSH-Px and FA profile was assessed using a general linear model (Minitab v18.0) which included effects of diet and tissue type. The degree of unsaturation of the samples was quantified using PI (Erickson, 1992), and relationship between GSH-Px activity, FA profile and PI was determined using linear regression (Minitab v18.0).

**Results** In both tissues, dietary Se supplementation increased ( $P < 0.001$ ) GSH-Px activity. There were Se by tissue interactions ( $P < 0.05$ ) for 14:0, 16:0, 18:0, cis-9 18:1, 18:2 n-6, 20:3 n-6 and

20:5 n-3, whereby concentrations were higher in the thigh tissue of Se supplemented birds. There was no relationship between GSH-Px activity and individual FA in the tissues from birds offered the Control diet, which is reflected in the lack of any relationship between GSH-Px activity and PI in Control bird tissues. However, there was a positive correlation ( $r = 0.826$ ;  $P = 0.003$ ) between PI and GSH-Px activity in thigh tissue of Se supplemented birds, whereby GSH-Px activity increased with PI index (Figure 1).



**Figure 1.** Relationship between glutathione peroxidase activity and peroxidisability index in thigh meat from poultry fed diets containing two selenium (Se) concentrations (low – no supplemental Se, high – 0.35 mg/kg supplemental Se).

**Conclusions** Supplemental Se may have modified the FA profile of lipid rich muscle tissues, by increasing the tissue content of some FA. Furthermore, the positive relationship between GSH-Px activity and degree of unsaturation in lipid rich muscle tissue of birds supplemented with Se indicates that increasing susceptibility to oxidation in tissues may increase GSH-Px activity, but only in animals which are Se replete.

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# Sensitivity of weight gain composition and N excretion of finisher pigs to dietary protein and essential amino acids

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**Application** Reducing dietary crude protein levels whilst meeting essential amino acid requirements does not impair carcass composition but significantly reduces N excretion per unit body weight gain.

**Introduction** We previously showed that through using pure lysine, methionine, threonine, tryptophan, isoleucine and valine, reduced crude protein (CP) rations resulted in increased N use efficiency (Houdijk and Roehe, 2019). We speculated that N excretion and thus environmental footprint should be reduced, if N balance is not impaired. Here we tested this hypothesis by assessing impact of reducing CP levels whilst meeting essential AA requirements on weight gain composition as a proxy for N balance, and used the outcome to estimate N excretion per unit body weight gain.

**Material and methods** A total of 72 118-d-old pigs (Danish Duroc × (Large White × Landrace)) were put into pens of 3 males or 3 females, with averaged body weight of 84.7±1.2 kg. Pigs were fed ad lib one of three iso-energetic diets for 25 days: high CP (HP), low CP (LP), or LP with added AA (LP+AA) to the same level of standardised ileal digestible essential AA as in HP. Resulting CP levels were 167, 149 and 151 g/kg for HP, LP and LP+AA, respectively from d 118 to d 132 (NE: 9.4 MJ/kg), and 159, 140 and 143 g/kg, respectively from d 132 to 143 (NE: 9.3 MJ/kg). Diets HP and LP+AA met finisher pig AA requirements, whilst the AA to CP ratio in HP and LP was constant. The average weighing pig for each pen was CT scanned one wk before trial start (at BW of ~78 kg), and the same pig was scanned at trial end (at BW of ~119 kg). Carcass composition was measured from the CT images (muscle, fat and bone mass) and used to calculate muscle, fat and bone mass per unit body weight gain (BWG). N balance was assumed to be 30% for the control pigs in order to calculate N excretion. Data were analysed using a 3×2 factorial ANOVA, accounting for the effect of diet, sex and their interaction. This work was approved by SRUC's Animal Welfare and Ethical Review Body (ED AE 05-2018).

**Results** The HP, LP and LP+AA pigs grew 1498, 1469 and 1546 g/day (s.e.d. 34 g/day; P=0.009; Houdijk and Roehe, 2019). Between the two scanning points, muscle and bone decreased

from 63.3±0.4% to 59.9±0.4%, and from 6.4±0.1 to 5.9±0.1, respectively, whilst fat increased from 15.8±0.4 to 19.8±0.4%. Feeding treatment and sex did not interact for any of the parameters. Table 1 shows that dietary treatment did not affect the contents of muscle, fat and bone per unit BWG. However, male pigs gained more bone than female pigs (52.5 vs 43.9 g/kg; s.e.d. 1.9 g/kg; P<0.001) and fat (599 vs 474 g/kg; s.e.d. 20 g/kg; P<0.001), whilst muscle gain was similar (281 vs 265 g/kg; s.e.d. 16.3 g/kg; P=0.330). Both N intake and N output were significantly smaller for LP and LP+AA pigs than for HP pigs.

**Table 1.** Muscle, fat and bone, N intake and N output, all in g/kg BWG of pigs fed high protein (HP), low protein (LP) or LP diets with amino acids as in HP (LP+AA) for 25 days from ~78 to ~119 kg live weight.

Dietary treatments	Parameters (g/kg BWG)				
	Muscle	Fat	Bone	N intake	N-excretion
HP	284	526	50	67.2	47.1
LP	267	546	47	57.5	37.3
LP+AA	269	537	48	58.7	38.5
s.e.d.	20	25	2	1.6	1.6
Probabilities	0.653	0.728	0.293	0.001	0.001

**Conclusion** Compositional gain was similar between HP, LP and LP+AA treatments. Therefore, at the assumed 30% N intake retention for the control pigs, this data would support the view that ensuring appropriate levels of essential amino acid levels in reduced protein diets would concur with a reduction in N excretion. Within the parameters of this study, N excretion reduced by 11.1% for each unit of CP reduction achieved. At assumed N intake retention for the control pigs of 20% and 40%, the latter would be 9.7 and 13.0, respectively.

**Acknowledgements** We thank Kirsty McClean, John Gordon, Claire Anderson, Andrea Bosio, Jo Donbavand, Mark Brims, Agnieszka Fruto, Mhairi Jack and Marianna Farish for technical support, and the BBSRC funded N-Circle project for financial support (BB/N013484/1). SRUC is supported by RESAS.

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# Investigating links between post-mortem carcass inspection outcomes and tail biting injuries in pigs: a potential welfare surveillance tool?

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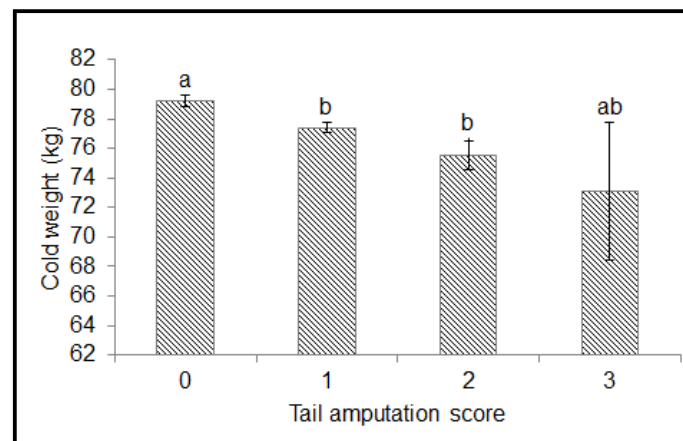
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**Application** It is important to better understand the link between post-mortem (PM) inspection outcomes and on-farm data in order to use carcass inspection to monitor health and welfare status of pigs on farm.

**Introduction** Recent research suggests slaughterhouse inspection can facilitate surveillance of farm animal health and welfare (Pandolfi et al., 2018). However, carcass data represent the accumulation of disease and injury over an animal's entire life history, and it can be difficult to attribute the causes of lesions found on carcasses to specific diseases or welfare issues on farm (van Staaveren et al., 2017). This study used data collected from a disease-free pig unit with a complete record of undocked pigs' health and welfare. We aimed to investigate further the connection between data collected on live animals and at slaughter, focusing on the impact of tail biting. **Material and Methods** A total of 1,344 pigs from three different experimental trials were included in this study. All pigs (Landrace × Large White) were reared from birth to slaughter in the Teagasc Pig Research Facility (Moorepark, Ireland) during 2015 to 2018. This facility has a high-health status, and no outbreak, symptom or reduced production performance were verified during the study period. No tail docking or castration were performed on the pigs in this study. Tail lesion and tail amputation were scored on individually identified pigs every two weeks from weaning (4wk of age) until slaughter (20–22wk of age), using the scoring systems described by Chou et al. (2019). Tail lesions were scored PM after scalding. Lungs, hearts and livers of 728 carcasses were assessed and lesions scored on individually identified pigs by a certified veterinarian using a scoring system described in Rodrigues da Costa (2018). The carcass report of individual pig included cold weight and condemnation records. Data were analysed using SAS 9.4. Logistic regression was used to examine the relationship between live/PM tail scores and the PM data. Linear mixed modelling was used to test the effects of live/PM tail scores, visceral lesions and carcass condemnation on cold carcass weight. Spearman's rank correlation was also used to examine on-farm and PM data.

**Results** Pigs with higher live and PM tail amputation scores were more likely to be scored positive for pleurisy ( $P < 0.01$ ), but not for other lung lesions or pericarditis. A higher probability of carcass condemnation was found when the worst live tail lesions were higher, and when PM tail amputation scores were higher ( $P < 0.05$ ). Carcass condemnation and higher PM tail amputation scores reduced the cold carcass weight ( $P < 0.001$ ; Figure 1). There was a moderate correlation between live and PM tail amputation score ( $r_p = 0.51$ ,  $P < 0.001$ ), but a weaker correlation between live and PM tail lesion ( $r_p = 0.22$ ,  $P < 0.001$ ). Figure 1. Effect of post-mortem tail score on carcass weight (LSMeans±SEM  $P < 0.001$ ).

Figure 1



**Conclusion** The results suggest a link between tail biting and the incidence of pleurisy as well as carcass condemnation. PM tail amputation score was also linked to reduced cold carcass weight, suggesting the negative impact of tail biting on economics. Tail amputations scored in the slaughterhouse reflected the severity of tail amputation on-farm; however, the correlation was much weaker for tail lesion scores. Tail lesions experienced during a pig's life may fluctuate and heal, becoming more difficult to detect by carcass examination. Therefore, tail lesions scored on the carcass may underestimate the severity of the actual tail biting events on farm. Carcass inspection can be a useful tool to monitor health and welfare, however, disease prevalence on-farm also need to be considered when studying the effects of tail biting on carcass characteristics. **Acknowledgement** This project was co-funded by the Department of Agriculture, Food and the Marine in Ireland and Teagasc Walsh Fellowship.

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# Effect of bactofort® on nutrient digestibility and intestinal pH of broiler chickens and its potentials as replacement for antibiotics

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**Application** Dietary supplementation of multi-strain probiotic (Bactofort®) had little improvement in the nutrient digestibility of broiler chickens; it however decreased pH acidity of the gastrointestinal tract.

**Introduction** Antibiotic growth promoters (AGPs) are routinely administered at lower doses than therapeutic to improve birds' performance due to improved feed efficiency (Peterolli et al., 2012) but the development of antibiotic resistant bacterial strains and residual effects of these feed additives in eggs and meat have led to various health hazards to consumers. However, increase in frequency of drug residues present in commercial poultry products (Mensah et al., 2011) has been a major concern to health-conscious consumers. The feed manufacturers are adopting new forms of natural feed additives that are the products of modern science. In view of this, an experiment using a selected multi-strain probiotic (Bactofort®) which consists of a fungal species - *Saccharomyces cerevisiae* and three other bacterial species - *Lactobacillus acidophilus*, *Enterococcus faecium* and *Bacillus subtilis* on nutrient digestibility and pH acidity of the gastrointestinal tract of broiler chickens was conducted.

**Materials and Methods:** Two hundred and fifty five week-old broiler chicks were assigned to five dietary treatments containing 0, 25, 50 and 75g Bactofort® for treatments 1 to 4 respectively while treatment 5 contained Oxytetracycline. Each treatment was replicated thrice in a completely randomized design (CRD) with 17 birds per replicate. The diets were isocaloric and isonitrogenous and formulated to meet the standard nutrient requirements of the broiler chickens. Digestibility parameters were determined at week 7 from three selected birds in each replicate which were kept in individual cages for total faecal collection using the standard methods described by A.O.A.C. (2005). Intestinal pH of the crop, proventriculus, ileum, jejunum and caeca was determined using a digital pH meter at the finisher phase. All data obtained were subjected to analysis of variance (ANOVA) of SAS (2002). Significant ( $P < 0.05$ ) difference between treatments means were compared using Duncan Multiple Range Test.

**Results** Significant differences ( $P < 0.05$ ) were observed in all digestibility parameters (dry matter, crude protein, crude fibre, ether extract, ash retention and nitrogen free extract). Birds fed 25g Bactofort® had the best digestibility in all the parameters

measured (DM, CP, CF, EE, and NFE) however, not significantly different from those fed control and antibiotic diets. This implies that addition of the probiotic to a little extent increased the efficiency of digestion and nutrient absorption processes of the intestinal tract of the birds. Evidently, pH acidity of proventriculus and caecum decreased with addition of Bactofort® however, there was an increase in pH acidity of proventriculus and caecum of birds fed 25g Bactofort® compared to the control. This could be as a result of the presence of the commensal bacteria such as *Lactobacilli* which produce a wide variety of short chain fatty acids (SCFAs), which are bacteriostatic for a subset of bacterial species either directly or by reducing pH of the intestinal environment. Birds fed 25g Bactofort® seems to have more favourable pH for microbial growth.

**Conclusion** Dietary supplementation of Bactofort® at 25g/100kg of diet had little effect on nutrient digestibility of birds at 7 weeks of age compared to the control and antibiotic diets. It however, decreased pH acidity of the gastrointestinal tract. Conclusively, supplementation at this level may replace in-feed antibiotics and obtain similar or even better digestibility and favourable environment for microbial growth.

**Acknowledgements** The authors acknowledge the research and technical staff of Animal Science Department, A.B.U, Nigeria.

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**Table 1:** Apparent Nutrient Digestibility (%) of Broiler Chickens (7weeks) Fed Diets Containing Varying Levels of Bactofort®

Parameters (%)	Inclusion Levels of Bactofort®				Oxytet	SEM
	0g	25g	50g	75g		
Dry Matter	73.06a	76.48a	68.46b	68.47b	73.29a	3.283
Crude Protein	76.77a	78.28a	72.03b	70.75b	75.14a	2.913
Crude Fibre	67.33ab	65.83ab	54.02b	55.93b	72.10a	4.560
Ether Extract	90.53a	88.75a	84.73ab	83.75b	85.68ab	1.668
Ash	68.18b	77.09a	66.76b	54.30c	63.34b	4.29
Nitrogen Free Extract	72.32a	75.07a	67.70b	70.05a	73.33a	3.061

a, b, c = Means with different superscript on the same row differ significantly ( $P < 0.05$ ); Oxytet = Oxytetracycline; SEM = Standard Error of Means.

**Table 2:** Intestinal pH of Broiler Chickens (7weeks) Fed Diets Containing Varying Levels of Bactofort®

Parameters (%)	Inclusion Levels of Bactofort®				Oxytet	SEM
	0g	25g	50g	75g		
Dry Matter	73.06a	76.48a	68.46b	68.47b	73.29a	3.283
Crude Protein	76.77a	78.28a	72.03b	70.75b	75.14a	2.913
Crude Fibre	67.33ab	65.83ab	54.02b	55.93b	72.10a	4.560
Ether Extract	90.53a	88.75a	84.73ab	83.75b	85.68ab	1.668
Ash	68.18b	77.09a	66.76b	54.30c	63.34b	4.29
Nitrogen Free Extract	72.32a	75.07a	67.70b	70.05a	73.33a	3.061

a, b = Means with different superscript on the same row differ significantly ( $P < 0.05$ ); Oxytet = Oxytetracycline; SEM = Standard Error of Means

# The effect of dietary fish oil prior and during experimental dextran sulfate sodium challenge on clinical scores and colonic histopathology in grower pigs

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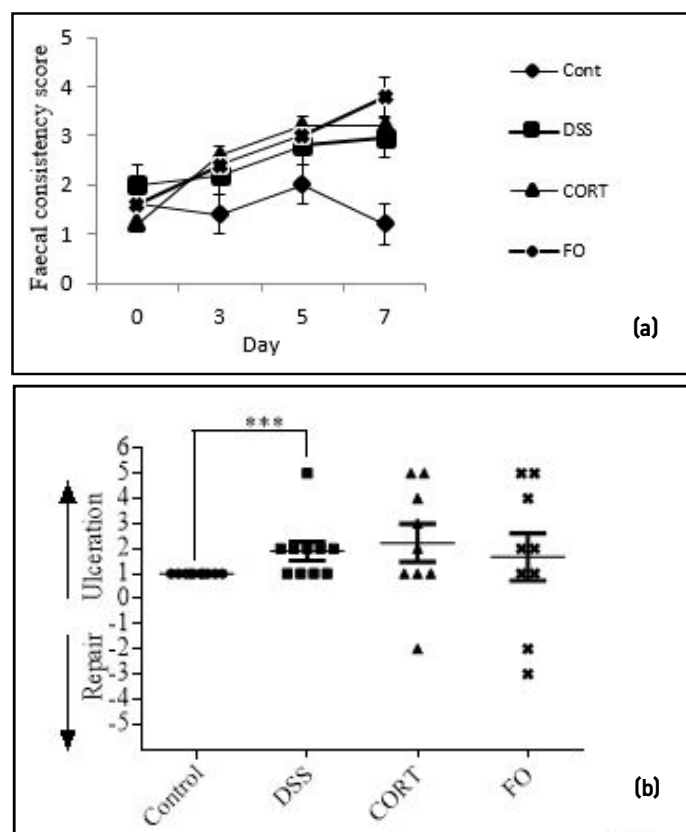
**Application** The pig is a potentially valuable model for human inflammatory bowel disease (IBD) and evaluating dietary supplements such as fish oil. Dextran sulfate sodium (DSS) elicits many of the features associated with IBD. In this study dietary fish oil exacerbated clinical scores and colonic inflammation associated with a DSS challenge in pigs.

**Introduction** Inflammatory bowel disease describes a family of debilitating gastrointestinal conditions. The incidence of diagnosis is increasing (Danese & Fiocchi, 2011) and partly attributed to environmental triggers including diet (Hou et al., 2011). Treatment options may include the use of glucocorticoids, however long term treatment with glucocorticoids is undesirable. The porcine large intestine is a potentially useful model to study inflammatory bowel disease-like conditions and evaluate prophylactic and therapeutic strategies. Oral gavage with dextran sulfate sodium (DSS) induces comparable features of inflammatory bowel disease in porcine colon including epithelial damage, goblet cell loss and immune cell infiltration (O'Shea et al., 2016). Fish oil contains greater amounts of polyunsaturated fatty acids (PUFA) relative to other oils and is reported to have immune-modulatory effects that may be beneficial for inflammatory conditions in the gastrointestinal tract. However the literature show conflicting reports and merits further investigation. The objective of this study was to evaluate the role of a dietary fish oil prior and during a DSS challenge on measurements of growth, faecal consistency, and colon pathology scores in pigs.

**Material and methods** Ethical approval was obtained from University College Dublin Animal Ethics Committee in accordance with Irish legislation (SI no. 534/2012) and the EU directive 2010/63/EU. Forty pigs weighing were assigned to 1 of 4 experimental groups and housed in pairs. Experimental dietary treatments were offered from day of weaning for 33 days until commencement of the DSS dosing period (d0) and throughout until conclusion of the study (d7); 1) Basal diet + distilled water (Control); 2) Basal diet + DSS (DSS); 3) Basal diet + DSS + corticosteroid; (CORT+DSS) and 4) Basal diet + DSS + Fish Oil (FO+DSS). Prednisolone (Prednidale; Dechra Veterinary Products), a glucocorticoid was co-administered with DSS in treatment 3 at 1 mg/kg bodyweight. Fish oil was supplied in treatment 4 at a concentration of 20g/kg at the expense of soy oil. The pigs were weighed on day of weaning, and during DSS-dosing (d 0, 3, 5, 7). Pairs of pigs were scored for faecal consistency from d 0 to d 7. On d 7 pigs were euthanized and a section of the proximal colon was stained with haematoxylin

and eosin for histopathological assessment. Data were analysed as a complete randomized design using the GLM procedure of SAS. Histopathological score data were analysed using Fisher's exact test with the FREQ procedure of SAS Least square means between experimental groups were compared using preplanned contrast statements as follows; 1. DSS vs. Control; 2. CORT+DSS vs. DSS group; and 3. FO+DSS vs. DSS group. The pig or a pair of pigs served as the statistical model.

**Results** The growth rate of pigs was 452, 184, 0.006 and 0.068 g/day for the Control, DSS, CORT+DSS and FO+DSS from d 0-7 respectively. The growth rate was lower ( $P < 0.05$ ) for the DSS versus Control. The CORT+DSS and FO+DSS were lower but not different when compared with the DSS group. Faecal consistency score and proximal colon pathology score are presented in Figure 1. DSS worsened faecal consistency and induced ulceration in the proximal colon. FO or CORT did not improve measured variables when compared with DSS



**Figure 1 (a)** Impact of treatments on faecal consistency where a score of 0 indicates solid, well-formed stools and 5 indicates severe diarrhoea & **(b)** histopathology score in the proximal colon of pigs.

**Conclusion** Pigs treated with DSS exhibited impaired growth rate, worsened faecal consistency and evidence of ulceration in the proximal colon. Therapeutic treatment with a glucocorticoid steroid or dietary exposure to FO did not alleviate DSS associated inflammation. **(a)**

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# Egg production and egg quality of Japanese quails fed diets containing varying levels of sun-dried cassava peel meal supplemented with honey as a replacement for maize

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A Twelve weeks feeding trial was conducted to determine the effect of feeding honey-flavoured sun-dried cassava peel meal diets on egg laying performance of Japanese quails. 120 six-weeks- old female Japanese quails were randomly assigned to four dietary treatments with three replicates of ten birds in a completely randomized design experiment. Four diets were formulated, Honey-flavoured sun-dried cassava peel meal replaced 0, 50, 75 and 100 % maize and tagged T1, T2, T3 and T4 respectively. Data were collected on hen-day egg production, hen-housed egg production, average egg weight, total feed intake, feed conversion ratio, cost of feed per kilogram, total cost of feed intake, cost per crate, average egg number and egg quality characteristics. All data generated were subjected to one way analysis of variance (ANOVA) using SPSS 2007,

Duncan Multiple Range Test was used to separate means where differences occurred. Results showed significant ( $p < 0.05$ ) differences among the treatment means for hen-day and hen-housed egg production with T2 recording significantly ( $p < 0.05$ ) higher values than the control. There were significant ( $p < 0.05$ ) differences among the treatment means for egg length, egg shape index, yolk weight, shell thickness and Haugh unit but no significant ( $p > 0.05$ ) differences were recorded for egg weight, egg width, yolk height, albumen weight and albumen height. Cost of producing 1 kg of T1 diets was expensive (N221.25) than the other treatments while cost producing 1 kg T4 diets was the least expensive (N145.2). Significant differences ( $P < 0.05$ ) occurred among the treatment means for total feed intake and total cost of feed intake with T2 recording higher cost than other treatments. It was concluded that Japanese quails responded positively sun-dried cassava peel meal supplemented with honey up to 50 % replacement level for maize without any adverse effects on hen-day, hen- housed egg production and egg quality parameters.

Parameters	T1	T2	T3	T4	SEM	LS
Average feed intake (g)	13.86c	18.80a	17.98b	18.08b	0.59	*
Hen-day production (%)	69.92c	85.79a	78.57b	72.90b	2.48	*
Hen-housed production (%)	49.82c	73.06a	66.33b	64.37b	2.58	*
Feed conversion ratio	1.22a	1.72b	1.77b	1.65b	0.07	*
Cost of feed per kg (N)	220.5a	185.6b	150.8c	115.8d	11.75	*
Haugh Unit	89.73a	89.28ab	89.37ab	88.94b	0.12	*
Shell thickness (mm)	0.22a	0.21b	0.20b	0.20b	0.002	*
Egg length (mm)	2.52b	2.61ab	2.72a	2.72a	0.03	*



# The effect of winning or losing an aggressive interaction on vocalisations in the domestic pig

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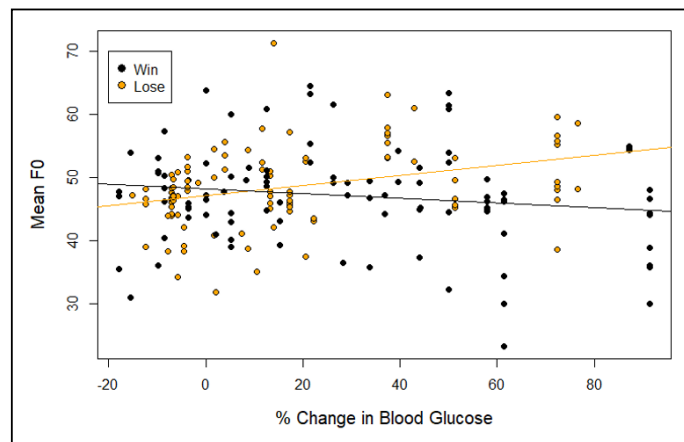
**Application** Vocalisations could be used as a non-invasive welfare indicator for commercial pig populations.

**Introduction** Vocal correlates of emotion indicate the emotional state of the caller, and may mediate social interactions. Physiological processes act on mammalian vocal anatomy to produce reliable vocal correlates of physiological state (Briefer 2012). We analysed domestic pig (*Sus scrofa domestica*) vocalisations following a staged contest, to test the association of 8 acoustic parameters with fight outcome, physiological fight costs, and aggressiveness as a personality trait.

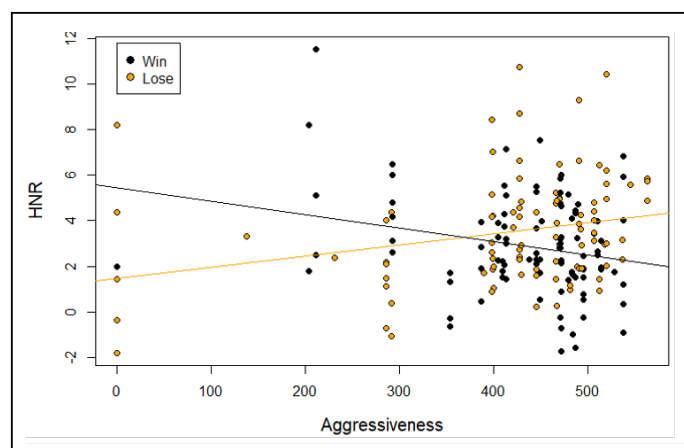
**Material and methods** The work on animals was approved by SRUC's Animal Ethics Committee (no. ED AE 21-2014) and the UK Government Home Office legislation (project licence PPL60/4330). A total of 80 male and female 9 week old pigs (Large White – Landrace sow x American Hampshire boar) were included in this study. Contests were staged between pairs of unfamiliar, juvenile pigs in an experimental contest arena. Contest dyads were balanced for sex and relative body weight. Weight ranged from 18kg to 39.4kg. Average contest length was 285 seconds; contests were stopped once a winner was identified. Acoustic recordings were made immediately following the contest while pigs were separated in holding pens. Recordings were processed and analysed in PRAAT software (Friel et al. 2019). Only grunt call types were analysed. Number of vocalisations analysed from each pig ranged from 0 to 11, with an average of 3 per pig. PRAAT analysis was used to measure seven acoustic parameters; fundamental frequency (F0), F0 variance, energy quartiles q25, q50 and q75, harmonic to noise ratio (HNR) and call duration. Blood samples were collected immediately pre and post contest from an ear vein, to investigate proportional change in blood glucose. Aggressiveness was estimated using the resident-intruder test as per Camerlink et al. (2015). The resident-intruder score was subtracted from the maximum attack latency to calculate an aggressiveness score.

Data were analysed using linear mixed effect (LME) models with contest outcome, change in blood glucose, aggressiveness, weight pairing, sex pairing, and the presence of an observer as fixed effects. Pig identity was included as a random factor to control for repeated observations of individuals. Interaction terms between contest outcome and aggressiveness, and contest outcome and percentage change in blood glucose were initially included in all models, but subsequently removed if not significant.

**Results** A total of 97 'winner' and 102 'loser' vocalisations were analysed. Vocalisation structure showed no direct association with fight outcome, but there was a significant interaction between fight outcome and physiological fight costs, and aggressiveness. There was an interaction effect between fight outcome and change in blood glucose on mean F0 (LME  $p = 0.01$ ,  $t = -2.63$ ,  $SE = 0.044$ ,  $est = -0.11$ ,  $df = 39$ ). Contest losers with a higher change in blood glucose had higher mean F0, while contest winners with a higher change in blood glucose had lower mean F0.



**Figure 1:** The interaction effect between fight outcome and change in blood glucose on mean F0, including linear trend lines. There was also an interaction between fight outcome and aggressiveness on harmonic-to-noise ratio (LME  $p = 0.006$ ,  $t = -2.80$ ,  $SE = 0.003$ ,  $est = -0.010$ ,  $df = 85$ ). Contest losers with higher aggressiveness scores produced vocalisations with higher tonality, while contest winners with higher aggressiveness score produced vocalisations with lower tonality.



**Figure 2:** The interaction effect between fight outcome and aggressiveness on harmonic to noise ratio, including linear trend lines.

**Conclusion** These results suggest that winner and loser vocalisation structure are affected differently by physiological fight cost and aggressiveness. Further research is required to elucidate how this relates to emotional state. These findings highlight the importance of accounting for recent experience and individual personality traits, when investigating vocalisations.

**Acknowledgements** This research was funded by the Biotechnology and Biological Sciences Research Council (BBSRC). SRUC receives financial support from the Scottish Government. We are also grateful to Irene Camerlink and Marianne Farish for their help with practical data collection.

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# Can supplementary magnesium reduce stress and aggressive behaviours in pigs during mixing?

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**Application** Supplementary dietary magnesium may be an effective way to improve pigs' welfare, health and overall production performance by reducing stress and aggressive behaviours.

**Introduction** Commercially farmed pigs experience stressful events throughout their lifecycle, for example weaning, mixing, transport and slaughter stress. Stress and aggressive behaviours are detrimental to the pig's welfare as well as performance and production (McLamb, et al., 2013; Hambrecht, et al., 2005). In industry, supplementary dietary magnesium is sometimes added to pig feed to try and alleviate an outbreak of aggression or stress (O'Driscoll, et al., 2013). However, the scientific evidence to support this strategy has mixed results. Therefore, the main aim of this study was to investigate whether supplementary dietary magnesium may be an effective technique to reduce stress and aggression during a mixing event. Phytase was also included in this study as previous research has shown that it may improve the uptake and bioavailability of magnesium (Rimbach, et al. 2008).

**Method:** 240 pigs were weaned at 28 days of age and assigned to pens of five balanced for sex, origin litter and weaning weight. Two focal pigs per pen were selected based on weight to ensure there was a high and low weight focal pig in each pen of five (96 focal pigs). All animals received a standard dietary regime for the first 20 days post weaning before beginning one of four trial diets; (A) control, (B) magnesium phosphate (0.15%), (C) phytase (0.03%) or (D) both magnesium phosphate (0.15%) and phytase (0.03%). After two weeks on the trial diets, mixing occurred resulting in pens of 10. Pig and feed weights were recorded on the first day of the trial diets, the day before mixing and the end of the trial, to enable the calculation of performance parameters. Each focal pig was lesion scored on a weekly basis using a five point scoring system (Stevens, et al. 2017). Saliva from each focal pig, as well as pooled pen faecal samples were collected for two consecutive days before the trial diet began (for the saliva the first day was for habituation). Following this, samples were collected the week before, day after and week after mixing. Both saliva and faecal samples were analysed for cortisol using ELISAs.

**Statistical Analyses:** All analysis was carried out using Rstudio. Lesion scores were separated into tail score, ear score (sum of both ears) and body score (sum of all main body areas: left and right flank, hindquarters, shoulders and back). Friedman tests were used to assess body score, ears score and tail score in relation to a diet and time point (before or after mixing). Two-way ANOVAs were used to analyse performance data (average daily gain, average daily feed intake and weight). Salivary cortisol was analysed using a general linear model. Faecal samples are currently being analysed.

**Results** Body, ear and tail lesions were significantly higher after mixing ( $P < 0.01$ ). Pigs receiving the phytase diet (C) had significantly more body lesions than both the magnesium diets (B -  $P = 0.009$ ; D -  $P = 0.011$ ). There was no significant difference between diets for the number of ear and tail lesions. Dietary treatment did influence the average daily gain (ADG) with the phytase diet having a significantly higher ADG in comparison to the control ( $p = 0.041$ ) and magnesium diet ( $p = 0.007$ ). There was no significant difference between the diets in salivary cortisol level. Faecal cortisol results to be determined.

**Conclusion** Overall supplementary magnesium may have some welfare benefits for commercial pigs during stressful events.

**Acknowledgements** This work was funded by BBSRC and Primary Diets formulated and supplied the feed.

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# Real-time lactate concentration analysis in pig faeces as a potential gut health indicator

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**Application** Measuring real-time lactate concentrations in pig faeces as a potential indicator of gut health.

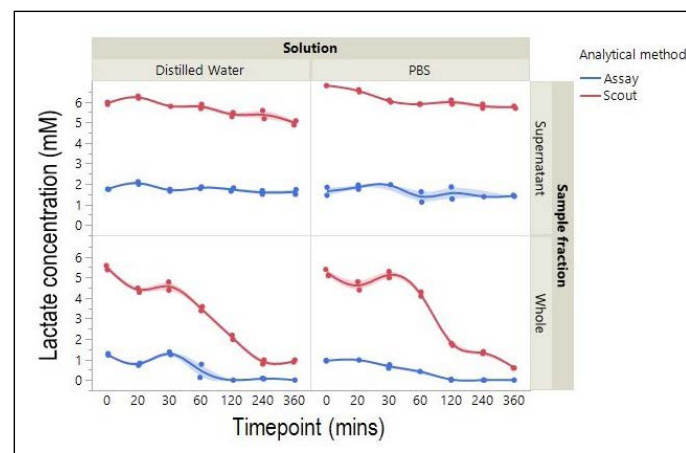
**Introduction** Lactate is the major fermentation end-product of lactic acid producing bacteria such as *Lactobacilli*. Bacterial species such as *Eubacterium hallii* utilise lactate & produce butyrate which is deemed beneficial for gut health, highlighting the importance of lactate in bacterial cross-feeding when assessing butyrogenic effects. Lactate is a metabolic intermediate that is quickly utilised by bacteria, thus is seldom detected in the faeces of healthy subjects or at low concentrations (<3mM) (Hove, et al., 1994). However, in sufferers of ulcerative colitis & other gastrointestinal disorders, faecal lactate concentrations up to ~100mM have been detected (Hove et al., 1994), suggesting lactate accumulation & inability of the bacterial community to utilise it at a sufficient rate. This posed the question as to whether the detection of faecal lactate accumulation could be an indicator of bacterial community imbalance & a potential gut health indicator. The UK pig industry is under increased pressure to reduce antimicrobial usage, thus research into potential non-invasive markers of gut health status is timely. It is however unknown if lactate can be detected in pig faeces & how quickly it is metabolised by faecal bacteria. This pilot trial aimed to establish a method to detect lactate in pig faeces & investigate how quickly a known concentration of lactate would be utilised by faecal bacteria.

**Material & Methods:** A faecal sample was taken from a 96kg finisher pig. Faeces was immediately aliquoted (2.5g) into either 10ml of distilled water (n=16) or 10ml of phosphate buffer solution (PBS; n=16). These samples were equally split between two sample fraction groups 'Whole' or 'Supernatant'. Lactate concentration was measured at 7 timepoints; 0, 20, 30, 60, 120, 240 & 360 mins. 'Whole' samples (Water, n=8; PBS, n=8) were spiked with 5mM of lactate at 0 mins. At each subsequent timepoint, the sample was centrifuged for 5 mins at 2000xg, supernatant was collected & immediately frozen on dry ice. 'Supernatant' samples (Water, n=8; PBS, n=8) were first centrifuged for 5 mins at 2000xg & then supernatants were collected & spiked with 5mM of lactate. Samples were immediately frozen at each experimental timepoint on dry ice. Lactate concentrations were measured in duplicate at each timepoint using two analytical methods;

firstly, a handheld Lactate Scout device (SCT) was used to measure real-time lactate concentrations immediately prior to freezing. After thawing, lactate concentrations were also measured using a colorimetric assay (Biovision-K627-100) following deproteinisation. Data were analysed using a repeated measures mixed analysis (JMP-v14).

**Results** There was a significant three-way interaction between timepoint, sample fraction & analytical method (Figure 1;  $P < 0.05$ ). At 0 mins, the SCT device correctly measured the spiked lactate concentration (~5mM) in both the 'Whole' and 'Supernatant' samples, however the assay measured concentrations 4 times lower at the same timepoint in both 'Whole' and 'Supernatant' samples. Lactate concentration in the 'Whole' samples decreased to ~0.76mM over 360 mins when measured by the SCT device, and was undetectable after 120 mins with the assay. Lactate concentration in the 'Supernatant' samples remained constant over time around the initial spiked level when measured by both the SCT and assay. There was no significant effect of solution type on lactate concentrations ( $P > 0.05$ ).

**Figure 1:** Lactate concentration over time.



**Conclusion** The assay failed to measure 5mM of spiked lactate, suggesting elements within the faecal matter interfered with the assay despite sample deproteinisation. The SCT results showed that when faecal bacteria remained in contact with lactate in the 'Whole' samples, the majority of lactate was utilised by 360 mins, however, when lactate was added to the 'Supernatant' samples its concentration did not decrease over time suggesting that centrifugation removed the majority of lactate-utilising bacteria. PBS did not stop the bacterial utilisation of lactate. In summary, the SCT is a suitable real-time method for measuring lactate concentrations compared to the colorimetric assay which failed to measure the spiked lactate concentration in the presence of faeces. The process of collecting faeces for lactate analysis must be rapid & the use of a buffer to halt microbial activity may be required to ensure true values are obtained. Further research & validation is required.

**Acknowledgements** AB Vista funding & EKF-Diagnostics (SCT).

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# What is the current significance of compromised pigs on commercial farms in N.I. in terms of mortality and impaired performance?

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**Application** This study found that on commercial farms in NI the average growth rate of low birth weight pigs was 64g/day lower than that of average birthweight piglets. Furthermore the most common cause of death for low birthweight pigs was starvation and most common stage of death was during lactation. This data will help science and industry at large understand the profile of compromised piglets on commercial farms and lead to strategies to maximise their value.

**Introduction** Increases in litter sizes within commercial pig production have led to elevated numbers of low birthweight pigs with higher pre-weaning mortality, lower weaning weights and reduced lifetime performance (Fix et al, 2010). However an accurate measure of how these animals perform on commercial farms in comparison to heavier littermates in terms of growth performance, mortality and weight at slaughter age using current data is lacking. This study aimed to assess the growth of low and average birthweight piglets from birth to slaughter as well as establish the most common time and cause of death for both weight categories.

**Material and methods** This study followed the performance of 328 low birthweight (Low BW; <1kg) and 292 average birthweight (Av BW; 1.25-1.75kg) piglets across 4 commercial and 1 research farm from birth to slaughter. All farms complied with Red-Tractor Assurance Pig Standards. Normal management practice was not interfered with. Each animal was individually weighed at birth and assigned a numbered ear tag for individual identification. Pigs were weighed at 4, 8, 12, 17 and 22 weeks of age. Parameters of animal origin (e.g. litter size, birth mother) and management (e.g. pen size, diet) were recorded throughout production. All animals which died during the study had a date of death and weight recorded, and all post-weaning mortalities underwent post-mortem analysis.

**Statistical analysis** Data was analysed as a linear mixed model using a REML estimation method. Birth mother parity, number of piglets born alive in each litter, number of still born piglets in each litter, gender, fostering and total litter size were fitted as fixed effects while farm number and birth-mother ID were fitted as random effects.

**Results** Birth mother (BM) parity, born alive (BA) in litter or still born (SB) in litter had no significant effect on performance or mortality ( $P>0.05$ ). As expected, at weaning Av BW pigs were heavier than Low BW piglets, with a difference of 1.16kg (Table 1). This weight differential increased to 5.74kg by 12 weeks of age and 9.08kg by week 22. Animals which had been cross-fostered were significantly lighter at weaning (7.0kg vs 7.5kg), 8 weeks (17.0kg vs 17.9kg) and 12 weeks (33.3kg vs 35.3kg) of age. However this may have been influenced by the majority of cross-fostered animals being of Low BW (57.2% vs 42.8%). Pre-weaning mortality of Low BW pigs was over three times greater than for Av BW pigs, with post-weaning mortality also significantly greater for the Low BW animals. The average age of death was also significantly lower for Low BW pigs. Starvation and sows lying on piglets were the major causes of pre-weaning mortalities in Low BW animals accounting for 49% and 28% of deaths respectively. With Av BW pigs, 30% of pre-weaning mortalities were unknown, with a further 22% of deaths due to over-lying by the sow and 13% caused by scouring. No clear differences were identified in the causes of post-weaning deaths between Low BW or Av BW pigs.

**Table 1** Effect of birthweight on animal mortality and performance.

	Low BW	Av BW	SEM	BW (P Value)	Sex (P Value)	Fostered (P Value)
Birth weight	0.92	1.51	0.008	<0.001	0.861	0.231
4 week weight	6.76	7.92	0.149	<0.001	0.015	0.01
8 week weight	15.71	19.11	0.313	<0.001	0.006	0.036
12 week weight	31.58	37.32	0.554	<0.001	<0.001	0.022
17 week weight	58.24	65.72	0.909	<0.001	<0.001	0.266
22 week weight	91.71	100.79	1.193	<0.001	0.714	0.717
Age of death (days)	29.55	47.08	7.68	0.024	0.422	0.109
Pre-wean mortality (%)	20.89	6.08	-	<0.001	0.531	0.072
Post-wean mortality (%)	11.6	7.91	-	<0.001	0.271	0.576

**Conclusion** This work quantified the inferior performance of Low BW compared to Av BW pigs on a number of commercial farms in Northern Ireland. Compromised animals weighed almost 10kg less at slaughter age. Whilst cross-fostering was associated with reduced weaning weights and post-weaning growth this may have been due to the majority of fostered pigs being of low birth weight. Death of Low BW pigs occurred significantly earlier in production. Furthermore, as starvation accounted for almost half of the pre-weaning mortalities amongst Low BW animals, improving lactation nutrition should be the primary focus of intervention strategies which aim to minimise the negative impact of these animals on herd performance and profitability.

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# Essential Oils Can Improve Pig Gut Health Following Ileitis Challenge

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**Application** Oregano Essential oil was shown to be a promising natural supplement to support pig health following ileitis challenge.

**Introduction** With increasing pressure on the pig industry to reduce the use of anti-microbials whilst improving animal performance and welfare, there is a growing interest in sustainable alternatives. oregano (*Origanum vulgare*) contains the compounds carvacrol, thymol, -terpinene and -cymene, which possess both anti-microbial and anti-oxidant activity (Gheisar and Kim, 2018) which may confer benefits in times of dysbiosis. Ileitis, or proliferative enteritis is primarily caused by *Lawsonia intracellularis* and represents an economic risk to ~95% of swine herds (Armbruster et al., 2007) with either sub-clinical performance loss or clinical signs of diarrhoea and acute outbreaks that can result in sudden death. This trial aimed to investigate the benefits of using a commercially available oregano essential oil (OEO) product in combination with the standard vaccination program to see if there were any improvements in health and performance of pigs under ileitis challenge.

**Material and Methods** The study was conducted with SSUI (Swine Services Unlimited Inc., USA) to evaluate the potential benefits of an OEO product alongside a current ileitis vaccination program. All piglets were vaccinated with modified-live Enterisol® Ileitis vaccine (Boehringer Ingelheim Vetmedica, Georgia, USA) at 6 weeks of age by oral gavage. The OEO was provided in the feed (5% OEO on an inert carrier (Orego-Stim®, Anpario plc, UK). A total of 96 high health LW x LR piglets were enrolled onto the study day 0, at approximately 28 days of age (weaned at 21 days of age and fed standard starter diet for 1

week) and randomly allocated, 8 slatted pens of 4 pigs (blocked for gender and body weight) to 1 of 3 dietary treatments: Control, no additives; OS1, OEO included at 0.5kg/t throughout; OS2, OEO included at 1kg/t throughout. All pigs were given Enterisol® Ileitis vaccine at 6 weeks of age (day 14), and at 9 weeks of age (day 35) were challenged orally with ~1x10<sup>9</sup> *Lawsonia intracellularis* total dose per pig. At 13 weeks of age (day 67) all pigs were euthanized and necropsied for gross lesion scores in ileum, jejunum, cecum and colon, on the basis of the severity of mucosal thickening (0-normal, to 4-severe). Clinical scores 2 to 4 were considered indicative of clinical ileitis. Faecal scores and performance data were measured throughout the trial. Faecal consistency was scored on a scale of 0 to 3, with abnormal results considered to be those of 2, mild diarrhoea or 3, profuse projectile diarrhoea. All data was analysed using JMP®, Version 13. SAS Institute Inc., Cary, NC, 1989-2019.

**Results** The inclusion of OEO (OS2) reduced the risk of pigs exhibiting lesions, evaluated by relative risk analysis, (rr=0.58) when compared with the vaccinated control (Fig. 1). OEO (at both inclusions) also lowered the risk of abnormal faecal scores, evaluated by relative risk analysis, (rr=0.91) (Fig. 2), thereby reducing clinical signs of damage following ileitis challenge. ADG was numerically improved by the addition of OEO following ileitis challenge compared to the vaccinated control group (Fig. 3). Mortality was not significantly affected following ileitis challenge.

**Conclusion** The addition of oregano essential oil showed the ability to improve performance and gut health of pigs challenged with *Lawsonia intracellularis* when used in conjunction with vaccination. These benefits are interesting and warrant further investigation to understand the possible mode of action.

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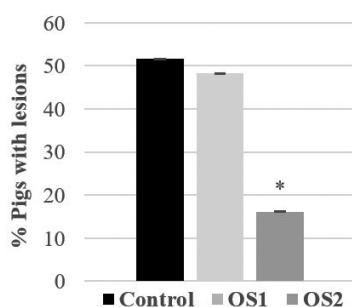


Fig.1 Percentage of pigs showing gross lesions, \*denotes reduced relative risk. With S.E bars

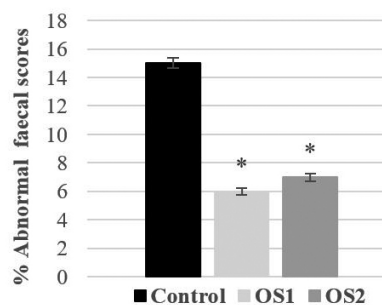


Fig. 2 Percentage of pigs with abnormal faecal scores. \*denotes reduced relative risk. With S.E bars

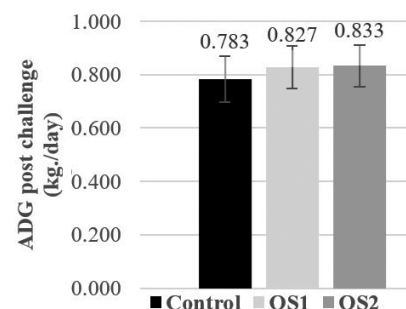


Fig. 3 Average daily gain (ADG), following challenge, from day 35-67. With S.E bars



# Genotypic characteristics of antimicrobial resistant *Escherichia coli* isolated from the caecum of broiler chickens

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**Application** The *E. coli* isolated from older (29-35 d old) birds are of two main multilocus sequence types which carry the *mdfA-1* gene but are otherwise not resistant to antibiotic. If their prevalence in the chicken caecum could be encouraged at a younger age, it may make the young (8 d old) bird less susceptible to antibiotic resistant disease.

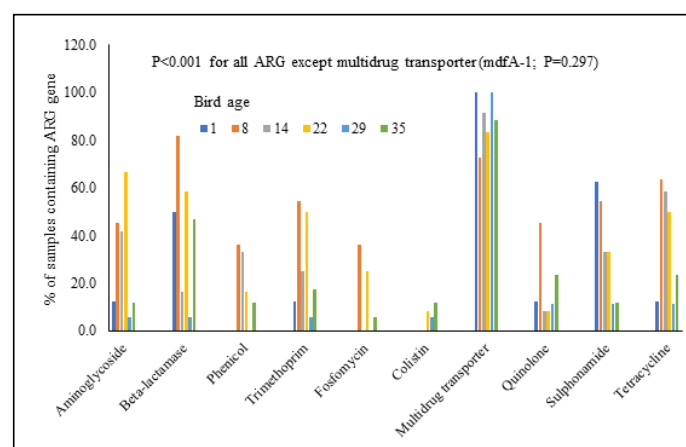
**Introduction** The growing prevalence of antimicrobial resistance (AMR) by bacteria is a concern for both human and animal health. *Escherichia coli* (*E. coli*), found in the intestinal tracts of all animals and birds, has been widely used as a sentinel species for the determination of AMR. Probiotics such as *Lactobacillus* sp. and yeasts may help prevent the proliferation of pathogenic bacteria and potentially AMR in the gut through mechanisms such as competitive exclusion, neutralization of toxins, bactericidal activity or enhanced immune competence. The aim of this experiment was to determine the effect of a yeast (*Candida famata*) and a bacterium (*Lactobacillus plantarum*), administered in the drinking water, on the prevalence of AMR status of *E. coli* throughout the life of broiler chickens through whole genome sequencing (WGS).

**Material and methods** Male, day-old chicks (Ross 308) were used and *Candida famata* (isolated from a chicken) or *Lactobacillus plantarum* (isolated from a pig) was administered via the drinking water. Water was provided either untreated (CON) or with *C. famata* (CF; 108 CFU/ml) or *L. plantarum* (LP; 105-108 CFU/ml) in water hoppers on two days each week for 35 d. Birds were sacrificed every week and samples of caecal digesta were taken. 87 separate isolates of *E. coli* taken from caeca throughout the study were isolated and their genomic DNA extracted using Pure-gene yeast/bact Kit B (Qiagen, Venlo, Netherlands) and standard protocols for fresh samples of Gram-negative bacterial cultures were followed. All extracts were submitted to Quadram Institute Bioscience to determine the genotype of individual *E. coli* isolates by whole genome sequencing (WGS). Antibiotic resistant genes (ARG) were identified by reference to the Resfinder database. The percentage of isolates carrying different ARG was calculated, and the effect of bird age and treatment determined using the Chi squared test.

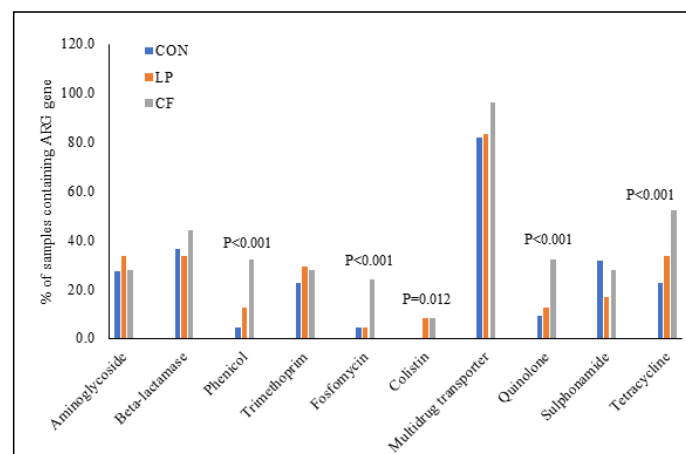
**Results** The profile of ARG in *E. coli* isolates was significantly affected by bird age (Figure 1), with ARG generally declining after 8 d of age. Administering yeast was associated with an increase in the carriage of ARG for phenicol, fosfomycin, quinolone and tetracycline,

while ARG for colistin was increased when either *Candida famata* or *Lactobacillus plantarum* was added (Figure 2) Nearly all samples isolated carried the multi drug transporter gene *mdfA-1*, and this was not affected by bird age or treatment. -lactamases (primarily *blaTEM*), were isolated from 80% of samples at 8 d, but 47% of samples at 35 d. Tetracycline resistance genes (*tetA*, *tetB* and *tetJ*) were present in 64% of isolates at 8 d and 24% of isolates at 35 d. A total of 20 different multilocus sequence types (MLST) were identified, with a further 19/78 isolates being unknown. At 1 d old, most isolates were MLST 48, and this was the most common type (14/78) throughout the study and was isolated from birds throughout their life. Greatest diversity was observed when birds were 8-22 d old; when birds were 29 and 35 d old the most common isolates were MLST 48 (20% of isolates) and 1665 (26% of isolates). These isolates carried the *mdfA-1* gene, but carried no other ARG.

**Figure 1.** Effect of bird age on the presence of ARG for different antibiotic classes in *E. coli* isolates



**Figure 2.** Effect of treatment on the presence of ARG for different antibiotic classes in *E. coli* isolates



**Conclusion** Birds are most susceptible to colonization with antibiotic resistant strains of *E. coli* at the end of the starter phase (around 8 d old). In the absence of antibiotic exposure, the carriage of ARG by *E. coli* in the chicken caecum declines through the grower and finisher phases.

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# Is it just about grazing? UK citizens have diverse preferences for how dairy cows should be managed

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**Application** Grazing, comfort, and health and welfare were equal top priority for dairy cows in a survey of UK citizens who ranked a range of options using choice 'trade offs'. However, six characteristically distinct groups within the sample ranked preferences differently. Follow-up interviews confirmed that complex rationales and varying concepts of 'naturalness' underpinned the range in people's choices. These findings indicate diversity in preferences and understanding about how cows are managed, and opportunities to address the current disconnect between dairy farming and its different publics through improved communication, marketing, or changes to farming systems.

**Introduction** Conflicting views between the dairy industry and its publics about how dairy cows should be managed (Cardoso et al., 2018), alongside a rise in availability of dairy alternatives and criticism over welfare and climate change concerns, challenge future markets for milk producers. Publics value animal welfare as well as naturalness and grazing (Lusk and Briggeman, 2009; Bazzani et al., 2016) but the relative importance of specific aspects of management and the reasons for these preferences are yet to be established.

**Material and Methods** 2,054 UK citizens were asked through an online survey to rank 17 attributes relating to dairy cow management and milk production using the novel application of 'best worst scaling', a discrete choice methodology that allows a trade-off between items (Louviere and Woodworth, 1991; Finn and Louviere, 1992). Follow-up qualitative interviews on a subset of participants identified the rationales underpinning different choices.

**Results** Hierarchical Bayesian analysis of survey results revealed (i) access to grazing; (ii) cow health and welfare; and (iii) cow comfort were of equal top importance. However, six citizen groups within the sample, identified through latent class analysis, expressed significantly different priorities. Each group had different indicative characteristics, as established through a multinomial logistic model. Despite this heterogeneity, interviews confirm wide underlying support for concepts of a natural life, grazing and outdoor access, but with overlapping and conflicting definitions.

**Conclusion** Being outdoors and the physical act of grazing are seen as the natural way to keep dairy cows despite resignation that other systems exist. If the sample reflects the wider population, there may be opportunities for the dairy industry to improve communication about positive practices, develop new dairy product markets, and/or consider changes to dairy farming systems to better meet different citizens' needs.

**Acknowledgements** The authors gratefully acknowledge funding from AHDB.

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# Encouraging farmer change; does cognitive dissonance impact adoption of best-practice recommendations for sheep lameness control?

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**Application** Understanding farmer-centric barriers to best-practice uptake in UK sheep flocks is essential to secure adoption of optimal management practices when controlling lameness. Cognitive dissonance appears to affect decision-making.

**Introduction** Significant advances have been made in our understanding of lameness treatment and control in sheep, particularly footrot which accounts for 70% of lameness in English flocks (Winter et al. 2015). Best practice recommendations have evolved in-line with research; foot trimming, perceived as a mainstay in traditional sheep husbandry, is now ill-advised due to infection risk and permanent hoof damage (Winter et al. 2015; Kaler et al. 2010). Despite this, in 2015, 56% of farmers reported to trim lame sheep for treatment (Reeves et al. 2019). Understanding why farmers maintain suboptimal traditional practices is integral to facilitating best-practice uptake. Continuing to perform deep-routed behaviours could be explained by cognitive dissonance; when an individual experiences conflict between beliefs, attitudes or behaviour, leading to inconsistency between what the individual knows and how they act (Festinger, 1961). The aim of this study was to explore foot trimming practice in UK flocks; apply cognitive dissonance theory to farmer behaviour and better appreciate decision-making processes.

**Material and methods** A survey was utilised to investigate lameness management. Eligibility to complete the survey was restricted to UK sheep farmers; sampled voluntarily through self-selected sampling. The survey (paper and online) was administered by social media, email, newsletters and via UK industry stakeholders. Survey participants could consent to a follow-up interview. Principles of Grounded Theory analysis were applied to the data collection and analysis of twelve qualitative interviews. Telephone or face-to-face semi-structured qualitative interviews were conducted, using a standardised and contextualised interview guide. Interviews were audio-recorded and transcribed verbatim. Farmers were initially convenience sampled, with later interviewees selection by theoretical sampling, characteristic of Grounded Theory, facilitating theoretical category emergence from the continuous, iterative

process between analysis and data collection. Survey data was checked for duplications, cleaned and coded as appropriate for statistical analysis. Descriptive statistics using Pearson Chi-Squared tests were performed in Genstat (VSN International, UK). Thematic analysis of interview transcripts was conducted in NVivo 12 Plus (QSR International).

**Results** In total, 532 UK sheep farmers completed the survey. Almost a fifth (19.9%,  $n = 106/532$ ) of farmers routinely foot trimmed, on average trimming, twice annually (95% CI: 1.8-2.3). Pedigree flocks ( $p < 0.001$ ) and those with  $\leq 250$  ewes ( $p < 0.001$ ) were more likely to routinely trim. Nearly half (46.8%,  $n = 249/532$ ) of farmers would trim misshapen claws without signs of active infection and 51.3% ( $n = 273/532$ ) would trim when treating lame sheep. Commercial flocks ( $p < 0.001$ ) and those with  $> 250$  ewes ( $p < 0.001$ ) were more likely to trim lame sheep. Farmers aged  $\leq 35$  years were more likely to trim lame sheep than older farmers ( $p = 0.013$ ). Interview thematic analysis indicated 1) farmers are knowledgeable of best-practice recommendations relating to avoiding routine trimming and trimming lame sheep, but 2) make an informed decision to continue with this practice. Farmers expressed reluctance to leave misshapen feet; a major influence in deciding to trim regardless of lameness status. As traditional practice, foot trimming was habitually performed, which farmers found difficult to discontinue.

**Conclusion** Despite best-practice recommendations, farmers routinely and therapeutically foot trim. Routine trimming is conducted by smaller-scale flocks, but trimming lame sheep is most associated with large, commercial flocks. Younger generation farmers are more likely to trim lame sheep, indicating that best-practice recommendations are not influencing new entrants into the farming industry. Cognitive dissonance may impact decision-making, affecting the uptake of best-practice, where suboptimal behaviours, such as foot trimming, are still favoured and performed, despite the negative implications on foot health, recovery rates and time.

**Acknowledgements** With thanks to Perry Foundation, MSD Animal Health and Harper Adams University for funding.

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# The vicious circle of vaccines updates I: Deeper insights into the intrinsic limitations in veterinary vaccines outcomes (an article review)

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**Application** The improvement of the vaccine's design has global importance. Developing of newly designed vaccines would help in better combating either the never-ending deluge of outbreaks or vaccines' potency in the veterinary field.

**Introduction** Vaccines are worthy to be considered as one of the greatest breakthroughs in the history of biological science. They had offered us a preparedness state, and vaccinated hosts became more protected against pathogens that were once terrifying. However, vaccines seem to be losing their pushing force with several viruses that keep a stagnant situation with no actual improvement for their field situation. For that, we preview the intrinsic limitations of vaccines that cause impediments to their outcome leading to the eventual need for an update. Such intrinsic limitations are inherent within the vaccine context, however, they may be less apparent in the experimental conditions due to utilizing the conventional parameters. Thus, intrinsic limitations in the vaccine protective activity were fully discussed and explained.

**Intrinsic limitations** There are some conventional intrinsic limitations to be described in any vaccine's shortcomings. For instance, live attenuated vaccines hold the potential of reversion to virulence, while inactivated vaccines hold the risk of the inadequate immune response (Vetter et al., 2018). Moreover, losing antigenic integrity has been observed in many cases that reflecting on the efficient immune response which elicited by a specific vaccine depends on the generation of memory pool of B and T cells toward the vaccine antigens and in turn their corresponding pathogen antigens. Thus, any deviation for the antigenic profile between the vaccine and original pathogen would entail differences in the immune response. Several issues could influence the antigenic profile of the vaccine including inactivation step (either by formaldehyde or beta-propiolactone), antigen/adjuvant interactions, and/or different folding/post-translational modifications due to expression in a different host system.

Furthermore, immunosuppressive domains could implicate this limitation. It would be noticed with the LAVs where the original virus poses immunosuppressive domains however they did not lose their effect during the random attenuation process. For instance, recent findings indicated the ability of IBV for inhibiting nitric oxide (NO) production; which is a key player for several antimicrobial functions (Amarasinghe et al., 2017). Therefore, if the IBV-LAV did not suffer mutations in its' immunosuppressive domains during attenuation, then the vaccine would preserve the immunosuppressive effect leading to a compromised immune response to the same vaccine or other field infections. From this perspective, the intensive usage of live attenuated vaccines would be worrisome and need further revisiting for their evaluation parameters.

Finally, immunodominance (ID) plays one of the most significant roles in immune evasion (Angeletti and Yewdell, 2018). The immunodominant epitopes are mostly the more abundant and more accessible on the intact virion that matches with the globular head epitopes (Victoria and Wilson, 2015). This generates an antigen hierarchy in which the immunodominant epitopes seize most of the immune response and form the vast majority of the B and T cell repertoire pool (Angeletti et al., 2017). Unfortunately, the surface globular head proteins are more likely to be hypervariable regions, so as the viral drift antigenically the protection would diminish (Mallajosyula et al., 2015). This limitation could be observed with all the current commercially utilized vaccine platforms including IVs, LAVs, recombinants, and/or split vaccines.

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# Human- animal relationships: An investigation using housed pregnant dairy and beef origin suckler heifers

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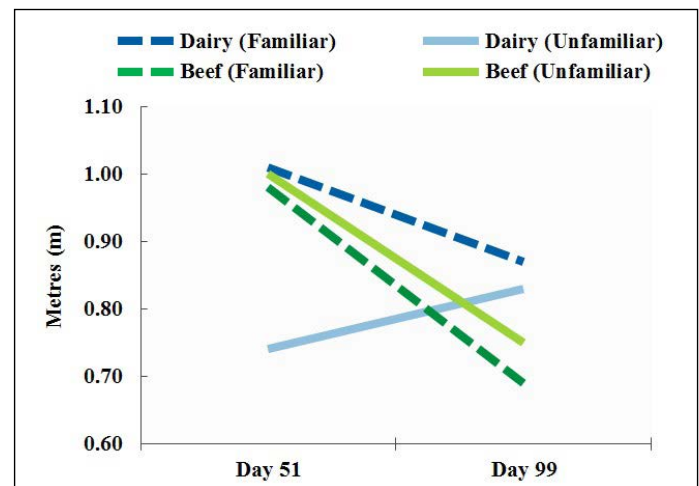
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**Application** The avoidance distance test is an accessible measure of the human-animal relationship of housed pregnant dairy and beef origin suckler heifers.

**Introduction** The human-animal relationship (HAR) can be defined as the perception between the human and the animal, which develops and expresses itself through their mutual behaviour. The quality of the HAR is fundamental to the safety of handlers and the welfare of animals. The level of fearfulness of animals is determined by the experiences the animal has gained, in association with their individual genetic disposition. The objective of this study was to assess the fear/HAR responses over time in housed pregnant suckler heifers using three behavioural tests; crush agitation (CA), exit speed from the crush (ES) and avoidance distance at the feed face (AD). **Material and methods** During routine weighing and body condition scoring the CA and ES of dairy (n=20) and beef (n=43) origin housed pregnant heifers were recorded consecutively, on days (d) 43 and 86 post-housing. The CA was scored using the methods of Stockman et al. (2012) by the same observer at both time-points. The ES was obtained from videos which were recorded as animals exited the crush. A score was assigned to each animal based on latency time (seconds) and distance (metres, m) travelled to join peers. On d 51 and 99 post-housing, AD was measured by both a familiar and an unfamiliar human. The human, positioned two meters from the feed face, approached the animal slowly with their left arm raised laterally from the hip, at a 45 degree angle. The withdrawal distance of each animal was recorded using a laser distance measure. If the animal did not withdraw, it was recorded whether or not the animal could be touched by the human. If the animal remained at the feed face and was touched by the human, an AD of 0 m was assigned. If the animal withdrew from the feed face as the human attempted to touch them, an AD of 0.05 m was assigned. Data were statistically analysed using PROC MIXED; the model included the fixed effect of breed, with time as a repeated measure (SAS 9.4). Results are presented as means (+/- SEM).

**Results** No difference ( $P>0.05$ ) was found between dairy and beef origin heifers in CA and ES at either time point. Mean (SEM) AD to the unfamiliar human was greater for beef than dairy-origin heifers on d 51 (1.00 (0.048) v. 0.76 (0.085) m,  $P=0.01$ ), but there was no difference ( $P>0.05$ ) between the breed types on d 99 (0.75 (0.081) v. 0.83 (0.130) m,  $P>0.05$ ). In contrast, on d 51 AD to the familiar human was similar for both beef and dairy origin heifers (0.98 (0.069) v. 1.01 (0.080) m,  $P>0.05$ ) whereas on d 99 it was lower in beef than dairy-origin heifers (0.69 (0.073) v. 0.87 (0.097) m,  $P=0.02$ ).

**Figure 1** The AD (m) for dairy and beef origin heifers for familiar and unfamiliar humans on d 51 and 99 post-housing.



**Conclusion** Beef-origin heifers habituated to both the familiar and unfamiliar humans, whereas the dairy-origin heifers only habituated to the familiar human, over time (Figure 1). From the three investigated tests, AD proved to be a more sensitive measure of the HAR and how cattle perceive humans, whether they are familiar or unfamiliar.

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# Sire Influence on hatchability and heritability estimates of Quails in a humid tropical environment

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**Application** Quails are generally reared for egg and meat production. Information on sire influence relating to hatchability and heritability estimate of Japanese quails found in Nigeria is comparatively scanty.

**Introduction** The Japanese quail was brought to Nigeria in 1992 [NVRI, 1994]. The young male begins to crow at 5-6weeks old. Males also have cloacal glands, a bulbous structure located at the upper edge of the vent which secretes a white foamy material. This unique material can be used to access the reproductive fitness of the males.

**Materials and methods** Study on Japanese quail was undertaken to determine sire influence on hatchability and estimation of heritability values among three strains of the bird in a humid tropical environment in Nigeria. Fertility and hatchability traits; body weights (BW), Shank length (SL), thigh length (TL), Breast length (BL), body length (BL), keel length (KL), and Wing length (WL) at 2nd,6th,10th weeks of age, were measured on 300 crossbred progeny from 270 cinnamon brown dam, mated with three different strains of sire namely; cinnamon brown (CB), Panda white (PW), and Silver brown (SB) in the mating ratio of 1:4. The data were subjected to analysis of variance appropriate for completely randomized block design (CRBD), and significant means separated with Duncan's multiple range tests. Heritability estimate was done using the sire component equation (Becker 1984).

**Results** The average per cent fertility (71.64 - 75.76%), hatchability (50.41 -57.17%), per cent dead in germ 55.00 -69.08%), dead in shell (11.20 - 18.25%) were significantly ( $P < 0.05$ ) better in PW – sired progeny , while piped (13.06 – 20.11%), brooding (3.00 – 4.67%) and rearing (1.00 – 1.33%) mortality showed no significant different ( $P > 0.05$ ) among the three sired – progeny. The analysis showed that at week 2,6,10 , SB- sired progenies had superior heritability estimates in most of the linear traits and recorded lower indeterminate values as compared with other sired – progenies . Moderate to high heritability estimates (42 % -83%) obtained for body weight at ages 6 and 10 weeks among the three progenies suggests

that selection for body weight or growth rate in Japanese quail should be carried out at 6th and 10th weeks of age and that PW – sired progeny be selected for better hatchability for enhanced breeding programme.

**Conclusion** PW – sired progeny be selected for better hatchability for enhanced breeding program for both egg and meat production in the strains.

**Acknowledgements** we appreciate the Department of Animal Breeding and Physiology MOUAU for financial assistance.

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**Table 1:** Influence of sire on fertility and hatchability of three strains of Japanese quail

Parameter	CB×CB	PW×CB	SB×CB	SEM
Fertility%	71.64 <sup>b</sup>	75.76 <sup>a</sup>	72.30 <sup>ab</sup>	1.36
Hatchability %	54.08 <sup>b</sup>	57.17 <sup>a</sup>	50.41 <sup>c</sup>	3.79
Dead in germ %	69.08 <sup>a</sup>	55.00 <sup>b</sup>	61.51 <sup>ab</sup>	10.28
Dead in shell %	11.20 <sup>b</sup>	12.22 <sup>b</sup>	18.25 <sup>a</sup>	3.24
Piped %	13.06	15.56	20.11	2.90
Brooding mortality %	4.67	3.00	3.33	0.47
Rearing mortality %	1.33	1.00	1.00	0.13
Laying mortality %	0.00	0.00	0.00	0.00

a,b Means across rows did differ significantly at ( $P < 0.05$ ); SEM= Standard error of the mean. CB×CB=Cinnamon brown x Cinnamon brown, PW×CB=Panda white x Cinnamon brown,

**Table 2:** Heritability estimates ( $\pm$ SE) of body weight and linear body parameters among the three strains of Japanese quail at week 2, 6, and 10

Week	Parameter	CB×CB	PW×CB	SB×CB
2	Body weight	0.04±0.46	*	*
	Shank length	*	0.21±0.59	0.31±0.66
	Thigh length	*	0.36±0.70	0.63±0.86
	Breast width	0.12±0.52	*	0.63±0.86
	Body length	0.86±0.97	*	0.79±0.94
	Keel length	0.45±0.75	*	0.01±0.43
	Wing length	*	0.15±0.55	*
6	Body weight	0.31±0.66	*	0.42±0.73
	Shank length	0.25±0.62	*	0.00±0.00
	Thigh length	0.22±0.38	*	0.46±0.76
	Breast width	0.81±0.95	*	1.01±1.29
	Body length	*	*	0.83±1.57
	Keel length	*	*	0.25±0.62
	Wing length	*	*	0.57±0.82
10	Body weight	0.88±0.98	*	0.43±0.74
	Shank length	0.36±0.70	*	0.00±0.00
	Thigh length	0.28±0.64	0.56±0.82	1.14±0.52
	Breast width	*	0.76±0.92	*
	Body length	*	*	*
	Keel length	0.22±0.59	*	0.67±0.88
	Wing length	*	*	0.47±0.77

\*Non estimable heritability, CB×CB=Cinnamon brown x Cinnamon brown progeny. PW×CB=Panda white x Cinnamon brown progeny SB×CB= Silver brown x Cinnamon brown progeny.

# The effect of inclusion of an oregano essential oil in the milk of calves on *Cryptosporidium* shedding

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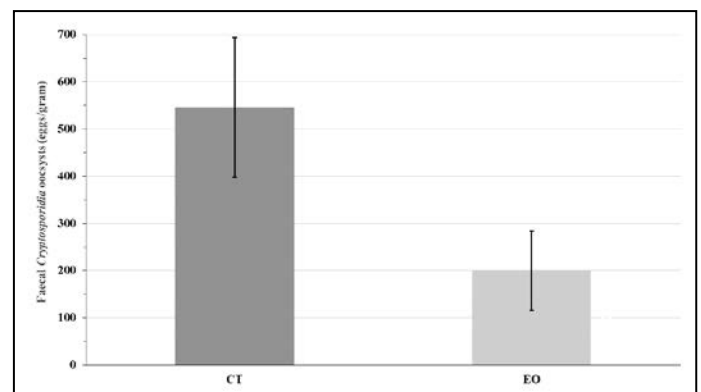
**Application** Cryptosporidiosis is a major cause of calf diarrhoea and morbidity. The inclusion of oregano oil in the milk of calves in the first 2 weeks of life may help reduce the incidence of faecal shedding of *Cryptosporidium parvum*.

**Introduction** *Cryptosporidium parvum* (*C. parvum*) is the major cause of neonatal enteritis in dairy calves in the first 2-3 weeks of life, being the sole causative organism in 37% of outbreaks according to the Veterinary Investigation Diagnosis Analysis (VIDA) database and was found in a further 20% of calves with diarrhoea (Thomson et al., 2017). Moreover, unsaleable, waste milk from the dairy herd containing antibiotic residues is commonly offered to male calves as a source of low cost milk and has been associated with increases in antimicrobial resistance (AMR) (EFSA, 2017). Considerable efforts have been made to reduce on-farm antibiotic use including the use of essential oils (EO). The effect of EO on AMR in calves has been discussed previously (Ray et al., 2019). Even though EO are used to improve animal health, whether or how EO influence Cryptosporidiosis in young cattle remains unexplored. Therefore, this project aimed to evaluate if there was any effect on *C. parvum* oocyst shedding when calves were fed waste milk containing antibiotic residues with and without EO.

**Material and methods** Within 72 hrs of birth experimental calves (5 male calves/treatment) were assigned to one of two dietary treatments: control (CT; offered waste milk with antibiotic residues) and Essential Oil (EO; as CT + Oregano EO (Orego-Stim Liquid, Anpario plc) for the first 10 d). Milk was offered in two daily feeds of 2.5 L per feed, with or without 5 ml of EO included in the milk (2 ml/L). After 10 d EO supplementation all calves received the same diet, without EO, and were offered the same housing and management conditions. Faecal samples were collected from the rectum on day 0, 3, 7, 10, 14, 21 and weaning. Fresh faecal samples were used to count *Cryptosporidia* oocysts by using the flotation technique. Data were subjected to ANOVA using a General Linear Model (Minitab); the model included treatment and day as fixed effects and calves nested within treatment as a random effect. Treatment means were separated by Tukey's test and statistical significance was declared at  $P < 0.05$ .

**Results** *C. parvum* oocysts were not detected in any faecal samples collected on days 0, 3 and after 21 days. There was no treatment x day interaction. The overall mean faecal counts of *C. parvum* oocysts were reduced in calves receiving EO supplementation (548 vs. 200 eggs/gram;  $P = 0.02$ ). The reduction in faecal shedding of *C. parvum* in the CT calves with time is typical of *C. parvum* shedding in calves. All calves in the CT group exhibited faecal shedding of *C. Parvum* oocysts at 2 or more time points whereas 1 EO fed calf had no shedding of *C. parvum* throughout the study and two calves had only one time point with shedding.

**Figure 1** Mean (of all timepoints) faecal oocyst counts in dairy calves fed whole milk twice daily without (CT) and with (EO) oregano essential oil included for 10 days.



**Conclusion** Despite the small number of calves, supplementation of the diet with EO in the first 2 weeks reduced the faecal count of *C. parvum* oocysts. Further work examining the effect of EO supplementation for a longer time period and with a larger number of animals merits investigation.

**Acknowledgements** The study was partly funded by Anpario plc. We also acknowledge the support provided by the School of Agriculture, Policy and Development and the Centre for Dairy Research, University of Reading. The student was funded under the scheme of Undergraduate Research Opportunities Programme (UROP), University of Reading.

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# Performance of dairy cows offered grass silages produced within either a three or four harvest system

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**Application** A four-harvest system can produce higher quality grass silage which increases cow dry matter intake and milk production without increasing concentrate intake compared to a three-harvest system.

**Introduction** Silage digestibility declines by 3.3 percentage units for each week delay in harvest date (Keady et al., 2013), while Huhtanen (2018) calculated that a one week delay in harvest can result in an extra 3.2 kg of concentrate dry matter (DM) being required to sustain the same level of energy corrected milk yield. As harvesting grass at a less mature stage can improve silage quality, there has been a move toward multi-harvest systems. More frequent harvesting increases silage production costs, so benefits must be obtained in either performance or concentrate sparing. This study investigated the effect of offering grass silages produced within a three- or four-harvest system, on the intake and performance of cows managed on a feed-to-yield concentrate system.

**Material and methods** Two treatments, comprising perennial ryegrass silages produced within either a four-harvest (4H) or three-harvest system (3H), were examined. Cutting dates for 4H were: 17 May, 25 June, 8 August and 11 September 2018, while cutting dates for 3H were: 29 May, 24 June, and 11 September 2018. Cows (n = 80; 56 multiparous and 24 primiparous) were allocated to one of the two silage systems at calving, and remained on experiment for 25 weeks. Within both 4H and 3H, each cow was offered silage from each harvest consecutively (harvest 1, followed by harvest 2, etc) for a pre-determined number of days (in proportion to the DM yield for each harvest). Silages were offered as part of a mixed ration containing approximately 8 kg concentrate/cow/d. Individual cow milk yields were calculated weekly. Milk yields not 'supported' by the intake of the mixed rations, were met by offering additional concentrates through an out-of-parlour feed system (at a feed rate of 0.45 kg/kg milk). Treatment effects on DM intake (DMI), milk yield and composition, body weight (BW), body condition (BCS) and energy balance (EB) were examined. Mean weekly data were analysed using REML, with cow as the experimental unit and week as the repeated measure.

**Results** Silage from harvests 1-4 (4H) had a metabolisable energy (ME) content of 12.1, 11.2, 10.7 and 10.8 MJ/kg DM, respectively, while respective values for harvests 1-3 (3H) were 10.9, 10.6 and 10.6 MJ/kg DM. Average protein content of silages was 168g/kg and 148g/kg DM in 4H and 3H, respectively. Silage DMI was greater with 4H than 3H ( $P < 0.001$ ), while concentrate DMI and total DMI were unaffected by harvesting system (Table 1). Milk yield and milk protein content were greater with 4H ( $P = 0.009$  and  $P = 0.004$ , respectively), while milk fat content was greater with 3H ( $P = 0.022$ ). Cows offered 4H silages had a greater milk fat yield, milk protein yield and milk fat + protein yield ( $P = 0.002$ ,  $P < 0.001$  and  $P < 0.001$ , respectively). Treatment had no effect on BW or BCS. Cows offered 3H silages had a more positive mean EB balance over the experiment than cows on 4H silages (5.57 v. 0.62 MJ/d;  $P < 0.001$ ).

**Table 1** Effect of silage harvesting frequency on cow performance.

	4H	3H	SED	P-Values:		
				Treatment	Week	Interaction
Silage DMI (kg/d)	10.4	9.5	0.30	<0.001	<0.001	<0.001
Total DMI (kg/d)	23.4	23.0	0.43	0.131	<0.001	0.172
Milk yield (kg/d)	39.7	37.3	1.08	0.009	<0.001	0.501
Fat (g/kg)	41.1	42.1	1.28	0.022	<0.001	0.587
Protein (g/kg)	33.6	32.9	0.45	0.004	<0.001	0.140
Fat + protein yield (kg/d)	2.94	2.75	0.087	<0.001	<0.001	0.516

**Conclusion** Increasing harvesting frequency from three to four harvests improved silage quality. Silage DMI, milk yield and milk fat + protein yield were increased within the four-harvest silage system.

**Acknowledgements** This project was funded by Department of Agriculture, Environment and Rural Affairs (DAERA) and AgriSearch (Farmers Levy).

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# Effects of storage time and storage temperature on the egg quality of Lohmann white and brown layers

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**Application** Eggs from different genotypes are stored under different temperatures, with different storage methods. However the storage method, environment and genotype affect the internal and external qualities and shelf life of eggs.

**Introduction** The acceptability of eggs by consumers or for industrial use is influenced by their quality (Stadelman, 1996), which can be measured by both internal and external characteristics. External quality is focused on egg weight, shell cleanliness, soundness of shell, shell texture, color and shape. These features are important to the processor as eggs with superior external qualities arrive in a better condition for the consumer (Sabbir et al., 2013). Therefore, a study was carried out to determine the effect of genotype, storage time and temperature on the internal and external qualities of chicken eggs.

**Material and methods** In a 2 X 3 X 5 factorial arrangement, 450 freshly laid eggs were used for the experiment, with 225 eggs from 2 genotypes, Lohmann brown and Lohman white, respectively. Fifty-six-week old layers were randomly allotted

to 3 temperature regime: refrigeration (50C), room temperature (220C) and high temperature (290C) and 5 storage time 0, 5, 10, 15 and 20 days with 30 eggs/temperature regime/genotype and 90 eggs/storage time/genotype. Eggs were labeled, weighed and analyzed for internal and external qualities and then, randomly stored in chambers for 0, 5, 10, 15 and 20 days under the 3 different temperature regimes 50C, 220C and 290C. Data obtained were subjected to three-way analysis of variance with genotype, storage period and storage temperature as fixed factors using the General Analysis of Variance procedure of GenStat. Means were separated using least significant difference (LSD) test at  $\alpha 0.05$ .

**Results and discussion** Results obtained showed no significant difference ( $P > 0.05$ ) between the two genotypes with regard to egg weight and shell thickness (Table 1). The eggs from the Lohmann brown, were however superior in haugh unit, an important determinant of egg freshness. Egg weight, albumen weight and haugh unit were significantly ( $p < 0.05$ ) affected by storage time. Storage temperature significantly ( $p < 0.05$ ) affected the egg weight, albumen height and haugh unit, which are important egg quality determinants. Higher haugh unit was recorded for refrigerated eggs.

**Conclusion** The results from this study showed that genotype, storage time and temperature affect egg quality. Lohmann brown eggs had superior internal quality, while egg weight, albumen weight and haugh unit decreased with increasing storage length. Refrigeration storage method showed better quality with increasing storage periods.

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**Table 1:** Effects of genotype on the external and internal egg quality, storage times and storage methods on egg quality

Parameters	Genotype									
	Lohman White	Lohmann Brown	SEM	Genotype	SL	ST	S X SL	S X ST	ST X SL	S X ST X SL
Egg weight/g	58.3	59.4	2.4	NS	*	*	*	NS	*	NS
Shell weight/g	6.1	5.9	0.1	NS	NS	NS	NS	NS	NS	NS
Shell thickness/mm	0.36	0.35	0.2	*	*	NS	*	NS	NS	NS
Albumen weight/g	32.1b	33.0a	0.7	*	*	NS	*	NS	NS	*
Albumen height/mm	5.2b	7.3a	1.8	*	*	*	*	*	*	*
Yolk weight/g	18.6a	17.9b	0.6	*	NS	*	NS	*	NS	NS
Yolk colour	4.2b	4.7a	0.2	*	*	NS	*	NS	NS	*
Haugh unit/%	67.7b	74.0a	1.4	*	*	*	*	*	*	*
Albumen pH	8.5	8.4	0.1	NS	*	NS	NS	NS	NS	NS

# Optimal management of dairy cattle farms in Greece: an economic efficiency analysis

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**Application** The identification of the most efficient intensive dairy cattle farms in Greece allows the description of the structure and the productivity of these farms and constitutes a practical tool for revealing the best observed management practices.

**Introduction** Nowadays, the European dairy livestock sector is characterized by the predominance of intensive production systems that depend on capital endowments, animals of high yields, purchased feedstuff and hired skilled labour (Britt et al., 2018). The prevalence of such intensive production systems is more evident in the dairy cow sector where large scale farms of entrepreneurial type have emerged, driven by the growing demand for milk, the abolishment of milk quotas and high fixed costs (European Commission, 2016). The economic performance of such intensive systems is heavily dependent on their management efficiency. The efficient use of the existing resources on these farms is crucial to their profitability and consequently to their competitiveness. In this context, the measurement of efficiency can be a practical decision tool for adopting management strategies that would induce dairy cattle farmers to increase their productivity.

**Material and methods** Through the application of a Data Envelopment Analysis (DEA) model on technical and economic data from 47 dairy cow farms, the efficient farms that utilize fully the existing technology of production were identified. The inputs used in the DEA model were: (i) farm size (number of dairy cows), (ii) human labour (hours), (iii) variable cost (€) and (iv) fixed capital cost (€). The output variable was gross revenue (€). These 47 farms were categorized on the basis of their estimated level of Technical Efficiency and the main technical and economic indicators were compared between efficient and inefficient farms.

**Results** Efficient farms (16 of the 47 farms) achieve higher gross margin and lower total production cost, rear the same number of cows with inefficient farms, but make better use of labour. Moreover, efficient farms cultivate more hectares of land, especially of irrigated land to mitigate market uncertainties. The analytical results indicate that feeding costs in inefficient farms are 13.5% higher compared to efficient ones although their milk yield is lower. The contribution of meat sales to farm income is higher in the efficient farms (6.7%), while the low share of subsidies in gross revenue verifies the market orientation of the sector.

**Table 1** Efficient vs Inefficient Farms

Technical indicators and economic results	Efficient average farm (n=16)	Inefficient average farm (n=31)
Land (irrigated equivalent in hectares)	0.275	0.247
Non-irrigated (ha/cow)	0.015	0.037
Irrigated (ha/cow)	0.260	0.210
Cows (number)	143.6	142.2
Milk yield (kg/cow/year)	8413	7675
Average milk price (€/kg)	0.425	0.435
Labor (hours/cow/year)	81.9	97.3
Family (hours/cow/year)	38.0	51.2
Hired (hours/cow/year)	43.9	46.1
Gross revenue (€/cow)	4061	3659
Total cost (€/cow)	3381	3967
Net profit/loss (€/cow)	680	-309
Gross margin (€/cow)	1818	1018
Milk cost (€/Kg)	0.41	0.48

**Conclusion** Cost analysis implies that dairy cattle farms should operate with increased variable cost and utilize their infrastructure at full capacity to decrease their fixed cost per cow. Farms should make use of other sources of income such as the meat produced on farm in order to increase their economic resilience and be more risk averse in an environment where margins to lower production costs in order to improve competitiveness in highly intensive farms have been narrowed down.

**Acknowledgements** This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-03989).

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# Assessing mineral provision from drinking water for nutrition studies: supply of zinc from galvanized troughs

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**Application** Due to the galvanization of drinking water troughs, zinc (Zn) content could substantially contribute to total Zn intake and needs to be considered in an animal nutrition experiment looking into mineral supplement effects.

**Introduction** Drinking water is a potential source of minerals for livestock. Before the beginning of a sheep nutrition experiment taking place at North Wyke-Rothamsted Research (Southwest, England), the micro-mineral contents of the drinking water provided in the small ruminant facility were analysed to test whether drinking water might be an important variable to consider.

**Material and methods** The water supplied for the studied site was classified as soft (total hardness level = 15 mg-calcium L<sup>-1</sup>) and was at pH= 7.86. There were 24 sheep pens with one drinking trough in each pen in the small ruminant facility. The water drinking troughs were cleaned up by a nylon-brush before sampling. Each water sample consisted of a 1-2 ml subsample from each drinking trough (bulked from 24 individual samples). The 24 subsamples were taken using a clean syringe and mixed together in a clean polypropylene tube as one sample. In total, three replicates were taken. The samples were filtered through Whatman no.42 filter paper before analysis by ICP-MS or ICP-OES.

**Results** The concentrations of cobalt (Co), selenium (Se) and iron (Fe) in the drinking water were close to or below the detection limits of analysis (Table 1). All the minerals had low concentrations in the drinking water, except for zinc (Zn) (1.82 ± 0.02 mg L<sup>-1</sup>). Assuming a sheep drinks 2-6 L of water daily, the sheep could consume 3.6-10.9 mg Zn per day from water alone. In our current sheep experiment, the sheep were fed a concentrate containing Zn in different forms at a requirement level (104 mg Zn kg<sup>-1</sup>). Extrapolating the daily intake of the concentrate (approx. 600 grams day<sup>-1</sup>), the Zn provision from the drinking water contributed circa 6 – 18 % of the daily Zn intake.

**Table 1** The concentrations of the micro-minerals in the drinking water samples

	Co (MS) (µg L <sup>-1</sup> )	Cu (MS) (µg L <sup>-1</sup> )	Fe (OES) (mg L <sup>-1</sup> )	Mn (MS) (µg L <sup>-1</sup> )	Se (MS) (µg L <sup>-1</sup> )	Zn (OES) (mg L <sup>-1</sup> )
Sample 1	0.01	2.33	0.010	3.88	0.05	1.810
Sample 2	0.04	3.36	0.011	4.35	0.03	1.851
Sample 3	0.01	1.91	0.012	3.92	0.03	1.811
Mean ± SD	0.02 ± 0.02	2.53 ± 0.75	0.011 ± 0.001	4.05 ± 0.26	0.04 ± 0.02	1.820 ± 0.020
Detection limits of the ICP-MS/OES	0.05	0.03	0.005	0.02	0.04	0.006

**Conclusion** The Zn concentration in the drinking water at the small ruminant facility (Rothamsted Research, North Wyke) provided a substantial supply, contributing ca. 6-18% of daily Zn intake (based on a current feeding trial). Therefore, Zn content in drinking water should be taken into consideration within animal nutrition studies which use galvanized troughs to fully determine Zn daily intake.

**Acknowledgements** This work was taken place at North Wyke-Rothamsted Research, supported by the Biotechnology and Biological Sciences Research Council (BBSRC). This work was also funded by Alltech Ltd, who provided funding for the lead author's PhD and the concentrate feed used in this study.

# A preliminary investigation to determine if selenium loss through exhalation in sheep supplemented with selenium yeast at NRC requirement could be quantified using a gas bag technique

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**Application** Se loss in exhalation from sheep supplemented selenium yeast at NRC required levels (0.2 mg kg<sup>-1</sup> diet) could be omitted in a full Se-balance trial as the levels were below detection.

**Introduction** Selenium (Se), when dosed to sheep at high levels (>2 mg kg<sup>-1</sup> BW), was found to be significantly lost through exhalation within four hours after dosing (Davis et al., 2013; Tiwary et al., 2005) quantified using a bag technique. Significantly higher Se was found from the sheep supplemented with sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) or selenomethionine (Se-Met) than from control sheep with no supplement. However, data on Se exhalation from sheep fed with Se at NRC requirement level (0.23 mg kg<sup>-1</sup> diet) using the same sampling technique is lacking. We therefore performed a preliminary trial to determine whether the bag collection technique is suitable for detecting Se exhalation from sheep supplemented Se at NRC level, to help inform future full Se-balance trials comparing organic and inorganic Se.

**Material and methods** Following the methods proposed by Tiwary et al. (2005), sheep exhalate was collected by a hand-made breath collection apparatus, encompassing a breathing mask connected to a non-rebreathing valve allowing the sheep to breath in normally from one side of the valve and to exhale out on the other side of the valve to a 3L collection bag. Two sheep were housed together in the small ruminant facility at Rothamsted Research, North Wyke for five days. During housing, the sheep had their diet individually titrated from a 100% forage diet to a 60:40 forage: concentrate diet. On the last housing day, sheep were fed a concentrate containing 0.6 mg-Se kg<sup>-1</sup>, in the form of Se-yeast (with Se-Met as the dominant form) to provide 0.23 mg kg<sup>-1</sup> of the total diet as recommended by the National Research Council (NRC) of the United States. A 2L exhalate sample was collected from each sheep at the following time points on the last day: before feeding, and 4 hours and 8 hours after the feeding of Se-containing concentrate. The collected exhalate sample was passed through an activated charcoal tube at 1L min<sup>-1</sup> flow rate for 2

minutes. The sampling timepoints and the associated sampling and analytical methodologies are according to Tiwary et al. (2005) that significant Se exhalations were observed. To analyse the Se sorbed on the charcoal of compartment I, the charcoal was extracted with 3ml 50:50 ratio of ethanol and water on a rotary shaker (200 rpm) for 2 hours. After the extraction, the extracts were centrifuged at 500 x g for 10 minutes. The ethanol extracts were mixed with distilled water at a volume ratio of extract: final analyte = 1:10 or 1:5 to evaluate potential matrix effects in ICP-MS analysis. The analytes were kept at 4°C and analysed within two days from sample extraction.

**Results** The results showed that none of the exhalates collected after 4 or 8 hours were different to the Se content of the controls (samples before Se-supplementation), and all the Se concentrations in the exhalate extracts were close to the detection limits of ICP-MS (0.05-0.1 µg L<sup>-1</sup>) (Table 1).

**Table 1** ICP-MS analysis of Se concentrations in the exhalate extracts and the calculated total-Se-content in the 2L- exhalate samples

Sample collection time	Sheep number	Se concentrations in the ICP-MS analyte (µg L <sup>-1</sup> )	Volume ratio of extract in the final analyte	Calculated Se in the 2L-exhalate sample (ug)
Before Se-feeding	Sheep 1	0.044	1:10	0.001
		0.091	1:5	0.001
	Sheep 2	0.043	1:10	0.001
		0.094	1:5	0.001
4 hours after Se-feeding	Sheep 1	0.037	1:10	0.001
		0.085	1:5	0.001
	Sheep 2	0.047	1:10	0.001
		0.071	1:5	0.001
8 hours after Se-feeding	Sheep 1	0.045	1:10	0.001
		0.112	1:5	0.002
	Sheep 2	0.045	1:10	0.001
		0.062	1:5	0.001

**Conclusion** Se loss through exhalation from sheep fed with Se-yeast at the NRC required level of 0.23 mg kg<sup>-1</sup> diet could not be detected over non-supplemented control animals. To measure the total exhalation of Se from standard diets more precisely, a chamber system that enables collecting exhalate for a longer period may be more suitable for the detection of low-level Se exhalation than the mask-collection method we used in this preliminary trial to determine if exhalate should be included in future trials.

**Acknowledgements** This work was taken place at North Wyke-Rothamsted Research, supported by the Biotechnology and Biological Sciences Research Council (BBSRC). This work was also funded by Alltech Ltd, who provided funding for the lead author's PhD and the concentrate feed used in this study.

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# Qualities of eggs from laying hens fed supplemental selenium and $\alpha$ -tocopherol

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**Application** Improved egg quality characteristics could be achieved through dietary supplementation with selenium and  $\alpha$ -tocopherol.

**Introduction** A change in quality of an egg can be achieved by several interventions. Nutrition and dietary manipulation are veritable avenues for appropriate improvement in egg quality indices. This research was aimed at investigating selenium and  $\alpha$ -tocopherol supplementation in laying hen diets on egg quality attributes.

**Materials and methods:** ISA brown pullets (n=192) at week 31 of age were assigned to six experimental diets. Basal diet (T1) which contained 2736 Kcal/Kg metabolisable energy and 17% crude protein but without supplemental selenium and  $\alpha$ -tocopherol while diets T2, T3 and T4, contained 0.5, 1.0 and 1.5 mg/kg supplemental selenium, respectively. Diets T5 and T6 were supplemented with 20 and 40 mg/kg  $\alpha$ -tocopherol, respectively. Each treatment comprised four replicates with eight birds per replicate. The pullets were allotted in a completely randomised design. Determination of egg quality was done using standard methods. At week 34, eggs were randomly sampled for egg quality characteristics. Egg weight (g) were measured using an electronic digital scale. Yolk was carefully separated from albumen to measure yolk and albumin weights using an electronic digital scale. Albumin length (mm) was measured using digital Vernier caliper, (Scott and Silverside, 2000). Albumen height it was measured with Vernier Caliper 1cm away from the edge of the yolk and the height of the sticky fluid on the tip was adjusted and the height read on the digital screen. Relationship between albumen height and egg weight was used to determine the Haugh Unit as described in the following equation (Haugh, 1937),  $HU = 100 \log_{10} (h - 1.7W^{0.37} + 7.6)$ . Where HU=Haugh Unit, h=observed height of the albumen in millimeters and W= egg weight in grams.

**Statistical analysis:** Data were subjected to analyses of variance (SAS, 2003) and means were separated using Duncan's multiple range test option of the same software at  $\alpha 0.05$ .

**Results** Dietary supplementation had no significant effects ( $p > 0.05$ ) on egg weight, albumin weight and Haugh unit. However, hens on T6 recorded significantly decreased ( $P < 0.05$ ) albumin length while those on T1 (98.81), T2 (94.89), T3 (96.93), T4 (96.20), and T5 had significantly higher albumin length. Yolk weight values for hens on T4 (13.86) and T5 (13.36) were similar to 14.48, 14.56 and 15.10 in hens on T1, T2, and T3, respectively. Ratio of yolk weight to albumin weight was increased with T2 (0.40), T3 (0.40) and T4 (0.41) mg/kg which was similar to 0.39 and 0.36 obtained in hens on T1 and T5 while hens on T6 had lower value (0.35).

**Conclusion** Egg albumin length, yolk weight, and yolk to albumin ratio was significantly lower in hens on T6. Selenium and  $\alpha$ -tocopherol supplementation in laying hens diets has no significant effect on egg HU. Further research should be carried out to ascertain the combination effects of selenium and  $\alpha$ -tocopherol on egg quality.

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**Table 1:** Quality characteristics of eggs from laying hens fed supplemental selenium and  $\alpha$ -tocopherol

Parameters	T1	T2	T3	T4	T5	T6	SEM
Egg Weight (g)	55.13	53.63	55.20	52.93	53.70	53.43	0.67
Albumin Weight (g)	37.33	36.00	37.70	33.73	36.66	36.46	0.70
Albumin Length (mm)	98.81 <sup>a</sup>	94.89 <sup>a</sup>	96.93 <sup>a</sup>	96.20 <sup>a</sup>	96.31 <sup>a</sup>	69.62 <sup>b</sup>	3.43
Yolk Weight (g)	14.48 <sup>a</sup>	14.56 <sup>a</sup>	15.10 <sup>a</sup>	13.86 <sup>ab</sup>	13.36 <sup>ab</sup>	12.74 <sup>b</sup>	0.26
Yolk Weight: Albumin Weight	0.39 <sup>ab</sup>	0.40 <sup>a</sup>	0.40 <sup>a</sup>	0.41 <sup>a</sup>	0.36 <sup>ab</sup>	0.35 <sup>b</sup>	0.01
Haugh Unit	75.32	70.38	81.21	73.08	77.05	81.41	1.65

# The effect of inclusion of oregano essential oil in milk replacer and of concentrate pellet size on feeding behaviour of dairy calves to 70 days of age

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**Application** The inclusion of 2ml/head/day of oregano oil (Orego-Stim Liquid) in the milk replacer of calves increased visits to the automatic milk feeder and concentrate feed stations which may indicate improved health status.

**Introduction** Diseased calves tend to visit feeders less frequently than healthy counterparts (Duthie, 2019). This suggests that feeding behaviour could be used as an early indicator of disease onset and Belaid (2019) found that calves began to visit milk feeders less frequently as many as 10 days prior to the clinical identification of disease. There is evidence that feeding behaviour patterns that developed in early life have the potential to persist, thus having longer term implications for health and welfare (Miller-Cushon, 2015). Oregano essential oil (OEO) is a flavouring compound and has potential to increase feeding behaviour and intake. There is limited data on the diameter of calf starter feed on intake patterns. Therefore, this project aimed to evaluate the effect of OEO and concentrate pellet size on feeding behaviour of dairy-bred calves up to 70 d of age.

**Material and methods** At 5 days of age, 70 Holstein-Friesian calves were assigned to one of four treatments in a 2 x 2 factorial arrangement comparing milk replacer (with or without 2 ml OEO per day (Orego-Stim Liquid, Anpario plc)) and concentrate pellet size (3 mm or 5 mm diameter), allocated according to birth weight and gender. All calves received their mothers' fresh (>1 hour) colostrum for 4 days and were offered milk replacer (150 g powder/L) and concentrates ad libitum via automatic feeders from 5 days of age. Milk replacer was offered at 6 L per day from day 5 to 33. At day 33 it was reduced to 2 L until day 56 (weaning). Calves were housed in straw bedded pens, with 15 calves per pen. Milk replacer intake was recorded daily from 5 to 56 days and concentrate intakes recorded daily from 5 to 70 days. Scales were placed at the concentrate feeders to record full bodyweight at each feeder visit. Forage was offered as straw pre-weaning and as silage post-weaning. Number and duration of visits to the automatic milk feeder and concentrate feeder were recorded. The direct and interactive effects of essential oil and pellet size were measured by REML analysis with variance components, using a linear mixed model, with pen as the random model (JMP 14.2.0).

**Results** There were no interactions between the inclusion of OEO and pellet size on any parameter; therefore, only the treatment effects are presented. Calves offered OEO in their milk visited both the milk ( $P < 0.001$ ) and concentrate feeders ( $P < 0.001$ ) more frequently. Calves offered 5mm pellets consumed a greater amount of feed per visit to the concentrate feeders (219.5 vs 203.0g/visit,  $P < 0.001$ ). Eating speed was greater in calves offered OEO ( $P < 0.001$ ) and in calves offered 5mm pellets ( $P = 0.002$ ). Due to the greater eating speed, visit time was shorter for calves offered OEO ( $P < 0.001$ ). Visit time was not affected by pellet type.

**Table 1** The effect of oregano essential oil (0 v. 2ml/d) and 3 or 5 mm diameter pellet on calf feeding behaviour.

	Milk Replacer Additive		Pellet Diameter		SED	P Value	
	OEO +	OEO -	3mm	5mm		OEO	Pellet
Milk feeder visits (n/d)	11.41	10.74	11.01	11.14	0.42	<0.001	0.514
Concentrate feeder visits (n/d)	6.68	6.13	6.44	6.36	0.99	<0.001	0.452
Eating speed (g/min)	37.61	34.58	35.02	37.17	1.71	<0.001	0.002
Visit time (min/d)	6.07	6.70	6.39	6.38	0.51	<0.001	0.947

**Conclusion** The inclusion of 2ml per calf per day of oregano essential oil in the milk replacer increased visit numbers to both milk and concentrate feeders as well as an increase in eating speed.

**Acknowledgements** The study was funded by DAERA and Anpario plc. We also acknowledge the support provided by the farm staff at the Agri-Food and Biosciences Institute, Hillsborough.

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# Post-weaning growth response and feeding behaviour of low and average birthweight pigs to sow lactation feed intake

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**Application** Low birthweight piglets have the capacity to achieve acceptable weaning weights when reared on sows offered a high feed allowance. This weight advantage at weaning was lost in the post-weaning period through a poorer feed conversion efficiency since the intake and feeding behaviour of low and average birthweight pigs was similar. This work demonstrates a need to understand the differences in the biological drivers of feed efficiency between low and average birthweight pigs, as well as the need to provide further interventions post-weaning to maximise the performance of compromised pigs.

**Introduction** Previous work has shown that when compromised pigs are reared on sows offered a high lactation feed allowance, they will achieve reduced mortality with weaning weights equivalent to average birthweight counterparts reared under commercial conditions (Hawe et al, 2019). This work aimed to assess if benefits achieved at weaning were retained through the growing and finishing stage.

**Material and methods** This two (birth weight) x two (lactation feed allowance) factorial arrangement study complied with the Animals (Scientific Procedures) Act 1986. Litters comprised of either 14 low birthweight (Low BW; <1kg) or 14 average birthweight (Av BW; ~1.5kg) piglets were reared by sows offered either a low (Low FA) or high (High FA) feed allowance (Hawe et al, 2019). It is worthy of note that the Low FA treatment during lactation was comparable to that used commercially. This study focused on the post-weaning period with animals remaining in their litter groups until slaughter at week 22 and performance monitored throughout the growing and finishing

stages. All animals followed a standard feeding regime post-weaning. Animals were individually weighed at week 5, 7, 12, 15, 17 and 22. Feeding behaviour was recorded on an individual basis using electronic 'FIRE' feeders between weeks 12 and 22.

**Statistical analysis** Data was analysed using a linear mixed model. Birthweight and feed allowance were fitted as fixed effects. Replicate, birth mother or foster mother were incorporated as a random effect. In certain instances 'treatment' was fitted as a fixed effect in order to compare and contrast each treatment group. A significance level of 0.05 was used.

**Results** There were no significant interactions ( $P>0.05$ ) between treatments on any performance parameters recorded (Table 1). The average liveweight and ADG was greater for Av BW pigs than Low BW animals throughout the trial ( $P<0.05$ ). Sow FA during lactation did not influence piglet weight or ADG post-weaning ( $P>0.05$ ). Relative growth from week 4-10 was higher for pigs from sows offered Low FA ( $P=0.006$ ). ADFI was greater for Av BW pigs from week 4 to 10 but was not influenced by BW from week 12 to 22. Av BW pigs recorded a superior FCR from week 12 to 22 ( $P<0.05$ ). From week 12 to 22, BW and FA had no effect on the time spent in feeder per day, feeder visits per day, average time of each feed interval or eating velocity ( $P>0.05$ ). However from week 17 to 22 Low FA pigs entered the feeder a significantly greater number of times per day ( $P=0.038$ ). Feed intake per interval during week 12 to 22 was greater for animals offered High FA ( $P=0.028$ ). Analysis comparing the individual sow treatments on pig performance found that, despite there being no difference in weaning weight, Low BW pigs offered a High FA had a lower ADG from week 4 to 10 than Av BW pigs offered a Low FA, resulting in a significantly lower piglet weight at week 7 ( $P=0.02$ ) and 12 ( $P=0.013$ ). Furthermore there was no significant difference in the weight, ADG or ADFI of Low BW pigs offered Low FA or High FA from week 7 to slaughter ( $P>0.05$ ).

**Conclusion** Pre-weaning benefits of enhanced sow lactation intake on piglet weight were not retained at slaughter, with results suggesting compensatory growth occurred post-weaning. The 5.4% reduced slaughter weight of compromised pigs can be explained by their 2.2% reduction in FCR during the finishing period. Low BW pigs require post-weaning intervention in order to maximise lifetime performance. Furthermore, more focussed research on the biological drivers of FCR in compromised pigs is required.

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	Low BW		Average BW		SEM	Significance (P-value)	
	Low FA	High FA	Low FA	High FA		Birth weight	Feed allowance
Piglet week 4 wt (wean) (kg)	7.0	7.9	7.9	8.6	0.36	0.003	0.005
Piglet week 22 wt (kg)	99	100	103	107	2.7	0.003	0.11
ADG wk 4-10 (g)	428	423	463	465	10.1	<0.001	0.892
ADG (wk 12-22) (g)	965	979	994	1034	25.9	0.025	0.139
ADFI wk 4-10 (g)	607	632	691	698	23.3	0.006	0.498
ADFI (wk 12-22) (g)	2166	2184	2174	2256	61.1	0.366	0.241
FCR (wk 4-10)	1.53	1.55	1.53	1.57	0.033	0.756	0.39
FCR (wk 12-22)	2.25	2.23	2.20	2.18	0.030	0.017	0.417
Time feeding per day (mins)	68	65	67	65	2.1	0.714	0.141
No. times in feeder / day	8.3	7.7	9.4	9.1	1.09	0.39	0.255
Feed intake / interval (g)	335	354	299	333	24.1	0.296	0.028
Time in feeder / feed (mins)	10.1	10.4	9.1	9.3	0.69	0.219	0.339
Eating velocity (g/min)	34	35	35	37	1.6	0.221	0.156

**Table 1** Effect of piglet BW and sow FA on post-weaning performance and feeding behaviour during finish



# Comparison of the tannic acid equivalent in different varieties of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.)

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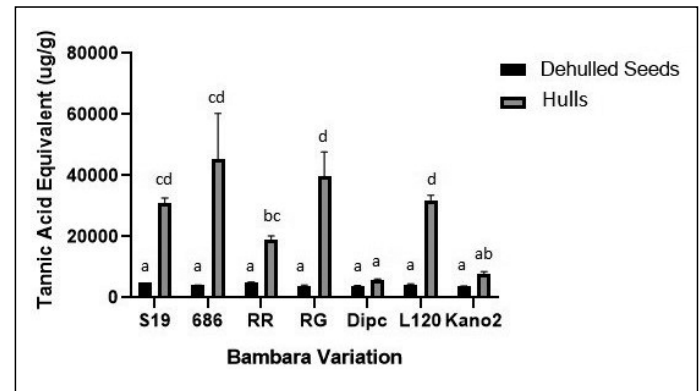
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**Application** Bambara groundnut seeds vary significantly in hull tannin content which may influence nutrient availability when used whole as an animal feed ingredient. Bambara seeds are grown and utilised locally with no extensive trading, meaning there is currently a low associated cost.

**Introduction** Globally, many under-utilised crops could provide a marketable alternative to highly used soybean. Drought resistant Bambara groundnut, is one such crop, with seeds containing 25% protein (Gulzar and Minnaar, 2017). A large number of different coloured varieties of Bambara exist. One limiting factor for their use is the presence of anti-nutritional factors; including tannins, which bind dietary protein and micronutrients. The study aim was to quantify the total phenolic contents, as tannic acid equivalents, within the different varieties of Bambara groundnut.

**Materials and Methods** Seven Bambara varieties (S19, 686 (black-coloured varieties), RG, RR (red varieties), Dipc, Kano2 (cream varieties) and L120 (tan variety)) were dehulled and the seeds and hulls ground prior to the study. To assess tannic acid concentrations total phenolic content was assessed. Ground samples were suspended at 25mg/ml in 0.1M NaOH, vortexed for 60s, then centrifuged for 3500g for 5 minutes at 25°C. The supernatants (or supernatant from hulls diluted 1:4 in 0.1M NaOH) were assayed in solution with a final concentration of 2.3% (v/v) Folin-Ciocalteu reagent and 86mM Na<sub>2</sub>CO<sub>3</sub>. A standard curve (0-0.2mg/ml) of tannic acid (Sigma- Aldrich) was used. Following 1 hour incubation at 25°C room temperature in the dark, the absorbance was read at 655nm. Data was analysed using a two-way ANOVA (Genstat 19th Edition) and a Bonferroni post-hoc test was carried out for significant results ( $p < 0.05$ ). Results There was a significant two-way interaction ( $p < 0.001$ ) between the type of sample (hull or dehulled seed) and the Bambara variety (Figure 1). There were significant differences between the hulls but not dehulled seeds, whilst in the same variety hulls had higher tannic acid equivalent than the dehulled seeds, the exceptions being DiPC and Kano2.

**Figure 1.** The tannic acid equivalent content of Bambara Groundnut seeds. The tannic acid equivalent was assessed by determining total phenolic content in the hulls and dehulled seeds. There was a significant two-way interaction ( $p < 0.001$ ), different letters indicate a  $p < 0.05$  for Bonferroni post-hoc test.



**Conclusion** There is a significant variation in the tannin content of Bambara varieties, determined as total phenolic contents, predominantly found in the seed hull. This could have a significant impact on the nutrient availability in this potential feed ingredient if used whole. As the cream varieties show the lowest tannic acid equivalents across the whole seeds, these would likely be the best for use in animal feeds, based on these results alone. In addition, the removed hulls may have uses as a by-product, as tannins have been found to reduce faecal egg counts in ruminants and halt larval development in vitro (Min and Hart, 2003).

**Acknowledgements** This work was funded by the University of Nottingham and BBSRC

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# Evaluation of the in vitro adaptation of bovine inoculum to tannins and their effects on ruminal parameters

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**Application** When testing an additive or food for an animal, the cost is drastically reduced if previously tested in vitro.

**Introduction** In vitro gas production bioassays have been used to simulate the ruminal digestion process as a viable alternative to determine the effect of additives and foods before being offered to animals in in vivo experiments. This is because they are fast, inexpensive and relatively easy, but often no significant results are found due to the slow action of the components of the additive or feed in the modulation of ruminal fermentation. The objective of this work was to evaluate the adaptation of the inoculum, not yet exposed to condensed tannins (CT), in an in vitro incubator, for later inoculation in the in vitro technique of gas production measured by a data logger.

**Material and Methods** Rumen inoculum (solid and liquid phase) was collected from 8 animal consuming a pasture diet. Rumen inoculum was pre-adaptation in vitro to the CT for 0, 24, 48 and 72 hours respectively.

**Statistical analyses** The experimental design was a completely randomized design, with 4 treatments (hours of adaptation) and 4 replications (inoculum). An ANOVA was performed using PROC GLM and obtaining means through LSMEANS. The Tukey test was used at  $P < 0.05$ .

**Table 1.** Averages, MSE and probability of degradability parameters, gas production and dry matter

Variables	Treatments			SEM P value	
	T24, h	T48, h	T72, h		
IVDMD, %	69.0	66.3	69.5	1.1	0.216
Gas 8h, mL/gDM	12.3	12.7	11.0	1.3	0.498
Gas 12h, mL/gDM	35.0	34.1	35.8	1.7	0.412
Gas: 16h, mL/gDM	58.9	50.8	56.7	2.3	0.090
Gas: 20h, mL/gDM	77.7ab	68.0b	73.4ab	2.6	0.0342
Gas: 24h, mL/gDM	92.1a	80.1b	85.0ab	2.9	0.004
Gas: 48h, mL/gDM	134.0ab	116.0c	124.9bc	3.7	<0.0001
Gas: 72h, mL/gDM	165.8ab	148.2b	155.2b	4.7	0.001
PF, mgDM/mL	3.5b	4.0a	4.1a	0.09	0.0003

\* Means followed by the same lowercase letter in the line do not differ statistically from each other, by the Tukey test at 5% probability. Source: Lobo, A. A. G.

**Results** There was no significant difference between Please restate your treatments with each P value for clarity. treatments (P value: 0.216, 0.498, 0.412 and 0.090, respectively) for the IVDM and gas production in the initial times (8, 12 and 16 h). However, after 16 h of incubation the gas production in the (P < 0.034), showing that the adaptation modified the fermentation profile. The gas production results agree that there was an increase in the DM partition factor (P = 0.003), which means that a larger amount of degraded DM was required to form 1 mL of gas. In addition, the results show the importance of adapting the inoculum to the substrate to be used, since the non-specificity can generate erroneous conclusions.

**Conclusion** In addition, the results show the importance of adapting the inocula to the substrate to be used, since the non-specificity can generate erroneous conclusions.

**Acknowledgements** All thanks to CNPq and FAPESP by their financial support.

# In vitro gas production and organic matter digestibility increased with inclusion of cassava leaves in concentrate diets for ruminants

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**Application** Gas production and digestibility of diets increased with increasing levels of cassava leaf inclusion. Formulation of cassava leaves into concentrate diets for ruminants results in better digestion of nutrients.

**Introduction** Availability of quality feed resource for ruminant feeding in the tropics is critically affected by an extended dry season which in turn reduce the productivity of the animals. This scarcity requires the use of concentrate supplements, although comes at a very high cost due to the use of ingredients that are competed for humans. Cassava leaves, though a crop residue often discarded after harvest, contains crude protein of about 24% and can be used as a protein source in the formulation of concentrate supplement for dry season ruminant feeding. Thus, this study tested the in vitro gas production and digestibility of concentrate diets formulated using cassava leaves and peels.

**Material and methods** Four (4) concentrate diets were formulated with cassava leaves included at 0%, 5%, 10% and 15% of the total diet. Other ingredients included in the diet were: cassava peels, maize cob, wheat ofal, dried brewers grain, bone meal, salt and sulphur. The experiment was set out as a completely randomized design with four (4) treatments. The in vitro gas production technique employed was that described by Menke and Steingass (1988). Rumen liquor was collected from four (4) White Fulani cattle maintained daily on the diets into warm insulated flasks, filtered through layers of cheesecloth and used as the source of inoculum. The inoculum was then mixed with sodium and ammonium bicarbonate buffer (35g NaHCO<sub>3</sub> plus 4g NH<sub>4</sub>HCO<sub>3</sub> per litre) at a ratio of 1:2 (v/v) to prevent lowering the pH of the rumen fluid which could result in decreased microbial activity. A 200mg sample of diet substrate was replicated eight times (n=8) for each treatment and were placed into 100ml calibrated syringes fitted with plungers. A 30ml of the buffered inoculums was then added to each syringe containing the ground samples and were then positioned in an incubator kept at 39°C. Blank syringes containing 30ml of the buffered inoculums only was included as control. Gas volume was recorded following; 0, 4, 8, 12, 16, 20 and 24 hours of incubation and methane (CH<sub>4</sub>) gas was determined. Post

incubation parameters such as dry matter digestibility (DMD), metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) (Getachew et al., 2000) were calculated.

**Statistical analysis** Data obtained were assessed for normal distribution and then subjected to one-way analysis of variance with significant means separated at P<0.05 (Duncan's multiple range test) using the SPSS 23 software.

**Results & Discussion** Gas production volumes values were greater (P=0.01) in diets containing cassava leaves at 5, 10 and 15% of total diet. The DMD, OMD and SCFA values increased with inclusion of cassava leaves, however ME values were not affected by cassava inclusion. CH<sub>4</sub> production increased (P=0.03) with inclusion of cassava leaves.

**Conclusion** Increased gas production and organic matter digestibility indicated the potentially high nutritive value of cassava leaves when utilized as a protein source in concentrate supplements for dry season ruminant feeding. The increased gas volume and percentage of methane gas produced with inclusion of cassava leaves should be further investigated for mitigation opportunities such as the use of phytogetic plants as additive.

**Acknowledgements** This research was carried out at the Laboratory of Animal Nutrition Department, FUNAAB, Nigeria.

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Parameters	Cassava leaf inclusion (%)				SEM	P-value
	0	5	20	15		
Gas volume (ml)	40.8 <sup>b</sup>	50.5 <sup>ab</sup>	58.3 <sup>a</sup>	56.3 <sup>a</sup>	2.068	0.010
DMD (%)	48.5 <sup>b</sup>	52.4 <sup>a</sup>	54.5 <sup>a</sup>	54.6 <sup>a</sup>	1.816	0.001
OMD (%)	39.0 <sup>b</sup>	49.5 <sup>b</sup>	53.8 <sup>a</sup>	50.8 <sup>a</sup>	0.895	0.025
ME (MJ/kgDM)	16.91 <sup>ab</sup>	14.65 <sup>c</sup>	16.01 <sup>b</sup>	17.41 <sup>a</sup>	0.238	0.000
SCFA (µmol/g DM)	0.46 <sup>b</sup>	0.57 <sup>ab</sup>	0.66 <sup>a</sup>	0.65 <sup>a</sup>	0.047	0.010
CH <sub>4</sub> (ml/200mgDM)	8.38 <sup>b</sup>	11.63 <sup>ab</sup>	17.11 <sup>a</sup>	12.98 <sup>ab</sup>	2.750	0.005
CH <sub>4</sub> (%)	20.6 <sup>b</sup>	23.0 <sup>ab</sup>	29.4 <sup>a</sup>	23.1 <sup>ab</sup>	1.379	0.035

# Evaluation of milk replacer protein composition on calf growth and performance

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**Application** Whey protein concentrate and skim milk powder are comparable sources of dairy protein in milk replacer diets for calves to 8 weeks of age.

**Introduction** Dairy ingredients are the main source of protein in milk replacers (MR); these include skim and whey produced as co-products from the processing of dairy products destined for the human food industry. Skim milk powder (SMP) is whole milk with the fat removed and contains 80% casein and 20% whey protein. Liquid whey is the fraction remaining following the precipitation of caseins. Ultrafiltration of whey produces whey protein concentrate (WPC). In the acid conditions of the abomasum, whole milk forms a clot due to the enzymes renin and pepsin acting upon the casein protein in digesta. MR with a high casein (SMP) content results in curd formation, whereas when whey is the primary source of protein, no curd forms. Previous research has shown no effect of curd formation on digestibility or performance in calves fed at low levels (ca. 600 g DM / d) (Terosky et al., 1997; Lammers et al., 1998). The aim of this experiment was to determine the effects of four dietary ratios of SMP and WPC as the primary protein source in MR offered at high levels of up to 1050 g DM / d.

**Material and methods** Holstein Friesian calves (n=80) were randomly assigned, according to birth date, gender and birth weight (range 40.8 to 41.7 kg) to one of four dietary treatments. All MR (26% CP, 16% fat, reconstituted at 150 g / L) diets contained 25.35% dairy protein that was contributed by different ratios of SMP to WPC: T1 0:98; T2 29:69; T3 58:40; and T4 86:11, with amino acids contributing 0.65% of the protein. All calves received 3.5 L colostrum within 2 h of birth and a further 2 L within 12 to 16 h of birth and continued to be offered twice daily (2.5 L per feed) until 5 d of age. The MR was offered at a rate of 5 L / d from 5 to 10 d, 7 L / d from 11 to 34 d, 5 L / d from 35 to 49 d and 2 L / d from 49 to 55 d of age. Calf starter (CS) (18% CP) and fresh water was available ad libitum from birth, while chopped straw was offered from 56 d of age. Individual intake of MR, CS and water were recorded daily and body weight (BW) was measured weekly. Health status was assessed daily (faecal score, 1=normal consistency; 2=slightly liquid; 3=moderately liquid; and 4=primarily liquid). Pneumonia was recorded when calves displayed visual indicators as described in the Wisconsin Scoring System. Data was analysed using GenStat (version 18.1, VSN International Ltd). Statistical models included the fixed effects of time and MR treatment along with additional fixed

effects of birth weight and gender and housing block fitted as a random term. Data was analysed using a linear mixed model (feed intake and growth data) and a generalised linear mixed model (health data). Significance was determined by comparing the F-statistic against the F-distribution or Wald test compared against a Chi-squared distribution, as appropriate.

**Results** There were no treatment differences in total intake of; MR (range 40.9 to 41.1 kg DM; P=0.08) between 5 and 55 d of age, CS (range 52.4 to 58.1 kg DM; P=0.42) and water (range 144 to 159 L; P=0.59) between 5 and 70 d of age. Mean CS intake across treatments was >1.5 kg DM / d per calf and mean BW across treatments ranged from 71.9 to 74.5 kg at weaning at 56 d of age and between 86.3 and 89.4 kg at 70 d of age. The ADG from 0 to 14 d and 14 to 56 d of age were not related to protein source (SMP:WPC ratio; P>0.10). In the post weaning period ADG ranged from 0.97 to 1.14 kg / d between 56 and 70 d of age across treatments. There were no significant differences in number of episodes of scour and / or respiratory illness between treatments. Mean faecal score was below 2 across the experimental period and was not affected by dietary treatment.

**Table 1** Effect of dietary treatment (ratio of SMP to WPC) on calf growth and health

	Skim milk powder: Whey protein concentrate				SED	P-value
	T1 0:98	T2 29:69	T3 58:40	T4 86:11		
ADG 0 to 14 d of age, kg/d	0.40	0.31	0.34	0.38	0.065	0.544
ADG 14 to 56 d of age, kg/d	0.63	0.61	0.66	0.64	0.035	0.478
Scouring episodes, No.	0.39 (0.15-0.98)	0.54 (0.23-1.13)	0.63 (0.27-1.44)	0.75 (0.34-1.66)	-	0.485
Respiratory episodes, No.	0.57 (0.26-1.26)	0.67 (0.31-1.44)	0.71 (0.33-1.50)	0.44 (0.19-1.03)	-	0.679

**Conclusion** Growth rate and health status was similar across treatments in both the pre and post-weaning periods. In this experiment, WPC and SMP were comparable sources of dairy protein in MR diets offered to calves at high feeding rates.

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# Mineral contents of plant parts of *pennisetum purpureum* harvested from selected locations in South-west Nigeria

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**Application** Mineral is a chemical element required as an essential nutrient by organisms to perform functions necessary for life. Minerals originate in the earth and cannot be made by living organisms. Plants get minerals from soil.

**Introduction** Forages continue to represent the single most important feed resource for livestock in developed and developing countries (Jung and Allen, 1995). Thus the nutritional composition of forages varies from time to time and location to location (De Leeuw, 1979). Minerals are inorganic substances derived by plants through their roots which are the contact points with the soil. The mineral elements absorbed by plants are then converted into plant products. The latter are then fed to livestock for conversion into animal products (Martin and Roberts, 2000).

**Material and Methods** The research was carried out at the Laboratory of Pasture and Range Management Department of the Federal University of Agriculture, Abeokuta. The grass was sourced from selected locations across south-west Nigeria. The plant were separated into two (2) parts (stem and leaf) and oven dried for laboratory analysis. The study was laid out in 6 × 2 factorial design comprising of six (6) locations (Ewekoro, Odeda, Ibarapa, Ido, Oriade and Ejigbo) and two (2) plant parts (stem and leaf) and data collected were subjected to analysis of variance and the treatment means were separated using Duncan's Multiple Range Test using SAS (1999) package.

**Result** There was significant difference ( $p < 0.05$ ) on the mineral contents of plant parts of *P. purpureum* harvested at selected locations in south-west Nigeria. The Calcium (Ca) content ranges from 3.82g/kg in *P. purpureum* harvested at Ewekoro to 3.92g/kg in *P. purpureum* harvested at Ejigbo and *P. purpureum* harvested at Ido had the highest (112.83g/kg) the Potassium (K) content with the lowest value (75.89g/kg) observed in *P. purpureum* harvested at Ewekoro. Meanwhile *P. purpureum* harvested at Ido had the highest (8.49g/kg) Phosphorus (P) content with the lowest value (5.18g/kg) in *P. purpureum* harvested at Oriade, The stem fraction of harvested *P. purpureum* recorded has the highest value (114.31g/kg and

6.54g/kg) of Potassium and Phosphorus content respectively but the leaf fraction of the harvested *P. purpureum* had the highest value (6.03g/kg) in Calcium content. Meanwhile the leaf fraction of harvested *P. purpureum* was higher (87.57mg/kg) in Iron content but lower (8.26mg/kg and 14.14mg/kg) in Copper and Zinc respectively (Table 1).

**Conclusion** There were variations in the mineral contents of *P. purpureum* harvested at the selected locations. The leaf fraction of *P. Purpureum* harvested at the selected locations had the highest value in Ca, and Fe contents and the stem fraction recorded the highest value in K, P, Cu and Zn contents. Interestingly, the observed mineral contents in this study will sufficiently support optimal ruminant production in Nigeria.

**Acknowledgement** My unreserved and unalloyed appreciation goes to my Mr. Idehen John for his numerous contributions toward the successful completion of this work.

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**Table 1:** Mineral contents of plant parts of *Pennisetum purpureum* harvested at selected locations in South-West Nigeria

Factors	Ca	K g/kg	P	Fe	Cu Mg/kg	Zn
<b>Locations</b>						
Ewekoro	3.82 <sup>f</sup>	75.89 <sup>f</sup>	6.21 <sup>c</sup>	70.06 <sup>d</sup>	6.53 <sup>d</sup>	22.97 <sup>a</sup>
Odeda	5.89 <sup>b</sup>	89.83 <sup>d</sup>	5.77 <sup>e</sup>	66.25 <sup>e</sup>	11.33 <sup>b</sup>	10.38 <sup>e</sup>
Ibarapa	3.99 <sup>d</sup>	96.76 <sup>c</sup>	6.46 <sup>b</sup>	64.22 <sup>f</sup>	16.11 <sup>a</sup>	16.97 <sup>c</sup>
Ido	4.04 <sup>c</sup>	112.83 <sup>a</sup>	8.49 <sup>a</sup>	75.61 <sup>c</sup>	7.25 <sup>c</sup>	15.13 <sup>d</sup>
Oriade	7.60 <sup>a</sup>	76.95 <sup>e</sup>	5.18 <sup>f</sup>	82.60 <sup>b</sup>	6.43 <sup>d</sup>	14.38 <sup>d</sup>
Ejigbo	3.92 <sup>e</sup>	110.64 <sup>b</sup>	6.11 <sup>d</sup>	108.24 <sup>a</sup>	6.18 <sup>e</sup>	19.51 <sup>b</sup>
SEM	0.51	9.17	0.55	4.36	1.26	1.13
<b>Plant parts</b>						
Leaf	6.03 <sup>a</sup>	73.31 <sup>b</sup>	6.20 <sup>b</sup>	87.57 <sup>a</sup>	8.26 <sup>b</sup>	14.14 <sup>b</sup>
Stem	3.73 <sup>b</sup>	114.31 <sup>a</sup>	6.54 <sup>a</sup>	68.09 <sup>b</sup>	9.68 <sup>a</sup>	18.98 <sup>a</sup>
SEM	0.36	4.14	0.41	3.92	1.05	1.03

a, b, c, d, e, f : Means in same column with different superscripts are significantly ( $p < 0.05$ ) different.



# Restricting access of dairy cows to pasture under wet soil conditions

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Application Support dairy farmers making the decision to turn dairy cows out to pasture under wet soil conditions.

**Introduction** Extending the season to maximise grazed grass in the diet of dairy cows implies turnout in early spring and late autumn when inclement weather can cause wet soil conditions. Grazing on wet soils can lead to poaching damage and potentially lower grassland productivity. Restricting access time (RA) to pasture can reduce poaching and maintain daily grass intake when access time is split into two grazing sessions per day (Kennedy et al., 2009). However, implications at system scale, particularly the impact on annual herbage production, have not been examined.

**Material and Methods** Daily measured volumetric soil moisture content (VSMC) was used to govern the grazing management of four dairy systems in the present experiment from 09/2013 to 09/2016 at Solohead Research Farm (52°51'N, 08°21'W). Dairy cows were turned out to pasture or housed at any stage throughout lactation depending on VSMC, measured with a dielectric soil moisture probe. Systems were: (FT70) grazing full-time at VSMC<70 %; (RA60) RA at VSMC between 60 and 70 % and grazing full-time at VSMC<60 %; (RA50) RA at VSMC between 50 and 70 % and grazing full-time at VSMC<50 %; (FT50) grazing full-time at VSMC<50 %. Otherwise all systems herds were housed full-time. Herds with RA were allocated two 4-hour grazing sessions per day after each milking and spent the rest of the day indoors with no other feed. Herds that were housed full-time received grass-silage ad libitum during lactation. All systems were compact spring-calving with a stocking rate of 2.51 cows ha<sup>-1</sup> (24 cows per system). Each spring cows were blocked based on lactation number and calving date before one cow from each block was randomly assigned to each herd. Cows were turned out to graze 3 days after calving in mid-February and housed full-time at the end of November. The length of the grazing season was measured in terms of days at pasture where one grazing day was defined as when all cows per system were out full-time. The number of days with RA per system was recorded and counted as equivalent to one full grazing day. Herbage production of all paddocks was measured before each grazing and before each harvest of grass-silage. Poaching damage was measured as soil surface deformation (SSD) and hoof print depth (HPD).

Differences between systems were analysed in a mixed model in SAS 9.4 with system as fixed effect and block as random effect. Paddocks were experimental units for herbage and poaching measurements, individual cows for milk production variables.

**Results** The grazing season length of FT50 averaged 204 days per cow per year and 255 days for FT70, RA60 and RA50. RA (RA60: 30 days, RA50: 50 days) was applied mainly during spring and in late autumn. Milk protein concentration was higher in FT70 compared with system FT50 in spring (36.6 vs. 34.6 g kg<sup>-1</sup>, s.e.m. 0.28, P<0.001) and summer (38.9 vs. 37.5 g kg<sup>-1</sup>, s.e.m. 0.35, P=0.04) but did not differ between systems in autumn (mean 44 g kg<sup>-1</sup> s.e.m. 0.46). Milk solids production (milk fat plus protein) was higher in FT70 than FT50 in spring but not on an annual basis (Table 1). Poaching damage was reduced with time spend at pasture per system (HPD: 3.9, 3.5, 3.1 and 2.0 cm s.e.m. 0.11; SSD: 18.0, 16.0, 14.8 and 10.1 cm m<sup>-1</sup> s.e.m. 0.46 for FT70, RA60, RA50 and FT50, respectively; P<0.001). Annual herbage production was not affected by system (mean: 16500 kg DM ha<sup>-1</sup>, s.e.m. 715.7, P=0.43).

**Table 1:** Effect of grazing system on milk solids production (kg cow<sup>-1</sup> in period)

	FT70	RA60	RA50	FT50	S.E.M.	P-value
<b>Spring</b> (calving to 31 May)	213.9 <sup>a</sup>	217.8 <sup>a</sup>	208.5 <sup>ab</sup>	193.5 <sup>b</sup>	9.81	0.02
<b>Summer</b> (1 Jun to 31 Aug)	173.5	175.1	167.6	173.8	5.51	0.36
<b>Autumn</b> (1 Sep to dry off)	118.3	116.3	117.4	115.2	5.11	0.94
<b>Total</b>	505.7	509.2	493.5	482.5	14.93	0.17

**Conclusion** Restricting access time to pasture lowered poaching damage but did not impact on milk solids or herbage production. However, RA had a higher labour requirement and, hence, the FT70 system is likely to be more profitable than the RA systems. A shorter grazing season (FT50) was least profitable because of higher silage production and housing costs and lower milk sales. Recording VSMC can be an effective decision support to reduce poaching damage in grazing systems.

**Acknowledgements** This work was supported by DAFM funding under RSF-11-151

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# Effect Terminal Sire line on piglets' growth performance pre- and post-weaning

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**Application** The differences observed among treatments in offspring performance pre- and post-weaning demonstrates that farmers should consider the terminal sire line as a management tool to maximize the output of their sow population.

**Introduction** Arguably, the main concern of pig producers remains the increase in growth efficiency. While a lot of research and trials are done to evaluate dietary, nutritional and management interventions; the election of the most suitable terminal sire to use in each particular farm is widely overlooked. For a given sow population, it has been observed that the use of different terminal sire breeds can influence the offspring growth and feed efficiency (Cámara et al., 2016; Vermulen et al., 2016). The objective of this study was to identify whether the use of different terminal sire lines would influence offspring growth and efficiency of a given sow population.

**Materials and methods** A study was conducted in a commercial farm involving 2,563 piglets born from 226 different sows (LW x LR), across 6 consecutive weekly batches. At insemination, sows were allocated to one of the three Terminal sires (T. Sire): semen from T. Sire A, T. Sire B, or T. Sire C. Treatment groups were balanced by parity. All sows followed the same management and were fed the same gestation diet (DE 12.4MJ, CP 15.4%). During lactation all sows also followed the same management and were fed the same diet (DE 14.7MJ, CP 16.6%). Any sow with poor condition and/or less than 10 live born piglets and/or more than 5 stillborn/mummified were excluded from the trial. Piglets were weaned at 28 days of age. During stage 1 (S1) of the nursery period (4 weeks), pigs were housed in pens (groups of 70, 0.24m<sup>2</sup>/pig). Reproductive performance of sows was recorded at farrowing. All piglets were weighed after weaning (start of S1) and at 8 weeks of age (end of S1). From all the piglets monitored, 1,779 were individually tagged (from 226 different sows) and weighed at birth and at weaning. All pigs were fed the same creep and link diets during S1. Feed intake at a pen level was recorded. Data was analysed using GenStat 19th edition. For pre-weaning data, the sow was used as the statistical unit, and for the post-weaning data, pen was used as the statistical unit. Birth weight and S1 start weight were used as co-variates for weaning and end of S1 weight, respectively.

**Results** Offspring performance results are presented in Table 1. Pre-weaning, including only the individually tag animals (n=1749), no treatment effect was observed on birth weight (P>0.05) nor on born alive (P>0.05). However, Terminal Sire B and C had higher weaning weight than T. Sire A (P<0.05, respectively). After weaning and during S1, including all monitored pigs (n=2563), treatments did not differ for the S1 start weight nor for the S1 end weight (P>0.05, respectively). A tendency for treatment effect was observed for ADG (P<0.10) but no treatment effect was observed for ADFI (P>0.05). Nonetheless, T. Sire B had lower FCR than T. Sire C (P<0.05). Table 1 Effect of terminal sire on offspring performance during lactation and stage 1 (S1, 4 to 8 weeks of age) ab values with different superscripts differ statistically (P<0.05)

**Table 1** Effect of terminal sire on offspring performance during lactation and stage 1 (S1, 4 to 8 weeks of age)

	T. Sire A	T. Sire B	T. Sire C	SEM	P-value
n° pigs/n° sows	656/75	493/75	600/76		
Born alive/litter	15.28	15.57	16.13	0.213	n.s.
Birth weight, kg	1.29	1.35	1.29	0.008	n.s.
Weaning weight, kg	7.24 <sup>a</sup>	8.10 <sup>b</sup>	7.80 <sup>b</sup>	0.056	<0.05
n° pigs/n° pens	854/12	857/12	852/12		
S1 start weight, kg	7.69	8.12	8.09	0.249	n.s.
S1 end weight, kg	15.88	17.00	16.80	0.376	n.s.
S1 ADG, kg	0.31	0.34	0.32	0.004	<0.10
S1 ADFI, kg	0.49	0.48	0.51	0.705	n.s.
S1 FCR	1.58 <sup>ab</sup>	1.45 <sup>a</sup>	1.62 <sup>b</sup>	0.028	<0.05

ab values with different superscripts differ statistically (P<0.05)

**Conclusion** The Terminal Sire line used in a given sow herd can influence offspring growth performance during lactation and feed efficiency during the first 4 weeks post-weaning. Farms differ in their dam lines and management, the appropriate election of a Terminal Sire could be an easy tool to maximize herd output.

**Acknowledgements** The authors gratefully acknowledge funding from JMW Farms Ltd. and DAERA

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# Initial particle size distribution consistency of mixed rations fed to Holstein-Friesian cattle on UK commercial dairy farms

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**Application** Dairy farmers need to consider mixer wagon choice to minimise the particle size distribution (PSD) variation of the initial dispensed mixed ration (MR). PSD consistency recommendations requires investigation for UK MR given the only recommendations available are based on North American rations.

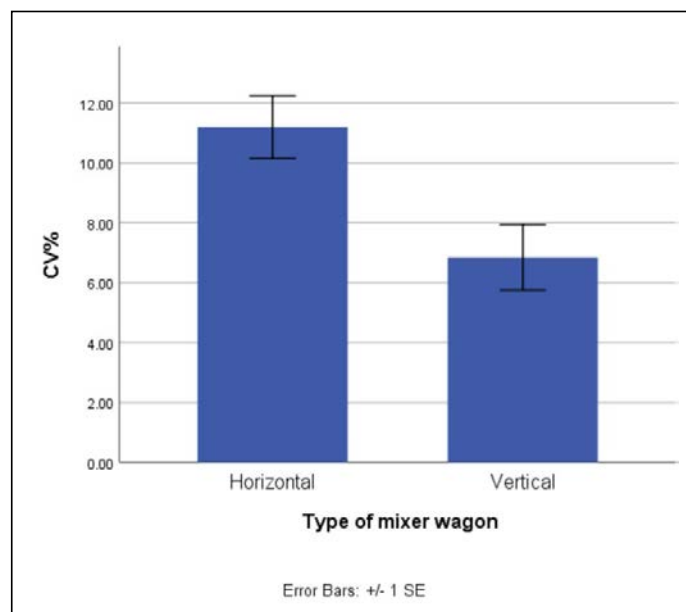
**Introduction** Mixed rations (MR) be this total mixed rations (TMR) or partial mixed rations (PMR) are now commonplace on UK dairy farms due to ever-increasing herd size and lactation yields (March et al., 2014). Rations should meet the cow's nutritional requirements although research has shown differences exist between the formulated ration and that actually delivered because of on farm mixing. This inconsistency, including PSD a key factor in the effective fibre aspect of the ration, has a negative effect on the performance and health of the cow (Zebeli et al., 2012). There is very little research in the UK surrounding the mix quality of MR and the reasons for such differences. Therefore, the aim of this study was to investigate the extent of PSD variation in the initial dispensed ration to dairy cows and the possible causes for this.

**Material and methods** MR samples were taken from different cow groups, including lactating and dry cows, present on five Holstein-Friesian dairy farms (n=12). For each of the MR loads, ten samples were taken equidistance along the feed bunk and a Penn State Particle Separator (PSPS) was used to determine PSD for each of these. Three PSDs were determined that included particle size >19mm, 8-19mm and > 8mm by using two sieves (19mm and 8mm) and a pan. The coefficient of variation (CV%) was calculated for each of the screenings (n=36) of the MR loads using the methodology described by Oelberg and Stone (2014). IBM SPSS Statistics (version 26) was used for data analysis. Association relationships between CV% of PSD and influencing ration factors were examined. Comparison of the CV% between horizontal and vertical mixer wagon type was made using an independent samples t-test.

**Results** Overall PSD inconsistency along the feed bunk of the initial fed-out MR was noted to be far higher than the North America recommendation of CV% being below 5%. There was no significant correlation with either dry matter %, forage % of the diet on a dry-matter basis, forage NDF% on a dry-matter

basis or wagon fill (fresh weight). However it was found the type of mixer wagon had a significant ( $P = 0.008$ ) influence on the spatial consistency of the mix, where the vertical wagon type had a lesser CV% (6.85%) compared to the horizontal mixer type (11.2%) (Figure 1).

**Figure 1** Influence of mixer wagon type on the PSD mix quality (CV%)



**Conclusion** Higher levels of spatial variation exist in MR on UK dairy farms when compared to North American rations. This may be explained with grass silage as the prominent forage UK MR, whereas this tends to be maize silage and alfalfa in North America. Although there was no associations found between key ration factors and PSD variation, the type of mixer wagon was found to be significantly influential. Rations mixed in vertical mixer wagon resulted in far greater initial spatial PSD consistency when compared to horizontal mixer wagons.

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# Herbage yield of two varieties of *Panicum maximum* as influenced by plant population density in South-western Nigeria

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**Application** Under and over plant population density affects the growth and dry matter yield of *Panicum maximum* varieties.

**Introduction** Forages have many potential benefits for livestock quality. The use of highly productive good quality grasses is necessary for improved animal productivity (Agishi, 1985). Improved grass varieties have been recommended for intensive livestock production in Nigeria due to their high forage production and nutritive value (Olanite et al., 2010). *Panicum maximum* is one of the most widely grown forages in the tropics. However, environmental effects on the plants can cause physiological deterioration and quality losses. Managing these losses is quite pertinent to achieving sustainable pasture crop production. As part of this maintenance, it is very important to maintain optimum plant population. Optimum planting population plays an important role in contributing to the high yield because thick plant population will not get proper light for photosynthesis and can easily be attacked by diseases and a very small population will also reduce the yield of the plants (Pookpakdi and Pataradilok, 1993).

**Material and methods** An area of land was cleared and was divided into 5×4 m plots such that there was a 1 m boundary between plots. The two varieties of *Panicum maximum* were established using two crown splits per hole. The study was laid out as a 2×3 factorial arrangement, in a split-plot design. This constituted of two *P. maximum* varieties (i.e. var. local and var. ntchisi) as the main plot and three plant population as the sub-plot (22,500 (low) 37,500 (medium), and 67,500 (high) plants/ha) replicated three times. The plots were well labeled with the treatments. The experimental area was kept weed free throughout the period of the experiment. Data were analyzed using the R statistical software. The grasses were harvested at 10 weeks after planting using a 1.0×1.0 m quadrat. In each quadrat area samples, the above ground vegetative plant material were harvested from 10 cm above ground level using a sickle. Following harvesting, the fresh weight was recorded and sub-samples of 500 g was taken. Subsample of 500g was also taken and separated into leaf and stem fractions and the proportion of each were calculated. The harvested grasses were oven dried at 65°C until constant dry weight was attained to determine dry matter percentage. The values obtained were used to calculate mean dry matter yields (kg/ha).

**Results** Cumulative fresh and dry matter yield, the leaf fresh and dry matter yield, as well as the stem fresh and dry matter yield increased at the highest plant population for the local variety only. There were no significant differences ( $p>0.05$ ) in the herbage yield of the Ntchisi variety planted at high or low plant population.

**Conclusion** At the highest planting population, the local variety produced higher yield than the Ntchisi variety.

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**Table 1:** Interaction of varieties × plant population on the herbage yield of *Panicum maximum*

Panicum maximum Varieties	Plant population (plants/ha)	Cumulative fresh yield			Leaf yield			Stem yield		
		Fresh	DMY	% DM	Fresh	DMY	% DM	Fresh	DMY	% DM
Local	22,500	3.51 <sup>b</sup>	2.33 <sup>b</sup>	67.17	1.44 <sup>b</sup>	0.91 <sup>b</sup>	63.06	2.07 <sup>b</sup>	1.42 <sup>b</sup>	69.68
	37,500	5.01 <sup>b</sup>	3.64 <sup>ab</sup>	70.67	2.06 <sup>b</sup>	1.30 <sup>b</sup>	62.52	2.94 <sup>b</sup>	2.34 <sup>ab</sup>	75.43
	67,500	8.42 <sup>a</sup>	5.51 <sup>a</sup>	65.17	3.48 <sup>a</sup>	2.15 <sup>a</sup>	62.00	4.95 <sup>a</sup>	3.36 <sup>a</sup>	68.27
Ntchisi	22,500	3.77 <sup>b</sup>	2.65 <sup>b</sup>	67.17	1.43 <sup>b</sup>	0.88 <sup>b</sup>	62.10	2.34 <sup>b</sup>	1.78 <sup>b</sup>	70.67
	37,500	3.09 <sup>b</sup>	2.18 <sup>b</sup>	70.67	1.19 <sup>b</sup>	0.73 <sup>b</sup>	62.97	1.90 <sup>b</sup>	1.45 <sup>b</sup>	68.53
	67,500	3.88 <sup>b</sup>	2.70 <sup>b</sup>	70.67	1.41 <sup>b</sup>	0.88 <sup>b</sup>	62.92	2.47 <sup>b</sup>	1.82 <sup>b</sup>	73.87
SEM		1.22	0.88	2.84	0.59	0.37	0.58	0.65	0.52	4.64

ab: Means on the same column with different superscript differ significantly ( $p<0.05$ )

SEM: Standard error of mean

DMY: Dry matter yield, % DM: Percentage dry matter

# Validation of piglet vitality scoring systems used in a commercial farm scenario

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**Application** A robust piglet post-birth vitality scoring system is important to assess the efficiency of management systems on a commercial farm.

**Introduction** Newborn piglet vitality can be used to assess the effectiveness of farm management systems and can be used to identify individuals that require assistance or euthanasia. Physiological and behavioural measures taken shortly after birth can be used to score a piglet's vitality. However, is it not always possible for personnel to be present during farrowing therefore, the objective of this study is to assess piglet vitality at birth, and at 6 and 24 hours after birth, to determine if a scoring system at 6 or 24 hours could be used to indicate piglet survival.

**Material and methods** This study was conducted on a commercial pig farm in Co. Tyrone. A total of 120 piglets, from nine randomly selected sows and gilts (Landrace x Large white) were used in the study. For each sow, parity, total piglets born, number of piglets born alive, number born dead, number of mummified piglets, farrowing date, farrowing start time and duration were recorded. For each piglet, time of birth, sex, weight and rectal temperature were recorded, and piglets were vitality scored as described in Table 1 (excluding movement). Scores were totalled, to give a combined vitality score out of 14. Piglets were then given a unique identification number on their back. At 6 hours and 24 hours old ( $\pm 4$  hours), living piglets were weighed and vitality scored for the characteristics of snout colour, muscle tone and movement (described in Table 1). Survival at 48 hours ( $\pm 4$  hours) after birth was recorded. Table 1. Description of the physiological and behaviour variables evaluated to score piglet vitality.

Table 1

Parameter	Score
Respiration	Absent = 0, Respiration Apneic for 15 seconds, with continued dyspnea and irregularity = 1, Strong respiratory efforts within 15 seconds and establishment of a normal rhythm = 2.
Muscle Tone	Flaccid = 0, Poor = 1, Good = 2.
Attempts to stand	None within 5 minutes = 0, Within five minutes = 1, Within one minute = 2.
Number of complete circles round an enclosure	Piglet not able to turn its body axis 360° from its initial orientation nor able to walk along the limits of the bucket = 0, Piglet can turn its body axis 360° from its initial orientation or walk along the limits of the bucket once within 30 seconds = 1, Piglet can turn its body axis 360° from its initial orientation or walk along the limits of the bucket at least twice within 30 seconds = 2.
Snout Colour	Cyanotic = 0, Pale = 1, Pink = 2
Meconium staining	Severe = 0, Mild = 1, Absent = 2
Udder stimulation	None within 5 minutes = 0, Within 5 minutes = 1, Within one minute = 2.
Movement	Piglet makes no attempt to escape from under tea towel = 0, Piglet escapes from under tea towel in 15-30 seconds = 1, Piglet escapes from under tea towel in less than 15 seconds = 2.

**Statistical analysis** Data were analysed using R version R-3.5.3. To compare the mean piglet vitality scores at birth, 6 hours and 24 hours old to survivability at 48 hours old, Welch's t-test was used for normally distributed data and Wilcoxon ranked sum test for non-normally distributed data.

**Results** Average sow parity was 2.88 and mean litter size was 20.56. The average number of liveborn piglets per litter was 19.0, with mean number of mummified piglets 0.33 per litter and mean number of stillborn piglets 1.38 per litter. Average farrowing duration was >4.5 hours. Of the 112 born alive 68 were male and 44 were female. A total of 104 piglets survived to 48-hours old, of the eight piglets that died, one had scour, one was euthanized, two piglets were crushed by the sow and the cause of death was unknown for the remaining four. The mean birth weight of piglets was 1.20kg, the mean rectal temperature at birth was 35.8°C and the mean vitality score at birth was 8.92. Piglet birthweight had a significant effect on survival to 48 hours old ( $P = 0.013$ ). Piglets that survived to 48 hours old tended to have higher vitality score at birth ( $P = 0.070$ ) and at 6 hours old ( $P=0.053$ ) than those that did not survive to 48 hours old. Piglets that survived to 48 hours old did not have a higher vitality score at 24 hours old than those that did not survive to 48 hours old ( $P = 0.123$ ).

**Conclusion** Piglet survival is a complex issue, affected by multiple factors, as such survival is inherently hard to predict. In this study, piglets that survived to 48 hours old tended to have higher vitality scores at birth and 6 hours old. The results also show that birthweight had a significant effect on survival to 48 hours old. Therefore, a combined measure of birthweight and vitality, taken before 24 hours old, could be used to predict survival. Alternatively, vitality score at 6 hours could be used on a commercial farm as predictor of survival. As the sample size in the study was small, validation of results in necessary with a larger sample size.

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# Labour saving associated with alternative Rising Plate Meter sampling techniques

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**Application** Reductions in time required to estimate herbage mass are likely to encourage the uptake of recording technologies by farmers and thus help drive improvements in pasture utilisation across the industry.

**Introduction** The profitability of pasture-based ruminant production systems is reliant on timely and accurate grazing management decisions (McSweeney et al., 2019). Precise monitoring of herbage mass in the unit of kg DM/ha can contribute to the relative success of these decisions (O'Donovan et al., 2002). Technology can aid accurate collection of this information, with many commercial tools already in common usage. Nonetheless, likelihood of these technologies being adopted by farmers is more often determined by factors such as cost, convenience and personal preference than accuracy of measurement (Lile et al., 2001). For this reason, Rising Plate Meter (RPM), which measures compressed sward height to estimate herbage mass, has become one of the most widely used methods globally. A 'pasture walk' with RPM involves multiple measurements from each paddock to make information spatially representative and, to satisfy this condition, manufacturers typically advise readings along a long 'W' transect. Anecdotal evidence in the UK suggests, however, that this protocol is often perceived as an excessive labour requirement and, as such, thought to be a contributing factor to the relatively low up-taking. Motivated by this observation, this study investigated the relative accuracy of measurements obtained through a straight diagonal walk, an alternative protocol that is considerably less labour intensive, with the higher-level aim of improving pasture utilisation rates across the livestock industry.

**Material and methods** The study was carried out at the permanent pasture farmlet (21.7 ha, 7 paddocks) of the North Wyke Farm Platform, a BBSRC National Capability grazing trial in Devon, UK (Orr et al., 2019). Data was recorded using a Jenquip EC20 Bluetooth Electronic RPM during the 2019 grazing season between March and October. Measurements were taken on a weekly basis with both 'W' and diagonal patterns recorded using the same equipment and operator (maps available on request). Species composition was dominated (>60%) by perennial ryegrass (*Lolium perenne* L.), with a smaller biomass of creeping bent (*Agrostis stolonifera*), Yorkshire fog (*Holcus lanatus*) and marsh foxtail (*Alopecurus geniculatus*). All statistical analyses were conducted using R version 3.5.1.

## Results

Across all seven paddocks and throughout the season, no substantial difference was observed in either estimated total pasture cover, or its within-paddock variation, between the two sampling strategies. On the largest paddock 'Burrows' (6.49 ha), for example, the correlation coefficient between the two methods was 0.95 ( $p < 0.001$ ), with the Mann-Whitney test not rejecting the null hypothesis that there is no systematic difference ( $p = 0.60$ ) (Figure 1). The average number of footsteps taken on diagonal pasture walks were estimated to be 404 paces compared to 1089 paces estimated for the 'W' pattern, a reduction of 63%.

**Conclusion** Herbage mass monitoring with RPM is likely to be similar under different walking patterns. Given the time and psychological constraints faced by farmers, recommending a diagonal pattern will save labour and potentially increase the likelihood of these techniques being used. Soil, climatic and topographical conditions under which this recommendation is valid are currently being investigated.

## Acknowledgements

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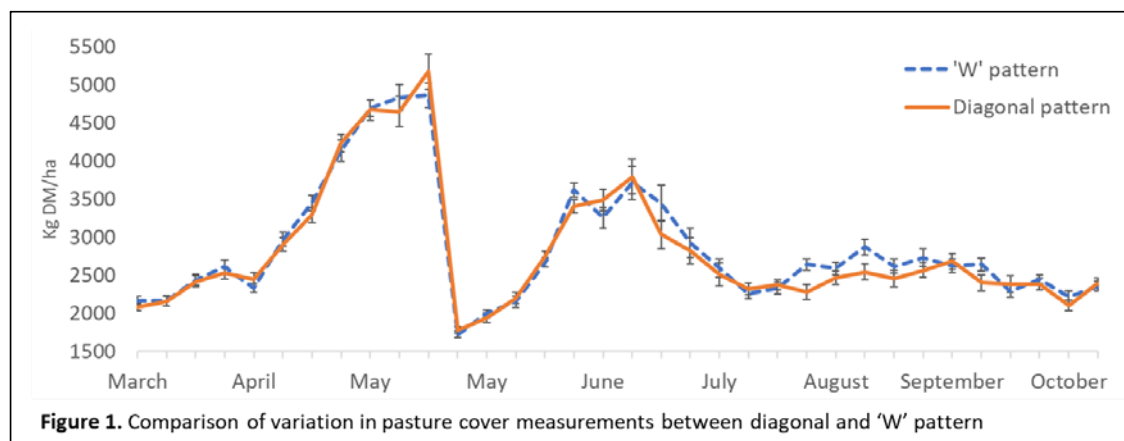


Figure 1. Comparison of variation in pasture cover measurements between diagonal and 'W' pattern

# Genetic relationships between ketosis and potential indicator traits

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**Application** Ketosis with subclinical and clinical symptoms is complex. The results showed that different information sources and traits can be used to improve the metabolic disease resistance of dairy cows.

**Introduction** In dairy cattle, the reduction of metabolic diseases by both, management and breeding, has gained in importance. Predictors from on-farm recording or laboratories may be valuable in this context (Pryce et al., 2016). The specific objective of this study was the genetic analysis of (sub)clinical ketosis and possible indicator traits.

**Material and methods** Within the project 'Efficient Cow', a one-year of extensive data collection was carried out in more than 150 farms keeping Fleckvieh, Brown Swiss and Holstein cows in the year 2014. All farms took part in the routine national health monitoring programme. In addition to routinely recorded data (e.g. milk yield, fertility, disease data), data of novel traits were recorded. Subclinical ketosis (KTEST) was detected by using the milk Keto-Test from ELANCO, which measures the milk  $\beta$ -hydroxybutyrate. The Keto-Test was performed twice during early lactation, at days 7 and 14 (0, 1 or 2 positive tests). Body condition score (BCS) was recorded by trained staff at each test-day on a scale from 1 (severe underconditioning) to 5 (severe overconditioning) in increments of 0.25. Fat-protein-ratio (FPR) was available for each test-day from the routine milk recording system. For this analysis, only the BCS and FPR of the first test-day were used. For FPR and clinical ketosis (KET), all available records between the years 2013 and 2015 were used. All other traits were recorded in 2014 only. Using mid infrared (MIR) spectral data from milk performance recording samples, LKV Baden-Württemberg has recently developed two alternative ketosis indicators (KetoMIR1 and 2; Werner et al., 2019). All MIR results during the first 100 days in lactation were used for the project year 2014. Ledinek et al. (2019) described farm characteristics and feeding systems. For this analysis, only the main breed dual purpose Fleckvieh was considered. Depending on trait, number of records ranged from 1 806 (KTEST) to 8 679 (KET yes or no). The pedigree comprised 20 313 animals. The model for genetic parameter estimation included the fixed effects of parity\*age at calving and year\*season of calving and the random effects of herd\*year, permanent environment and

animal additive genetic effects. Additionally the following fixed effects were considered for certain traits: type of recording (KET), days in milk (BCS, FPR, KetoMIR), scorer (BCS), and number of ketotests (subclinical ketosis).

**Results** Heritabilities ranged from 0.01 (KET) to 0.19 (KetoMIR1) and were in the expected range. Overall, genetic correlation estimates between traits were consistent, with the exception of the negative relationship between KET and FPR. KTEST had moderate to high positive genetic correlations to KET, FPR and KetoMIR. Correlations between KetoMIR and KET were lower than those between KetoMIR and KTEST. BCS was negatively correlated with all traits, which confirms that cows with a low BCS at the beginning of lactation are more susceptible to metabolic diseases.

**Table 1** Genetic parameters (heritabilities on the diagonal, genetic correlations on the off-diagonal) for clinical ketosis (KET), fat-protein ratio (FPR) and body condition score (BCS) at first test day, milk ketosis test (KTEST) and two alternative ketosis mid-infrared spectra estimates (KetoMIR1, KetoMIR2)

	KET	FPR	BCS	KTEST	KetoMIR1	KetoMIR2
KET	0.01	-0.41	-0.36	0.70	0.15	0.09
FPR		0.11	-0.25	0.22	0.60	0.81
BCS			0.16	-0.25	-0.43	-0.28
KTEST				0.07	0.41	0.45
KetoMIR1					0.19	0.66
KetoMIR2						0.09

**Conclusion** Metabolism with subclinical and clinical symptoms is complex. The results indicate that different information sources and traits can be used to genetically improve the metabolic disease resistance. BCS, mid infrared spectra and ketosis tests are promising auxiliary traits.

**Acknowledgements** The authors gratefully acknowledge funding from BMNT, the Federal Provinces of Austria, ZAR and its member organisations (Project Efficient Cow) and BMVIT, BMDW and the Provinces of Lower Austria and Vienna (COMET Project D4Dairy, handled by FFG).

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# In vitro methane inhibition in continuous culture

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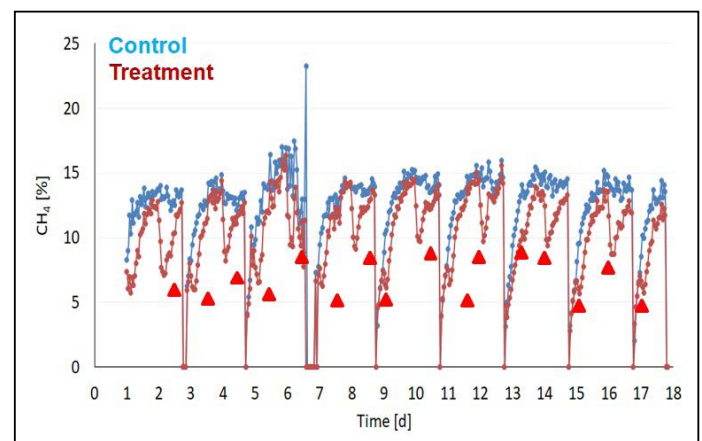
**Application** As methane inhibitors are developed, the need to study dosing regimens will become important. Dosing regimes may also affect the main VFA differently, having an impact on methane emissions.

**Introduction** The experimental compound 3Nitrooxypropanol (3NOP) is a methanogen inhibitor that has proven efficacy when mixed as a component of a total mixed ration fed to ruminant animals. However, other dosing systems may be needed for other feeding systems. 3-NOP leads to quick methane reduction following dosing, but, due to its mode of action, this is short lived (Hristov et al. 2015). We postulated that a continuous dosing regimen would have a longer inhibitory effect. This inhibitor is also a useful tool to study proxies that could be used as an alternative to methane measurement methods. The aim of the study was to analyse rumen metabolites in continuous flow fermentors to gain insights into how different dosing of a methane inhibitor affects them, as well as identifying rumen metabolites that could be used as proxies for methane emissions.

**Materials and methods** The study used a fully automated continuous rumen in vitro system (ACRIS). Rumen fluid to inoculate ACRIS was obtained from two fistulated, pasture-fed cows. The feed for ACRIS was a medium quality ryegrass pasture ground to pass a 2-mm sieve. The feed was dosed in two equal portions of 12 g, each over a 1-h period in the morning and afternoon. The treatments were 1) a single daily pulse dose of 3NOP before the morning feed, and 2) a continuous dose of 3NOP delivered via a syringe pump. The 3NOP was administered as CLOU-2 (20% 3-NOP, 80% 1,2-propanediol) in daily volumes of 0.8 ml. Control vessels received a pulse dose of 1,2-propanediol only, the solvent of 3-NOP in CLOU-2. A total of 6 vessels were used, 2 for each treatment, and the run was repeated in duplicate leading to 4 replicates for each treatment. The starting dose was 5 µmol/vessel/day and was increased depending on methane inhibition achieved, aiming for a reduction of approximately 30%, which is the level of inhibition reported from in vivo studies with this inhibitor (Dijkstra et al. 2018). The system was run for 21 days. Statistical analysis involved running an ANOVA comparing the 3 treatments in terms of both methane emissions and total VFA variability. Samples for metabolite analysis were taken from all vessels twice daily, before and after the pulse dosing of CLOU-2.

**Results** Applying CLOU-2 in a single pulse dose at a final dose of 7.5 µmol/d led to a stable 25% decrease in methane emissions. However, the direct effect of the methane inhibitor was very short and lasted only for a few hours before returning to control levels (Figure 1). The proportion of acetate in the pulse dosed vessels was lower over the whole experiment while the proportion of butyrate was increased. The effect on propionate was much less pronounced than butyrate. The continuous dose was increased in steps of 2.5 µmol/d day in order to reach the desired level of methane inhibition. Once this level was reached, the dose was maintained until the end of the run. Effect on methane were only observed when the concentration reached 17.5 µmol/d after 7 days. At this concentration hydrogen emissions increased and acetate proportions decreased ( $p=0.026$ ) while butyrate and propionate increased ( $p>0.05$ ). A more detailed analysis of metabolites is being undertaken and therefore will not be discussed currently.

**Figure 1** Pulse dose effect (red triangles) on methane emissions



**Conclusion** Continuous dosing required at least double the dose of pulse dosing to obtain the same level of inhibition. The two dosing regimens show similar effects at a VFA level. The results provide important information regarding slow release of inhibitors that can be adapted in future in vivo work. Acknowledgements The authors gratefully acknowledge funding from DSM. AgResearch and SRUC provided resources to undertake the study.

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# Can Blink Rate Testing Suggest Handling Stress in Commercial Sheep?

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**Application** This hypothesis suggests spontaneous blink rate analysis may be used to identify handling stress in commercial sheep, thereby improving livestock welfare and handling techniques.

**Introduction** In the commercial sheep industry, there is little research and few known facts relating to external behavioural indicators for stress in sheep. Existing research into stress identification relies on cortisol testing. Higher levels of cortisol in plasma and saliva are linked with elevated stress levels in sheep. Spontaneous Blink Rate (SBR) is a known method of recognising stress in humans, and recently, proven to be an effective stress recognition technique in horses (Roberts. et al. 2015). There has been no research into SBR for commercial sheep. This study investigates the value of SBR in identifying stress in sheep and if blink rate data is collectable. To test this theory, a comparative study using two handling systems (HS) with different designs was proposed. The first system was designed by Dr. Temple Grandin and demonstrated to reduce handling stress. The second system is a common design found on sheep farms across Britain.

**Materials & Methods** Previous studies into SBR in humans and horses used video recordings to collect data. Following this protocol and based on past research techniques, video recording analysis was the chosen SBR data collection method. The device used for testing was an iPhone SE, stabilised with a Manfrotto tripod. To gain enough data for comparative analysis, a paired form of study was chosen, using 19 mule sheep. Footage, once compiled, was observed and assessed for SBR variations between each HS. Resting spontaneous blink rate (RSBR) was later noted and assessed as a control group. The ewes were filmed moving through two different race systems and at rest, in a holding pen. Each HS was chosen to represent two different layouts for comparison, using a commercial flock. The first system incorporated a HS design with techniques advised by Dr. Temple Grandin (GHS), to encourage the calm controlled movement of sheep through the race, as seen in figure 1.

The second layout was a typical example of a standard handling system (SHS), used on farms throughout Britain and described in Figure 2.

In both handling tests sheep were filmed individually for two minutes. Each HS test lasted 45 minutes allowing the cohort to move from the gathering pen into the chute where filming took place. For both HS, the sheep were moved to gathering pens and left for a period of 40 minutes before HS tests started, to allow the sheep to rest. This follows the recommendation that livestock require a rest period post handling (Grandin, 1997). After the final handling test, sheep remained in holding pen for two hours before RSBR recordings were taken.

**Results** Highest SBR was recorded in SHS. Both GHS and SHS recorded a higher mean SBR than RSBR. The standard deviations are small compared with mean values, showing small variance within data, therefore, the reliability of the data is high. Mean and standard

deviation for each test were measured in GHS, (mean = 9.16, SD, 2.63). SHS (mean = 17.89, SD 4.95). RSBR (mean = 4.74, SD, 2.31).

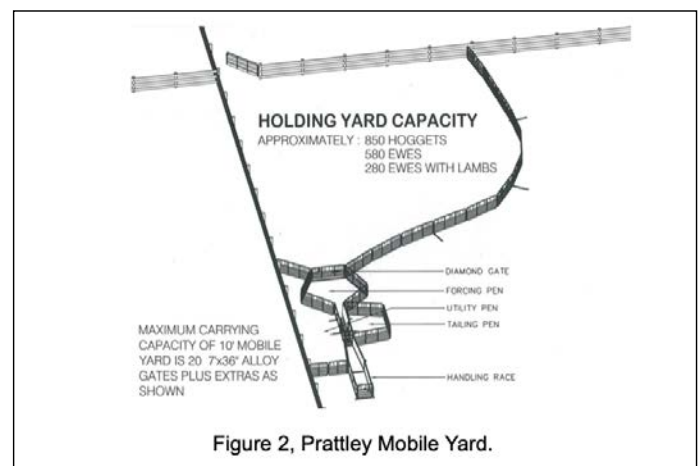
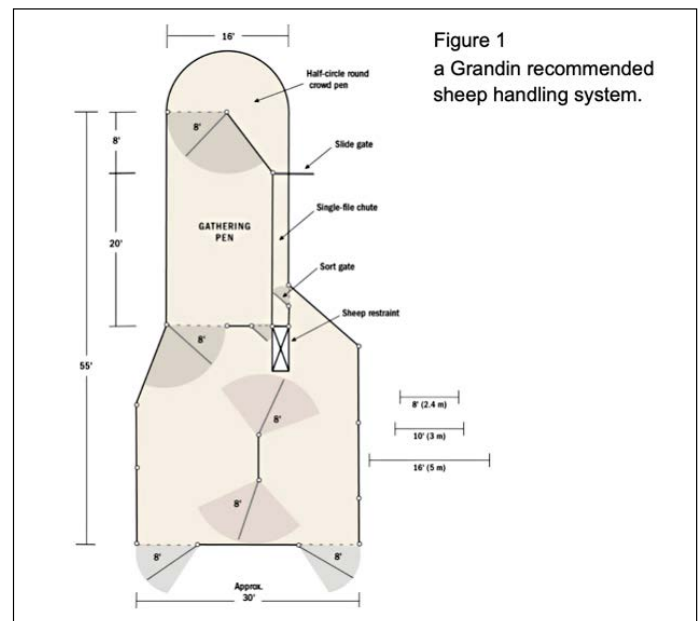
The T test shows a difference in condition between GHS and SHS which is significant and the probability it occurred by chance is  $p = 0.0005$ . ( $t = 6.97$ ,  $df = 18$ ,  $p = 0.0005$ ). Therefore, reject null hypothesis, accept alternative hypothesis; the difference between GHS and SHS is significant. An ANOVA test was conducted to compare SBR in GHS, SHS and RSBR. There was a significant SBR difference between the three environments ( $F = 76.42$ ,  $p = 0.00001$ ). Therefore, reject null hypothesis, accept alternative hypothesis; there is a significant difference between the three environments.

**Conclusion** The results from this study demonstrate the average GHS response, per subject, was half that of HS. RSBR was lower than each HS. Therefore, suggesting SBR may indicate handling stress.

**Acknowledgements** This project would not have been possible without the financial support offered by Universities Federation for Animal Welfare.

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# The ensilage of brown seaweed (*Saccharina latissimi*) as a feed source for ruminant livestock

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**Application** Ensiling might be a suitable method for preserving brown seaweeds as a livestock feed.

Introduction Livestock grazing uses a quarter of the planet's ice-free land, and one-third of croplands are used for livestock feed production. Seaweeds might be a suitable alternative for ruminant feeding in coastal areas, or countries closely connected to the sea, such as Ireland and Great Britain. Within this context, brown seaweeds (Phaeophyceae) are attracting high attention given their wide availability, ease of harvest and the content of polyphenols (phlorotannins) which have antioxidant and antibacterial properties (Wang et al., 2009). Nevertheless, seasonal harvesting oriented to maximise biomass and nutritional content implies the challenge of preservation as seaweeds degrade rapidly due to their high content of water (70–90%). Ensilage might be a suitable method of preserving brown seaweed as a livestock feed.

**Material and methods** Samples of *Saccharina latissimi* were randomly collected from different areas of a sheltered beach in Bangor, Co. Down, Northern Ireland in July 2017. Seaweeds were washed, wilted for 24-hours and cut (<5cm). In total, 33 subsamples (100g) were formed. The first three subsamples were immediately frozen, freeze-dried and milled through a 1mm-sieve to determine the chemical composition prior to ensiling (day 0). The rest were randomly assigned into each of the 2 silage treatments: natural silage without inoculant (90c); and silage with a commercially available, lactobacillus-based inoculant (90i). 15 bags for each treatment were vacuum-packed, and the bags were stored in the dark at room temperature (18–20°C) for 90 days. 5 samples/treatment were analysed immediately after opening for fermentation characteristics (pH, ammonia-N (NH<sub>3</sub>-N), lactic and acetic acid). Fresh (day 0) and ensiled seaweed were analysed for dry matter (DM), ash, Neutral Detergent Fibre (aNDF), Acid Detergent Fibre (ADF), crude protein (CP) (Nx5.0) (Angell et al., 2016) and total phenolic content (TPC). In vitro true dry matter digestibility (IVTDMD) was determined by incubating the silage in buffered rumen fluid for 48h according to the DaisyII incubation method, followed by NDF digestion in an ANKOM 200 Fibre Analyser. The data were analysed using JMP (14.0) using one-way analysis of variance (ANOVA). Statistically significant differences were further analysed using a post hoc test (Tukey HSD).

**Results** The ensiling process resulted in a reduction in ash, NDF and ADF fractions independently ( $P>0.05$ ) of the use of inoculant. In contrast, 90i samples showed an increase of the WSC (75g/kg DM) compared to day 0 (57g/kg DM) and a lower decrease ( $P<0.05$ ) of the CP than 90c samples. After 90 days, there was no change in the NH<sub>3</sub>-N and IVTDMD, which remained higher than 90%. The lactic acid content of *S. latissimi* silages (45–60g/kg DM) was comparable to grass silage (Driehuis et al., 2001); the high lactic acid to acetic acid ratio (>14) suggested the ensilage process followed a homolactic fermentation. No effect of inoculant ( $P>0.05$ ) was observed on fermentation characteristics. Results suggest that epiphytic bacteria were able to ferment the seaweed structural carbohydrates (NDF/ADF) to ensure a suitable pH decline (<4). The content of ash, although reduced during the ensiling process, should be monitored when intended for animal feeding.

**Table 1.** Chemical composition, fermentation characteristics and effect of inoculant (90c= untreated; 90i= inoculated) on *S. latissimi* after 90 days ensiling (g/kg DM unless stated otherwise)

S. latissimi					
	Day 0	Day 90		SEM	P
		90c	90i		
DM (g/kg)	190	205	202	3.62	ns
Ash	242 <sup>a</sup>	156 <sup>b</sup>	151 <sup>b</sup>	7.75	<0.001
CP	60.3 <sup>a</sup>	41.1 <sup>c</sup>	47.4 <sup>b</sup>	0.78	<0.001
WSC	56.6 <sup>b</sup>	63.6 <sup>ab</sup>	75.2 <sup>a</sup>	3.68	0.028
aNDF	385 <sup>a</sup>	86.4 <sup>b</sup>	86.4 <sup>b</sup>	16.63	<0.001
ADF	145 <sup>a</sup>	59.7 <sup>b</sup>	66.3 <sup>b</sup>	5.64	<0.001
TPC	0.71 <sup>a</sup>	1.62 <sup>a</sup>	1.31 <sup>a</sup>	0.07	0.009
IVTDMD (%)	94.6	95.1	94.0	0.62	ns
pH	-	3.90	3.91	0.05	ns
Lactic Acid	-	56.6	54.9	2.69	ns
Acetic Acid	-	3.93	3.76	0.44	ns
NH <sub>3</sub> -N (g/kg N)		12.2	13.0	3.93	ns

Means within the same row with different superscripts are significantly different ( $P<0.05$ )

**Conclusions** *S. latissimi* showed suitable ensilability characteristics even without the use of a lactobacillus-based inoculant and therefore can be explored further as an ensiled livestock feed.

**Acknowledgement** The authors gratefully acknowledge funding the Department for the Economy Northern Ireland (DfE) and Dave Davies from Silage Solutions.

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# Effect on sunflower seed and sunflower seed silage in the milk composition in dairy ewes

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**Application** High oil content foods, like sunflower seeds (SFS), induce milk fat depression when it is included in the diet. The silage of sunflower seeds (SFS silage) improve nutrients disposition in rumen, without decrease milk fat content and increases nutrients composition on milk.

**Introduction** Nowadays, exist an increasing interest to provide high quality milk products to satisfy the new demands. The supplementation with SFS is an strategy to improve the quality on milk (Beuchemin et al., 2009). Despite its high lipid content improves the composition of fatty acids in milk; the decrease of fat milk and yield is a negative effect. The fermentation silage process, improves the nutrients disposition in the rumen, which led to an increase of the milk composition. The aim of the present study was to examine changes in the composition of milk with the inclusion of SFS silage in contrast to the SFS (SFS), so to improve on milk quality without affecting the milk production.

**Table 1.** Milk yield (kg/d) and chemical composition (g/100 g) of the milk in dairy ewes supplemented with sunflower seeds

Item	Control diet	SFS diet	SFS silage	SEM	Treatment	Period	TxP
Milk Yield,kg/d	0.6431	0.7893	0.6260	0.1452	0.6958	0.6884	0.0038
FCM, kg/d	0.633	0.720	0.527	0.1405	0.8819	0.2776	0.0206
FPCM, kg/d	0.601	0.695	0.517	0.1333	0.8408	0.3431	0.0234
Protein	4.23 <sup>b</sup>	4.38 <sup>ab</sup>	4.58 <sup>a</sup>	0.1057	0.0438	0.0061	0.5106
Fat	5.78	5.69	4.79	0.4386	0.8103	0.0106	0.0138
Lactose	4.00 <sup>b</sup>	4.15 <sup>ab</sup>	4.34 <sup>a</sup>	0.1008	0.0429	0.0059	0.4974
NFS	8.93 <sup>b</sup>	9.29 <sup>ab</sup>	9.72 <sup>a</sup>	0.2297	0.0275	0.0052	0.2218

**Material and Methods** Six multiparous East-Friesian sheep into its first stage of lactation, were allocated into individual metabolic cages. According to a double 3 x 3 Latin square design, with 21 days experimental periods, 16 adaptation to the diets and 5 sampling days. The control diet, which consists of maize silage ad libitum and concentrate supplementation, in a ratio 60:40 respectively; control diet plus sunflower seed (SFS) supplemented with 5% in the whole diet a ratio (60:35:5, respectively) and control diet supplemented with SFS Silage 5% in the whole diet a ratio (60:35:5). The sheep had continuous access to water and were milked at 1500 h every day. The chemical composition of the milk, Fat corrected milk (FCM, 5% fat) and fat and protein corrected milk (FPCM) was determined.

**Results** The SFS diets was similar in milk yield and fat compared with the control diet ( $p < 0.05$ ). SFS silage diets increase protein, lactose and non-fat solids concentration, compared with control diet.

**Conclusion** The SFS silage diet proved to significantly increase in protein, lactose and non fat solids, in milk indicating an improve on nutrients availability, but also has an negative effect in milk yield production.

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# In vitro gas production and degradability of sunflower seeds (Helianthus annuus) silage and without silage for ruminant feed

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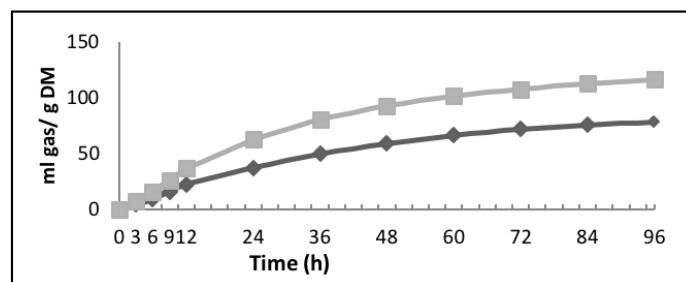
**Application** High oil content foods, like sunflower seeds (SFS), induce milk fat depression when it is included in the diet. The silage of sunflower seeds affects nutrients disposition in rumen.

**Introduction** The use of unprotected fats as oilseeds in the ruminant diet modifies the concentrations and molar proportions of volatile fatty acids and reduces gas production (Beauchemin et al., 2009), however, the way of presentation of the seed can influence these productions. The objective of this study was to evaluate the effect of sunflower seeds silage and without silage in in vitro ruminal fermentation.

**Material and methods** Sunflower seeds (SFS) silage (SFS silage, 0.7 L water / kg seed in fresh matter plus 10 ml of "Pulque" as additive) and without silage (SFS ctrl) were used, the chemical composition and In vitro gas production (Theodorou et al., 1994) was determined, 0.800 g of DM from sunflower seeds (SFS silage, SFS Ctrl) were used. Each sample was incubated in triplicate in glass flasks with 90 ml of buffer solution and 10 ml of sheep rumen fluid, three incubation periods were performed. Three sheep (42 kg LW) were used as donors of ruminal fluid, rumen fluid was extracted, filtered in triple cheese cloth gauze, and homogenized with CO<sub>2</sub>. The bottles were incubated in a water bath at 39 ° C. The volume of gas (ml of gas / g DM) as recorded at 3, 6, 9, 12, 24, 36, 48, 60, 72, 84 and 96 hours of incubation. The volume of gas accumulated from each sample was adjusted to the model proposed by France et al. (2000). After the incubation period (96h), the pH and the dry matter disappeared (DMD) were determined. A completely randomized design was performed, and a Tukey test was used when differences between treatments were observed (P <0.05).

**Results** In vitro gas production (ml gas / g DM) and DMD was lower (P <0.05) for SFS silage compared with SFS Ctrl. Table 1. In vitro gas production of sunflower seeds (SFS Ctrl) and SFS Silage

**Figure 1.** In vitro gas production for sunflower seeds silage (SFS Silage, u) and without silage (SFS Ctrl, n).



Variable	SFS Ctrl	SFS Silage	SEM	P value
pH	6.93b	7.07a	0.024	0.001
A	121.21a	85.91b	1.937	0.001
B	0.0336a	0.0265b	0.0006	0.001
C	-0.0169	-0.0114	0.0005	0.463
Lag time	0.451	0.435	0.0667	0.873
DMD	172.49a	132.40b	4.809	0.004

**Conclusion** The inclusion of sunflower seeds silage decreases gas production, and rumen degradation, with the possible absorption of nutrients in the intestine.

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# Effect of supplementing phytase (at commercial or a superdose level) in cereal/soya and by-product diets on growth performance and bone strength parameters in growing-finisher pigs

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**Application** Nutrient requirements for finishing pigs should be re-evaluated in relation to production performance. Phytase addition has no performance benefit. Although not significantly, phytase appears to numerically improve bone strength.

**Introduction** Phosphorus (P) is one of the most important nutrients in livestock diets. However, pigs are inefficient in their ability to utilise the P in feed as they lack the enzyme phytase to hydrolyse phytate-P. Subsequently, P is often supplemented in diets which may be costly and result in excess P excretion contributing to serious environmental issues. Previous work focused on improving the efficiency of P utilization in pigs has involved the supplementation of phytase in feed, resulting in improved growth (Zeng et al. 2014) and P-digestibility (Ketaren et al. 2007). The aim of this study was to examine the effects of supplementing phytase (at commercial or a superdose level) on the growth performance of pigs fed environmentally sustainable diets.

**Materials & methods** Dan Duroc pigs (n=80, 40 boars and 40 gilts) of similar genetics, husbandry background and bodyweight (41.3kg ± 0.6kg) were selected and assigned to mixed-sex pens (10 pigs/pen) at 10 weeks of age. Each pen was equipped with an MLP FIRE feeder which recorded individual feed intake and bodyweight at each visit. Pigs were allowed a two-week acclimatisation period to the feeding stations and were then offered one of four experimental diets. Each diet was offered to two pens resulting in 20 pig replications per diet. The experimental diets were; positive control 1 (PC1), a cereal/soya based diet + phytase at commercial levels (250 FTU, 0.01%). Positive control 2 (PC2), a by-product based diet + phytase at commercial levels (250 FTU, 0.01%). Negative control (NC), a by-product based diet without phytase and finally the negative control by-product based diet with a phytase superdose (NC+, 1000 FTU, 0.02%). Diets PC1 and PC2 were formulated to BSAS 2003 standards, containing nutrients at requirement levels (e.g. Finisher 1: DE 12.6 MJ/kg, CP 215g/kg, P 7.4g/kg and Ca 9.6g/kg. Finisher 2: DE 13.4 MJ/kg, CP 200g/kg, P 6.5g/kg and Ca 8.5g/kg). Whereas, the NC and NC+ diets contained reduced levels of these nutrients e.g. Finisher 1: DE 12.2 MJ/kg, CP 209g/kg, P 5.2g/kg and Ca 7.2g/kg. Finisher 2: DE 13 MJ/kg, CP 194g/kg, P 4.5g/kg and Ca 6.1g/kg). Finisher 1 formulations

of each diet were provided to pigs from 12-15 weeks of age while finisher 2 formulations were offered from 15-22 weeks of age. Feed intake and bodyweight data from the feeders were used to calculate average daily feed intake (DFI) and linear-regressed daily live weight gain (LWG) (according to MacNeil and Kemp, 2014) for each individual pig. This was then used to calculate feed conversion ratio (FCR) for each pig for the overall period of 12 to 22 weeks. Eight pigs per diet treatment were slaughtered at 22 weeks, metacarpals were removed and analysed for weight (g), diameter (mm), length (mm) and strength (max force, kg). Genstat (19th Edition) was used to conduct AVOVA's to examine differences in performance, bone weight, diameter, length and strength between treatment groups. Start weight was applied as a covariate for the performance parameters.

**Results** There were no significant differences in final body weight, DFI, ADG or FCR between the experimental treatments with average values of 109 kg, 2412 g/day, 1123 g/day and 2.16, respectively (Table 1). Bone strength parameters were not significantly affected by diet treatment with average weight, diameter and length of 27.8 g, 16.6 mm and 70.8 mm, respectively. Numerically, pigs offered the NC treatment had the lowest bone strength (216.9 vs. 232.7). Although non-significant, supplementing the NC diet with phytase superdose increased bone strength to a similar level as pigs fed PC treatments.

**Table 1.**

	PC1	PC2	NC	NC+	Sem	P
Final bodyweight (kg)	108.1	110.5	109.3	107.8	0.57	0.635
DFI (g/d)	2355	2484	2387	2422	56.1	0.403
LWG (g/d)	1150	1108	1130	1103	29.5	0.635
FCR	2.13	2.17	2.12	2.20	0.033	0.254
Bone weight (g)	29.3	28.1	26.7	27.4	1.01	0.293
Bone diameter (mm)	16.9	16.5	16.6	16.4	0.37	0.780
Length (mm)	73.0	70.4	70.1	69.5	1.12	0.154
Max force (kg)	234.1	236.5	216.9	227.6	10.8	0.587

**Conclusions** Overall growth performance was not affected by experimental diet or the use of phytase. The NC treatment resulted in similar performance as the PC treatments, indicating that nutrient supply was adequate in this formulation and may suggest that requirements for finishing pigs should be re-evaluated. Superdosing of phytase had no significant effect, although numerically improved bone strength when compared with the NC, which may be biologically important for pig welfare and suggestive of a positive effect of phytase on P and Ca metabolism. Digestibility studies are on-going, the results of which may support this conclusion.

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# Effects of red beet supplementation on growth and post weaning diarrhoea in weaned piglets

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**Application** Production wastes from red beet, are viable sources of alternate feed ingredient for weaned pig diet to alleviate post weaning diarrhoea.

**Introduction** Following the ban on the use of in-feed antibiotics in livestock production, other alternatives are being sought to alleviate post weaning diarrhoea (PWD) in pigs. Pharmacological levels of Zinc Oxide (ZnO) (2,000 to 4,000 mg/kg) is currently been used, but this will also be banned in the near future owing to concerns from environmental accumulation of zinc. Red beetroot has been found a good source of bioactive compounds such as; betalains and polyphenols as well as fibre. Beet bioactives have also being associated with strong antioxidant and anti-inflammatory properties. However, research related to the in-vivo effects of beet supplementation are largely insufficient. This study was conducted to evaluate the effects of red beetroot supplement in weaned pig diets on post weaning diarrhoea and growth performance.

**Materials and Methods** A 14-day feeding trial was conducted under an ethical approval granted by the Animal welfare and ethical review committee of the University of Leeds, UK (NO. 070510HM). Forty-eight piglets weaned at 28 days old were randomly allocated to one of four diet treatments balancing for weight, sex and litter origin (n =12). There were two pens per treatment and six pigs in each pen, each pig represented a replicate. The pens were housed in a temperature-controlled flat deck (135 cm X 135 cm) with open feeding troughs and nipple drinkers to allow access to feed and water ad-libitum. The experimental diet which was in mashed form was purchased from Primary diet, while the red beet powder was obtained from a commercial source and mixed into the feed on site. The pigs were fed with either a control diet (CON), ZnO supplemented diet (ZNO), 2% red beet (RB2) or 4% red beet (RB4) supplemented diet. The BW of pigs and feed intake per pen were measured on days 0,7 and 14 to calculate the average daily feed intake (ADFI), average daily gain (ADG) and the feed conversion ratio (FCR). While, diarrhoea occurrence was evaluated using a faecal score (1-5; firm to scour) provided in the established protocol at the trial site.

**Statistical analyses** Data collected were analysed using ANOVA and Duncan post-hoc test for significance between means (SAS Inst., Inc., Cary, NC, USA). An alpha level of 0.05 was used for determination of significance for all analyses.

**Results** There was a quantitative decrease in BW of pigs on diet CON and ZNO and an increase BW of pigs on diet RB2 and RB4 during the first week of the trial. However, this was not commensurate to the ADFI and resulted in the negative FCR observed for diet CON and ZNO. This could mean the animals had a form of post weaning stress and diarrhoea during the first week which was more severe in pigs on diet CON and ZNO compared to pigs on diet RB2 and RB4. Hence the pigs did not gain much weight though they were feeding. There was a significant difference ( $p < 0.05$ ) in the ADFI of the diets during the second week of the trial, which accounted for the increase in the BW of the animals. This could mean a level of post weaning stability (intestinal status and health) on the part of the animals. Although, the faecal scores were not significantly different between the diets at any time during the trial, pigs on diet ZNO had a lower faecal score. In sum, pigs fed the control diet and diets supplemented with red beet had similar responses.

**Table 1:** Effects of red beetroot supplement in weaned pig diets on post weaning diarrhoea incidence and growth

Period	Diets				SEM	Sig.
	CON	ZNO	RB2	RB4		
Pig weight (kg)						
Day 0	7.59	7.51	7.62	7.61	0.16	0.837
Day 7	7.31	7.16	7.70	7.63	0.21	0.430
Day 14	9.11	8.67	9.37	9.59	0.26	0.271
ADG (kg/pig/day)						
0-7 days	- 0.040	- 0.050	0.011	0.003	0.017	0.268
8-14 days	0.191	0.185	0.222	0.175	0.019	0.449
0-14 days	0.090	0.078	0.126	0.116	0.016	0.345
ADFI (kg/day/pig)						
0-7 days	0.107	0.112	0.168	0.169	0.014	0.137
8-14 days	0.283 <sup>ab</sup>	0.244 <sup>b</sup>	0.351 <sup>a</sup>	0.379 <sup>a</sup>	0.022	0.050
0-14 days	0.184	0.171	0.251	0.257	0.017	0.055
FCR						
0-7 days	-2.76	-2.57	0.21	0.19	1.04	0.425
8-14 days	1.51	1.37	1.58	2.09	0.15	0.165
0-14 days	2.16	2.38	2.18	2.21	0.22	0.802
Faecal Score						
0-7 days	2.35	2.07	2.50	2.36	0.12	0.339
8-14 days	2.28	2.00	2.14	2.28	0.07	0.209
0-14 days	2.32	2.04	2.31	2.32	0.08	0.293

Values represents mean, SEM: standard error of mean, Diets: CON (basal diet); ZNO (CON+ ZnO); RB2 (CON+2% red beet powder) and RB4 (CON+ 4% red beet powder). ADG- Average daily weight gain, ADFI- Average daily feed intake, FCR- feed conversion ratio. a,bDifferent superscripts in the same row denotes significant difference ( $p < 0.05$ ).

**Conclusion** Diet RB2 and RB4 have potential to moderate post weaning weight loss compared to diet CON and ZNO.

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# Impact of a sodium diformate and alkaloid-based feed additive in the lactation diet of sows on the performance of suckling piglets

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**Application** Sodium diformate and alkaloids appear to benefit the growth performance of suckling piglets, if the additive is included in the lactation diet of the sow.

**Introduction** Sustaining growth rate and optimal feed efficiency in pigs are key to their economic performance through to market. With mounting pressure on the costs of pig production in general worldwide, nutrition is increasingly under scrutiny. The application of organic acids and their salts in diets for pigs has been studied extensively for more than 50 years. They have proved especially effective in maintaining growth performance since the ban on antibiotic growth promoters came into effect in Europe. Several studies have also demonstrated benefits of diformates to diets for sows (Øverland et al., 2009). However, while the antimicrobial impact of organic acids and their salts, including potassium or sodium diformate, is mainly directed against Gram-negative bacteria, specific plant extracts containing isoquinoline alkaloids have been shown to have an antibacterial impact against various Gram-positive bacteria (Opletal et al., 2014). Despite the well-documented impacts of both additives, data on the combined impact in sows during late gestation and lactation, and their subsequent effects on suckling piglets under semi-commercial conditions has not yet been generated. This formed the impetus of this trial.

**Material and Methods** The study tested the efficacy of an agglomerate of sodium diformate with alkaloids, in multiparous sows at a research farm in Saxony-Anhalt, Germany. 40 sows were allocated to 2 equal groups and fed a commercial lactation diet from one week before farrowing until weaning. The study was conducted under veterinary supervision. The test diet contained 1.0% of the sodium diformate – alkaloid agglomerate (traded as Formi Alpha, hereafter referred to as DF-A). The lactation diet was fed according to a feeding curve, from the last week before farrowing to day 13 of lactation and thereafter ad libitum. All piglets were weighed at birth and weaning and their performance parameters recorded. Data were analysed using the t-test and a confidence level of 95% was defined for these analyses.

**Results** The number of piglets born alive, the weight of newborn piglets and the litter weight at birth were significantly ( $P < 0.05$ ) increased (Table 1), while the number of weaned piglets differed only numerically ( $P = 0.25$ ). At weaning, the body weight of piglets as well as the litter weight were again only numerically heavier. Here, piglet weight was more than 130 g greater and litter weight almost 4 kg heavier in the treated group compared to the negative control, while weaning age did not differ (25.7 days).

**Table 1** Performance parameters of suckling piglets during lactation of sows which were fed with or without 1% of the additive (DF-A) – data presented as Mean  $\pm$  S.D.

Parameter	Control	1% DF-A	P-value
Sow (n)	20	20	-
Feed intake per sow (kg/d)	5.83	6.53	n.d.*
Live-born piglets per sow (n)	16.4 $\pm$ 2.8	17.1 $\pm$ 2.4	0.22
Weight of live-born piglets (kg)	1.20 $\pm$ 0.14	1.30 $\pm$ 0.17	0.03
Total litter weight (live-born) (kg)	19.56 $\pm$ 3.26	21.90 $\pm$ 3.03	0.01
Weaned piglets per sow (n)	11.95 $\pm$ 0.59	12.15 $\pm$ 1.15	0.25
Weaning weight of piglets (kg)	6.91 $\pm$ 0.62	7.04 $\pm$ 1.27	0.34
Total weaned litter weight (kg)	82.44 $\pm$ 7.58	86.43 $\pm$ 19.16	0.20

**Conclusion** Feeding sows an agglomerate of dietary sodium diformate and alkaloids during late gestation and early lactation appears to have a beneficial impact on early post-natal growth in piglets, although in this case the weight at weaning increased only numerically.

**Acknowledgement** The help of the team from Livestock Feed Tests GbR in Jessen, Germany in providing the research facility and during the data collection is greatly acknowledged.

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Øverland M, Bikker P and Fledderus J 2009. Livestock Science 122, 241–247.



# An assessment of the effects of administration of an oregano oil liquid on the growth and faecal consistency of dairy-bred bull calves

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**Application** It is challenging to meet the growth targets of young dairy-bred, milk-fed calves. This pilot study suggests that administering an oregano oil liquid during early life may enhance growth without detrimental effects on health

**Introduction** Milk-fed dairy-bred calves are capable of achieving growth rates in excess of 0.8 kg/day (Bleach et al., 2005). However, many fail to achieve growth targets. One of the factors associated with poor growth rates during the milk-fed phase is neonatal calf diarrhoea (NCD), often caused by infectious microbes. Oregano essential oil constituents, carvacol and thymol, have been shown to have antimicrobial activity and to enhance growth of milk-fed calves (Froehlich et al., 2017). The aim of the present study was to investigate the effects of administering an oregano oil derived liquid during the first 10 days of life on the growth and health of dairy-bred calves to two weeks.

**Material and methods** Dairy-bred calves (n=20) were recruited to the study at birth. They were allocated to one of two treatment groups – Orego-Stim calves (n=10) were administered orally a 10 ml dose of Orego-Stim liquid (Anpario plc, Worksop, UK) once a day (at approx. 12:00) for 10 days after birth. Control calves (n=10) were untreated. Calves were removed from their dams at birth and offered 4 litres of colostrum. The calves were offered a further 2 litres of colostrum on day 0. From day 1 they were offered milk replacer (5.8 litres/day; 150g/l; Advanced Superstart, 23% CP, 20% oil) from teated bottles in 2 equal feeds with ad libitum concentrate (Vitastart starter pellets, 18% CP, For Farmers, UK). Water was also freely available throughout the study period. They were housed in individual, straw-bedded hutches ((2.5m<sup>2</sup> internal area, 1.8 m<sup>2</sup> external area; Calf-tel,

Hampel Animal Care, US). Liveweights (LWT) were recorded at birth, 7 and 14 days of age. Faecal scores (score 1 - firm to score 4 - watery) and cleanliness scores (score 1 - clean to score 4 - heavily soiled) were determined daily (Panivivat et al., 2004). Dehydration scores (score 1 - no dehydration to score 3 - severe dehydration; Walker et al., 1998) were determined daily. The incidence of NCD and respiratory disease were recorded. Calf LWT, daily liveweight gain (DLWG), faecal, dehydration and cleanliness scores were analysed using repeated measures ANOVA in Genstat (18th edition). The incidences of respiratory disease and NCD in the two groups of calves were compared using Chi-squared analyses.

**Results** One Orego-Stim and two Control calves died from rotavirus. There was no significant effect of treatment on the LWT (p=0.132) of the calves (Table 1). There was an effect of day (p<0.001) and a treatment by day interaction (p=0.003). There was no effect of treatment (p=0.111 or time (p=0.209) on DLWG but there was a treatment by time interaction (p=0.005). Overall DLWG were 0.2 kg/d higher (0.44±0.09 vs 0.24±0.10; p=0.047) in Orego-Stim calves. The average scour scores of the Orego-Stim and Control calves were 2.2±0.2 vs 2.5±0.2 (p=0.336), respectively in week 1 and 2.9±0.2 vs 3.2±0.2 in week 2 (p=0.439). There were no differences in the cleanliness scores and the dehydration scores of the calves. Overall, n= 6 Control calves were treated for NCD compared and n=3 Orego-Stim calves (p=0.178).

**Conclusion** The results suggest that there may be a beneficial effect of administering Orego-Stim liquid to calves during the first 10 days of life on their daily liveweight gains to 14 days of age. This finding warrants further investigation over a longer duration with a greater number of calves.

**Acknowledgements** The authors gratefully acknowledge contributions of the dairy unit staff.

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Live weight (kg)	birth	Week 1	Week 2	treatment	time	treatment x time
Orego-Stim	43.7±2.5	45.9±2.2	48.9±2.4	P=0.132	P<0.001	p=0.003
Control	41.9±2.2	44.0±1.6	45.5±1.7			
DLWG (kg/d)						
	0 to 7 days	7 to 14 day		treatment	time	treatment x time
Orego-Stim	0.32±0.11	0.51±0.10		P=0.111	P=0.209	P=0.005
Control	0.31±0.13	0.07±0.09				

**Table 1:** Mean (±sem) liveweight (kg) and daily liveweight gain (kg/day; DLWG) from birth to 14 days of age in calves given Orego-Stim liquid (n=9) or untreated Control (n=8) calves.

# Growth performance, nutrient digestibility, blood lipid profile, and fecal *Escherichia coli* and *Lactobacillus* counts on growing pigs fed with de-oiled lecithin emulsifier

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**Application** Digestibility and absorption of lipids are critical to ensure the maximum potential growth of pigs. It was reported that many factors could influence lipids digestion including animal age, genetic, lipase activity, and microbiota status, and diet composition. Exogenous emulsifier supplementation as a kind of nutritional bio-active additives may be helpful in growing pigs.

**Introduction** Lecithin, an excellent emulsifier, is usually available from sources such as soybeans, eggs, milk, and marine sources. It was reported that for a better nutrient absorption and utilization, emulsifier could help to lower the surface tension, increase the concentration of monoglycerides in the intestine, and promote the nutrient transport through the membrane. It was hypothesized that dietary supplementation of de-oiled lecithin (DOL) as exogenous emulsifier may be helpful in emulsification, consequently improving the nutrient absorption and growth of growing pigs. Therefore, the purpose of the present study was to investigate the effect of dietary supplementation of DOL as emulsifier on growth performance, nutrition digestibility, blood lipid profiles, and fecal *Escherichia coli* and *Lactobacillus* count in growing pigs.

**Material and methods** The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University, South Korea. A total of 75 crossbred growing pigs [(Yorkshire × Landrace) × Duroc] with an average

initial BW of  $24.97 \pm 1.42$  kg were used in a 6 week experiment. Pigs were randomly allotted to 3 treatments [5 pigs per pen (3 barrows and 2 gilts); 5 pens per treatment] based on BW and sex. Treatments were: 1) CON (basal diet), 2) TRT1 (basal diet + 0.1% DOL-60), and 3) TRT2 (basal diet + 0.1% DOL-97). The DOL-60 and DOL-97 contents were 60% and 97% of DOL, respectively. The diets were formulated to meet or exceed the recommendation of NRC (2012) for growing pigs. Pigs were weighed on a pen basis initially and finally, and feed consumption was recorded throughout the experiment. Average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G/F) were then calculated. Last 7 days, pigs were fed diets mixed with 0.2% chromic oxide as an indigestible marker to determine apparent total tract digestibility of dry matter (DM), nitrogen (N), and gross energy (GE). At the end of the experiment (week 6), two pigs (one barrow and one gilt) were randomly selected from each pen (10 pigs per treatment). Blood samples were taken from the pigs by jugular venipuncture using vacuum tubes. At the same time, fecal samples were collected from the pigs by via rectal massage. For nutrient digestibility analyzing, fresh fecal samples from per pen were mixed and pooled, and were stored together with feed samples in a freezer at -20°C until analysis. For fecal *E. coli* and *Lactobacilli* counts analyzing, fecal samples were pooled and placed on ice for transportation to the laboratory and analysis was immediately carried out. Data were subjected to analysis of variance as a completely randomized design using the general linear models (GLM) procedure of SAS.

**Results** Result indicated that the increased contents of DOL lead to higher ADG ( $P=0.037$ ), G/F ( $P=0.042$ ), digestibility of DM ( $P=0.0032$ ), N ( $P=0.0315$ ), and GE ( $P=0.0436$ ), and fecal *Lactobacillus* counts ( $P=0.0022$ ). Meanwhile, blood triglyceride ( $P=0.0717$ ) and total cholesterol ( $P=0.0543$ ) were trended to reduce. Furthermore, the fecal *E. coli* population were not affected ( $P=0.2043$ ).

**Conclusion** In conclusion, the supplementation of DOL in growing pig diets could improve the growth performance, nutrient digestibility and intestinal *Lactobacillus* count, thereby contributing to improved growth rate.

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**Table 1.** Effect of dietary supplementation of DOL on growth performance and nutrient digestibility in growing pigs.

Items	CON	TRT1	TRT2	SEM	P-value	
					Linear	Quadratic
ADG, g	720	743	755	7.04	0.0037	0.5124
ADFI, g	1658	1675	1661	17.12	0.9163	0.4575
G/F	0.4346	0.4438	0.4556	0.007	0.0420	0.8735
Dry matter, %	77.14	79.12	80.92	0.80	0.0032	0.9276
Nitrogen, %	74.56	76.82	77.45	0.89	0.0315	0.4624
Gross Energy, %	76.26	77.64	78.91	0.87	0.0436	0.9598

# Effect of protease derived from *Pseudoalteromonas arctica* supplementation on growth performance, nutrient digestibility, meat quality, noxious gas emission, and blood profiles in finishing pigs

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**Application** Feed costs represent a large proportion of the variable costs of swine industry. It is important to determining the profitability of a pig producer. There are limited studies on protease as a feed additive obtained from the fermentation of *Pseudoalteromonas arctica*.

**Introduction** Protease is a stand-alone enzyme, which is a newcomer in the feed enzyme market. There are limited studies on protease as a feed additive obtained from the fermentation of *Pseudoalteromonas arctica* (PPA). Therefore, the objective of this study was to assess the effect of graded levels of protease derived from PPA on growth performance, nutrient digestibility, meat quality, noxious gas emission, and blood profiles in finishing pigs.

**Material and methods** A total of 160 pigs (8 replicates with 5 pigs per pen) were used in this 10-week trial. Dietary treatment groups were as follows: CON (basal diet); TRT1 (Basal diet + 0.1% PPA); TRT2 (Basal diet + 0.2% PPA); and TRT3 (Basal diet

+ 0.3% PPA). The PPA used in this study was obtained from commercial company (Celltech). PPA is produced through a fermentation process by a strain of PPA with optimal pH of 11 and guaranteed to contain 624 unit/mg. The BW of finishing pigs was measured at the beginning, week 5 and week 10 of the experimental period, and feed consumption was recorded weekly on a pen basis during the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). On week 10, meat color, sensory evaluation, cooking loss, drip loss, pH, longissimus muscle area or water holding capacity was determined. And lean meat percentage, backfat thickness, the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), energy, the total protein, blood urea nitrogen (BUN) and creatinine was determined on week 5 and week 10. Faeces were collected by rectum massage on pen basis using a steel plate under the cage on week 5 and week 10 to determine fecal ammonia (NH<sub>3</sub>), hydrogen sulfide (H<sub>2</sub>S), and total mercaptans emission. Orthogonal polynomials were used to assess the linear and quadratic effects of increasing dietary concentrations of supplemental protease.

**Results** During week 1 to 5, pigs fed with different levels of PPA supplemented diet showed linear increase ( $p = 0.0109$ ) in the ATTD of N and linear decrease ( $p = 0.0021$ ) in the concentrations of serum total protein. During week 6 to 10, pigs fed with different levels of PPA supplemented diet showed a linear decrease in feed conversion ratio ( $p = 0.0338$ ). During the overall period, there was a linear decrease in feed conversion ratio ( $p = 0.0295$ ) associated with the inclusion of PPA.

**Conclusion** In conclusion, dietary supplementation with PPA diet has beneficial effects on growth performance, nutrient digestibility, backfat thickness and the concentrations of serum total protein.

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**Table 1.** Effect of dietary PPA supplementation on growth performance in finishing pigs

Items	CON	TRT1	TRT2	TRT3	SEM	<i>p</i> -value	
						Linear	Quadratic
Week 1-5							
ADG, g	800	816	841	831	15	0.0579	0.7983
ADFI, g	2241	2267	2308	2291	28	0.0965	0.8170
FCR	2.806	2.780	2.746	2.759	0.023	0.0752	0.9025
Week 6-10							
ADG, g	839	853	887	870	22	0.1389	0.6991
ADFI, g	2766	2783	2813	2831	47	0.4772	0.9177
FCR	3.300 <sup>a</sup>	3.269 <sup>ab</sup>	3.178 <sup>b</sup>	3.261 <sup>ab</sup>	0.038	0.0338	0.5238
Overall							
ADG, g	819	834	864	851	18	0.0944	0.7531
ADFI, g	2503	2525	2561	2561	36	0.2721	0.8769
FCR	3.059 <sup>a</sup>	3.029 <sup>ab</sup>	2.968 <sup>b</sup>	3.015 <sup>ab</sup>	0.028	0.0295	0.6396

# In ovo feeding of honey solution on gut histomorphometry of turkey poults

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**Application** Honey solution up to 5% fed in ovo enhanced utilisation of nutrient in turkey poults.

**Introduction** In turkeys, embryonic mortality occurs toward the end of the incubation period. Fast depletion of stored glycogen during the last phase of incubation forces the muscle protein of the growing embryo into gluconeogenesis which leads to poor tissue formation and hence, poor quality hatchlings and increased early poult mortality. Poultry producers experience early poult mortality of about 11.4% annually (Muhammad et al., 2010), which may be related to limited energy reserves and environmental stressors. In ovo feeding involves the administration of exogenous critical nutrients by the last phase of incubation into the amnion of developing chick embryos. In ovo administered nutrients are presented to the enteric tissues for digestion and absorption and then can be utilised or stored as energy. Honey which is rich in sugars is a potential carbohydrate source for growing turkey embryo. Honey contains about 200 substances, including amino acids, vitamins, minerals and enzymes, but it primarily contains sugar and water (Ajibola et al, 2007). Therefore, in order to augment the inherent nutrient in the amniotic fluid, this study was designed to evaluate the effect of in ovo feeding of honey on intestinal histomorphometric measurements in turkey poults.

**Material and methods** The in ovo feeding procedure was carried out at a reputable hatchery in Ibadan, Nigeria. The birds were managed post hatch at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Nigeria. Three hundred (300) viable 21-day incubated turkey eggs of comparable weight were allotted into five treatments containing 6 replicates 10 eggs per replicate. Treatment 1 was uninjected control, treatment 2 was distilled water injected control while treatments 3 to 5 had 5% glucose, 1% and 5% honey respectively. After hatching, poults were weighed, tagged and allocated to pens within their respective treatments. On day 21, two poults were sacrificed, dissected and jejunum harvested for gut histomorphological measurements (Iji et al., 2001). Data obtained were analysed with ANOVA (SAS, 2012) and means were separated using Duncan's Multiple Range Test at significant level of  $P = 0.05$ .

**Results** The effect of in ovo feeding of honey solution on jejunal histomorphometry of turkey poults at day 21 is shown in Table 1. Epithelial thickness of poults on uninjected control, distilled water and 5% honey were significantly ( $P < 0.05$ ) thicker than those on 5% glucose and 1% honey respectively. Deeper crypt was observed in birds on 5% honey while poults on 5% glucose had least crypt depth. Highest villi were recorded in birds on 5% honey compared to those on other diets. Poults with 5% glucose and 1% honey had identical villi which were higher ( $P < 0.05$ ) significantly than birds on uninjected control and distilled water. Highest villus height to crypt depth ratio was recorded in poults on 5% glucose compared with other dietary treatments. Goblet cell density of birds on uninjected, distilled water and 5% honey were similar and significantly ( $P < 0.05$ ) higher than those on 5% glucose and 1% honey respectively.

**Table 1** Effect of in ovo feeding of honey on jejunal histomorphometry of turkey poults (day 21)

Treatment	Uninjected control	Distilled water	5% Glucose	1% Honey	5% Honey	SEM	P-value
Epithelial thickness ( $\mu\text{m}$ )	100.18 <sup>a</sup>	107.74 <sup>a</sup>	65.50 <sup>b</sup>	75.95 <sup>b</sup>	107.06 <sup>a</sup>	3.45	0.001
Crypt depth ( $\mu\text{m}$ )	118.48 <sup>b</sup>	118.46 <sup>b</sup>	65.59 <sup>c</sup>	132.91 <sup>b</sup>	166.52 <sup>a</sup>	6.52	0.001
Villus height ( $\mu\text{m}$ )	400.96 <sup>c</sup>	400.38 <sup>c</sup>	513.55 <sup>b</sup>	559.34 <sup>b</sup>	831.75 <sup>a</sup>	30.9	0.001
Villus height/crypt depth	3.40 <sup>c</sup>	3.45 <sup>c</sup>	8.16 <sup>a</sup>	5.26 <sup>b</sup>	5.40 <sup>b</sup>	0.35	0.001
Goblet cell density ( $\text{mm}^2$ )	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.04 <sup>a</sup>	0.01	0.017

abc Means on the same row with different superscripts are significantly different ( $P < 0.05$ ). SEM= standard error of mean.

**Conclusion** In ovo feeding of honey solution up to 5% enhanced tissue development of turkey poults which may translate to improved nutrient utilisation.

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# The effect of micronisation on the in vitro digestibility of maize incubated with rumen inoculum

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**Application** The gas production profiles observed in this study indicate that micronisation of cereal maize may increase the in vitro digestibility of maize, suggesting that for Jersey cattle this technique might improve digestibility in vivo.

**Introduction** Cereal grains are extensively used as a major source of energy in cattle production systems due to their high starch content. Heat processing induces the gelatinisation of starch, which has been shown to improve microbial degradation in the rumen, enzymatic digestion in the small intestine and feed conversion rates (Campling, 1991). However, an extensive fermentation of starch in the rumen can cause a decrease in rumen pH (Sauvant et al., 1999) and consequent acidosis (Owens et al., 1998). Maize (*Zea mays*) naturally contains high amounts of starch (up to 73% of the dry matter content) and high metabolizable energy value (McDonald et al., 2011); thus, it is commonly fed to dairy cows and beef cattle for fattening (Campling, 1991). The aim of the study was to investigate the effect of micronisation on the in vitro fermentation and pH of maize incubated with rumen fluid inoculum.

**Materials and methods** An in vitro gas production technique was used to evaluate two forms of maize: untreated (UM) and micronised (MM). Substrates were ground to pass through a 1-mm dry mesh screen using an automated mill (Retsch Cyclone mill Twister) and four replicates of 1 g dry matter (DM) were incubated for 72 h at 39°C within gas-tight culture bottles according to the method of Theodorou et al. (1994). Rumen fluid and digesta were collected from two cannulated Jersey cows, mixed with the culture medium and used as the microbial

inoculum. The ANKOM RF Gas Production System was employed to monitor the gas produced from the fermentation and the pH of the resultant suspension after incubation was measured with the TRUEscience pH SMART Cap Kit. Cumulative gas production and rate of gas production data were analysed by a one-way ANOVA, whilst pH data were analysed using a two-sample T-test. Statistical analyses were performed using Minitab18.

**Results** In the early stages of incubation, the cumulative gas production (Fig. 1a) and the rate of gas production (Fig. 1b) from MM was higher ( $p < 0.05$ ) compared to UM. However, the extent of gas production at 72 h was similar for both substrates. The pH of the MM (6.13) was lower ( $P < 0.05$ ) than UM (6.19).

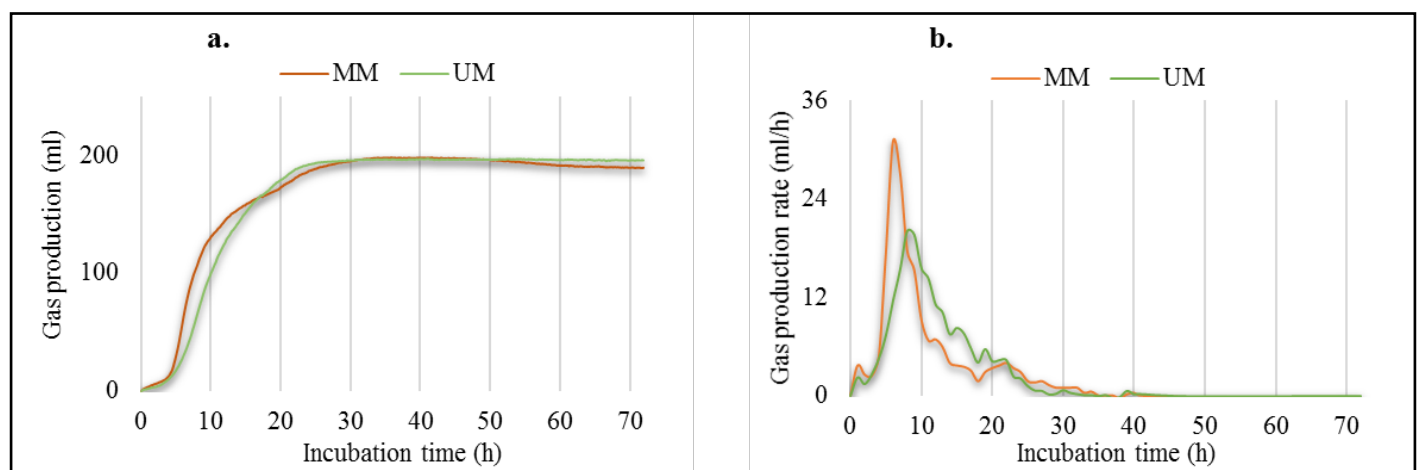
**Figure 1.** Cumulative gas production (a) and rate of gas production (b) from micronized maize (MM) and untreated maize (UM) incubated with a rumen fluid inoculum.

**Conclusion** Micronisation appears to increase the rate of maize degradation in the early stages of incubation, suggesting an increase in digestibility. However, the lower pH for MM compared to UM warrants further investigation, especially in the early stages of incubation. Given that this study was limited to using inocula from a single breed, further in vitro work using inocula from different breeds would be advantageous to examine putative breed effects, possibly due to the variations in the rumen microbiome.

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# Mineral and starch content in semi-aquatic plants: a potential dietary addition for captive Western Lowland Gorillas?

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**Application** Semi-aquatic plants such as *Typha latifolia* and *Phragmites australis* could be useful, locally sourced dietary additions for captive Western Lowland gorillas, and a potential source of enrichment for juvenile animals.

**Introduction** Wild western lowland gorillas have been reported to consume a range of semi-aquatic plants at times of the year with low fruit availability. Semi-aquatic plants are not commonly part of the diet for captive western lowland gorillas and they remain a relatively unexplored potential feed source for zoos. Little is known about nutrient requirements or mineral nutrition in captive primates so the aim of the current study was to evaluate these plants as a potentially useful dietary addition for captive gorillas. Minerals (Ca, P, Mg, K, Fe, Cu, Mn, Zn) and total starch content of samples of great reed mace (*Typha latifolia*) and common reed (*Phragmites australis*) were collected over February to June 2019 at Twycross Zoo and analysed, evaluating any effect of plant species, plant section and month of collection. In addition, preliminary behavioural observations assessed behaviour in relation to plant provision and to determine if captive gorillas deemed the aquatic plants palatable.

**Material and methods** Samples (n = 41) of *Typha latifolia* and *Phragmites australis* were harvested once a month in February, April, May and June 2019 from a nature reserve located at Twycross Zoo (Atherstone, UK). Individually labelled samples (~100g) were frozen (-20°C) and transported to laboratories at Sutton Bonington Campus, University of Nottingham for analysis. Samples were dried and ground (Ultra Centrifugal Mill; Retsch ZM 200) with a 0.5mm sieve. Mineral content (mg/kg) was analysed via Inductively Coupled Plasma Mass Spectroscopy (ICP-MS; Thermo-Scientific ICAPQ with Flatopole collision cell technology and energy discrimination), and a prior microwave digestion step. A sub-population of samples (n = 29) were also analysed for starch, representative across months of collection, plant species and plant section. Total starch content (g/kg) was analysed using the Megazyme Total Starch Assay Kit (AOAC Method 996.11). Samples (~100mg) were analysed in duplicate and read on a spectrophotometer at 510nm wavelength. Behavioural observations were also undertaken with a small number of animals; a male adult, three adult females and two juveniles, in accordance with previous published research (Birke, 2002) with scan-sampling over three consecutive days in July 2019. Animals were observed simultaneously over 45 minutes immediately following feeding plus additional supplementation (~400g) of the semi-aquatic plants. Behaviour was recorded across defined categories using an ethogram. Data were analysed using GenStat 19th edition (VSN International), with ANOVA comparing mineral and starch content. Factors investigated were plant species (*Typha latifolia* vs. *Phragmites australis*), plant section (whole plant, rhizome, stem and leaf and rhizome and stem) and month of sample collection (February, April, May or June) with level of statistical significance at P < 0.05.

**Results** *Typha latifolia* exhibited significantly higher concentrations of Ca, P, Mg and K than *Phragmites australis* although concentrations of Fe, Cu, Mn and Zn were not significantly different between species. Plant sections differed in concentration of Ca (P < 0.001), P (P < 0.001), K (P < 0.001), Fe (P < 0.001) and Mn (P < 0.001) - Table 1. Stem and leaf had the highest mean concentrations for Ca, P and K whereas rhizomes had highest mean concentration of Fe. Highest concentrations of Mn were in rhizome and stem sections. Starch analysis revealed no significant difference between plant species nor month of collection although starch levels were highest in rhizomes, and lowest in stem and leaf. Behavioural data revealed only the juveniles showed continued interest in the semi-aquatic plants (Table 2), particularly *Typha latifolia*.

**Table 1** Mean mineral content of plant sections of *Typha latifolia* and *Phragmites australis*<sup>1</sup>

	Plant section				s.e.d.	P
	Whole plant	Rhizome	Rhizome and stem	Stem and leaves		
Fe (ppm)	458 <sup>a</sup>	3061 <sup>b</sup>	1670 <sup>a</sup>	251 <sup>a</sup>	662.7	<.001
Mn (ppm)	402 <sup>a</sup>	240 <sup>a</sup>	1245 <sup>a</sup>	906 <sup>b</sup>	193.3	<.001
Ca (%)	0.47 <sup>a</sup>	0.46 <sup>a</sup>	0.59 <sup>a</sup>	1.42 <sup>b</sup>	0.181	<.001
P (%)	0.39 <sup>a</sup>	0.34 <sup>a</sup>	0.43 <sup>ac</sup>	0.53 <sup>bc</sup>	0.055	<.001
K (%)	2.79 <sup>a</sup>	1.54 <sup>a</sup>	2.63 <sup>a</sup>	4.58 <sup>b</sup>	0.627	<.001

<sup>1</sup>expressed on a DM basis. a-c Values within a row with different superscripts are significantly different.

**Table 2** Behavioural time budget of captive western lowland gorillas offered *Typha latifolia* and *Phragmites australis*<sup>1</sup>

Animal	Gender	Behavioural category				
		Stripping then consuming	Consuming only	Non-eating behaviour (e.g. play)	Other behaviour	Consuming other feed
Adult 1	Male	1	-	-	5	94
Adult 2	Female	-	-	-	35	65
Adult 3	Female	-	-	-	43	57
Adult 4	Female	-	-	-	11	89
Infant 1	Male	10	5	11	31	43
Infant 2	Male	13	9	31	34	13

<sup>1</sup>Data expressed as percentage of total observation time over 45 mins immediately post-feeding

**Conclusion** The study findings have highlighted significant differences in mineral content between the two semi-aquatic plant species, and within the specific plant sections. The limited behavioural data also suggests that these plants could be beneficial for captive gorillas, especially juveniles, as a form of nutritional/environmental enrichment. Analysis over a longer time period to further assess seasonality would be warranted in follow-up work along with future research to better understand biological availability of minerals to gorillas. Nonetheless, the results from the current study suggest that up to 400g/day of semi-aquatic plants such as *Typha latifolia* and *Phragmites australis* could be a potentially useful, locally grown supplement in captive gorilla diets.

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# Cereal vs. vegetable starch: Evaluation of nutrient digestibility and performance in extruded canine diets

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**Application** Despite increasing demand for grain-free diets, the current study suggests extruded canine diets formulated with wheat as the starch source are equally/more beneficial than equivalent diets based on potato as the starch source.

**Introduction** There has been an increasing trend towards more 'grain-free' diets in the pet food industry in recent years. This desire has been partly driven by owner beliefs that dogs should have a predominantly meat based diet, analogous to that consumed by their wolf ancestor, some owners incorrectly self-diagnosing wheat allergies in dogs, and partly as a result of owner demands reflecting human food trends, although there is little established scientific evidence of the benefits of grain-free diets for canines. The aim of the current study was therefore to better quantify the influence of starch source in extruded canine diets, comparing a cereal-based starch (wheat) with a vegetable based starch (potato) alternative, using a broiler chick assay. Two presentations of salmon were formulated as the main dietary protein sources, reflecting the popularity of this ingredient in pet food manufacture, commonly seen as a healthy, natural food choice associated with high quality aroma and flavour.

**Material and methods** All protocols and procedures were approved by School of Biosciences, University of Nottingham, Animal Welfare and Ethical Review Body (Ref:000137) and all birds were euthanised at the end of the study, with none entering the food chain. Parameters assessed were effect of starch source on Coefficient of Total Tract Digestibility (CTTAD) and animal performance, using a similar approach to previous published nutritional studies using a broiler chick assay to evaluate companion animal dietary materials (Fiacco et al.,

2017). Three test diets were formulated to compare the starch sources, with two differing presentations of salmon as the main dietary protein source; salmon meal or salmon protein hydrolysate (SPH) all processed in accordance with the ABP regulations. All dietary materials reflected those commonly used in canine diet manufacture and all diets were formulated to be similar with respect to FEDIAF guidelines, as is typical of pet food recipes. Test proteins were substituted on a total protein basis, reflecting a typical petfood approach and were iso-energetic. Diets (1; Salmon meal and wheat, 2; salmon meal and potato, 3; Salmon SPH and potato) were extruded prior to feeding and offered in meal form. A wheat/ SPH dietary combination was not evaluated in the current study as it is not truly reflective of that currently found in commercial practice within the pet food industry. Diets were fed to day-old male broiler chicks (n = 36), with each diet fed to six replicate pens of birds. Excreta was collected over Days 18-21 and all birds were euthanised on Day 23 to evaluate performance parameters and nutrient digestibility. Data were analysed using GenStat 19th edition (VSN International), with ANOVA comparing dietary starch source (wheat vs. potato) and protein source (Salmon meal vs. SPH) as the main factors tested. The level of statistical significance was set at  $P < 0.05$ .

**Results** Analyses (Table 1) revealed all diets to be highly digestible (CTTAD values all  $> 0.980$ ) and total tract digestibility of both starch and nitrogen did not appear to be affected by diet ( $P > 0.05$ ). Performance (ADFI and ADG) showed a consistent pattern in terms of starch source and protein presentation effects; these parameters were all superior for animals on a diet based on cereal starch as opposed to one formulated with potato starch ( $P < 0.001$ ).

**Conclusion** The study findings suggest that in terms of nutrient digestibility and performance, extrusion processing of canine diets formulated with wheat as the primary source of starch can be equally, or more beneficial than a potato starch-based diet. Beliefs by some owners that cereal starch is less beneficial do not appear to be evidenced by the findings across the parameters assessed in the current study. The results should help to inform the wider discourse around raw materials in pet food diets, and the demand for grain-free canine diets currently influencing the pet food market.

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	Starch source effect		Protein source effect		Meal	SPH	s.e.d.	P
	Wheat	Potato	s.e.d.	P				
ADFI (g/d)	129	81	11.2	<0.001	102	86	9.7	0.117
ADG (g/d)	82	30	6.2	<0.001	55	32	5.4	<0.001
CTTAD <sub>ST</sub>	0.992	0.987	0.005	0.307	0.987	0.992	0.004	0.265
CTTAD <sub>N</sub>	0.784	0.787	0.023	0.898	0.784	0.791	0.019	0.723

**Table 1** Mean performance and total tract digestibility of starch and nitrogen of broilers (Days 15 to 23) fed extruded canine diets containing differing starch and protein sources

# Neonatal calf diarrhoea affects lying behaviour in individually-housed dairy heifer calves

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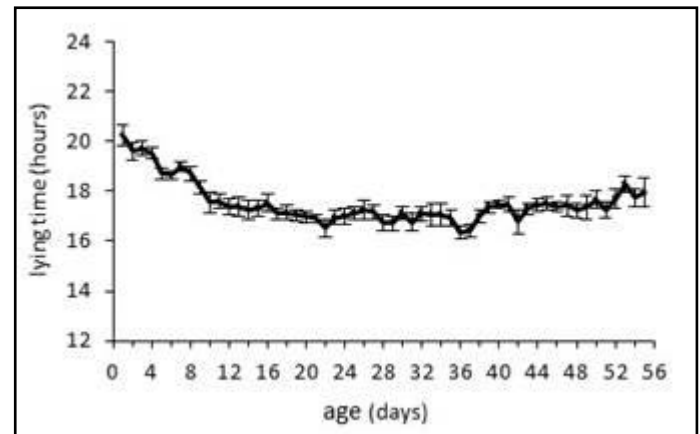
**Application** Lying behaviour of dairy heifer calves is affected by neonatal calf diarrhoea (NCD). The frequency and duration of lying bouts differ from those of healthy calves.

**Introduction** Neonatal calf diarrhoea (NCD) is one of the main causes of morbidity and mortality in milk-fed calves (Johnson et al., 2017). Outcomes are better where disease is detected early and animals are treated promptly. Previous studies reported the effects of NCD on lying behaviour of group-housed calves (Sutherland et al., 2018). The aim of the present study was to investigate whether accelerometers can detect the early signs of NCD in individually-housed calves.

**Material and methods** Holstein Friesian heifer calves (n=18) were recruited to the study between 16th January and 27th February 2019. The calves were removed from their dams at birth (day 0) and housed in individual, straw-bedded hutches (2.5m<sup>2</sup> internal area, 1.8 m<sup>2</sup> external area; Calf-tel, Hampel Animal Care, US). The calves wore calf jackets from birth until approximately 30 days. They were fed 4 litres of pasteurised colostrum (Brix reading >22%) from a teated bottle. From day 1 to 41 calf milk replacer (CMR; 150g/l, 23% CP, 20% oil; Wynngold Rich Cream, Wynnstay, UK) was fed in two feeds (3.8 litres per feed). The calves were weaned from day 42 with CMR reduced to 2.9 litres per feed and then further reduced to 2.9 litres per day from day 50 to 56. Starter pellets (18% CP; Vitastart, For Farmers UK), chopped straw and water were freely available throughout. Accelerometers (IceQubes, IceRobotics, Edinburgh UK; Bleach et al. 2016), were fitted by Velcro strap to the front left leg of each calf from day 0 to day 56. Data (time spent lying, the number and duration of lying bouts) were downloaded three times a week and exported from IceManager (IceRobotics, Edinburgh UK) to Excel for collation. Calves were monitored for NCD (faeces score 1 (firm) to 5 (watery)) and bovine respiratory disease (score 1 (normal) to 5 (severe)). NCD was defined as an increase in faecal score to  $\geq 3$  for  $\geq 2$  days. Respiratory disease was defined as a respiratory score of  $\geq 3$  for  $\geq 2$  days. Only one case of respiratory disease was observed. Calves with NCD were matched with healthy calves of similar age. The lying times and the number and duration of lying bouts per day, in healthy and NCD calves were compared by repeated measures ANOVA (Genstat version 19).

**Results** Overall, calves lay down for 20.0 $\pm$ 0.4 h/d (mean $\pm$ sem) on day one. By day 9 lying times had fallen to approximately 18 h/d (Figure 1). There was no effect of age on the number (p=0.244) or duration (p=0.198) of lying bouts. NCD was observed in 11 calves (61.1%) from day 9.7 $\pm$ 1.08 days lasting for 3.6 $\pm$ 0.36 days. Lying times were not affected by NCD. However, lying bouts were frequent (p=0.026) and longer (p=0.011) in NCD calves (see Figure 2A & B).

**Figure 1** Mean $\pm$ sem (n=18) lying times (hours/d) of



**Figure 2** Mean $\pm$ sem number of lying bouts (A) and length of



# Mineral contents of dried *F. thonningii* foliage and high quality cassava peel (HQCP) as feed for West African Dwarf Ram

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**Application** Unconventional feed are good sources of feed for ruminant animal (West African Dwarf Ram), however inappropriate or deficiency of nutrients- minerals commonly noticed in them limit their use as feed by farmers.

**Introduction** Seasonality in quantity and quality of forage disturb the performance of ruminant in the humid region of West Africa most especially during the period of dry season when the natural vegetation is low in nutritional value (Aye, 2007). This seasonality in forage supply sometimes leads to starvation and animal lost substantial measurable weight which eventually results to mortality (Tarawali, 1983). There have been efforts to search for alternatives such as unconventional feed and less expensive feed to mitigate this stress (Odeyinka et. al., 2003).

This study objective is to assess the concentration level of some minerals in Unconventional feed - Dried *F. thonningii* foliage and high quality cassava peel.

**Methodology** Firstly, experimental diets were sourced; Image1 (Dried *F. thonningii* foliage in polythene sacks) and Image2 (High quality cassava peel arranged on wooden pallets). Twenty West African Dwarf Rams selected for experiment were then divided into four groups of five West African Dwarf Rams, balanced for body weight and randomly allotted to the experimental diets in a completely randomized design.

Dried *Ficus thonningii* foliage was offered as supplement to high quality cassava peel at graded levels of 0, 20, 40 and 60%, and each supplemental level served as treatment T1, T2, T3 and T4 respectively. The control diet (T1) was enriched with 2% urea (to satisfy the minimum protein requirement for optimum rumen microbial functions). The urea was dissolved in water following the procedure of Finangwai and Dafur (2015) and the solution sprinkled over the peels and mixed thoroughly before feeding. The daily feeding of the supplementary diet (DFT foliage) was offered at 3% of the animal body weight at 9.00h and the basal diet (HQCP) and fresh water was offered ad libitum an hour later in a separate feeding troughs.

**Data Analysis** The milled samples of each fodder (dried *F. thonningii* foliage and high quality cassava peel) were replicated three times after which the data collected were averaged to give a single mineral content.

**Results** The results of some mineral compositions of dried *F. thonningii* foliage and high quality cassava peel tested for level of mineral concentration are presented in table 1. Dried *F. thonningii* foliage recorded higher values of minerals for calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg) than high quality cassava peel while the values of iron (Fe) and zinc (Zn) were higher in high quality cassava peel than dried *F. thonningii* foliage (Table 1).

**Table 1:** Mineral contents of Dried *F. thonningii* foliage and High quality cassava peel mash

Minerals (mg/kg)	Dried <i>F. thonningii</i> foliage	High quality cassava peel mash
Calcium	3.62	1.14
Phosphorus	5.60	1.47
Potassium	27.82	1.29
Magnesium	2.55	0.50
Iron	0.74	0.92
Zinc	0.07	0.09



**Conclusion** It was concluded that dried *F. thonningii* foliage and high quality cassava peel had mineral contents below the level for West African Dwarf Ram requirement.

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# Estimating methane emission rate from eructation peaks

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**Application** A method of measuring individual animal enteric methane emissions from ruminant livestock that is mobile, non-invasive and can fit into the animal's normal environment is of great interest for use on commercial farms. The amplitude of eructation peaks can provide a means of estimating individual animal emissions.

**Introduction** Historically most studies assessing methane emissions from cattle have been performed using respiration chambers, which is seen as the 'gold' standard for measuring emissions. However, respiration chambers are impractical for large-scale estimation of emissions from individual animals in national populations and on commercial farms. Availability of portable gas analysers has provided a means of measuring enteric methane emissions on commercial farms from large numbers of animals (Garnsworthy et al., 2012). The aim of this study was to investigate enhanced data filtering methods to identify and extract eructation peaks to estimate methane emission rate of individual cows.

**Material and methods** Methane spot measurements were obtained from 36 cows during 2,474 milkings, where cows were fed a ration containing between 39% and 70% forage. An infrared gas analyser (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK) was used to continuously measure the methane emitted by individual cows whilst being milked in a robotic milking station. The methane concentration was measured every second with clusters of peaks in concentration being identified from the time-series signal using peak analysis tools in MatLab Signal Processing Toolbox (version R2018a, The MathWorks, Inc., Natick, United States). The peak analysis software extracted the maximum amplitude and the rise time of the methane peak within each milking based on a minimum peak amplitude concentration of 200 ppm, three or more consecutive peaks (clusters) being identified and the minimum time between peaks between 20 seconds. The methane emission rate was extrapolated from the exponential response curve of the gas concentration by equation [1]: Emission rate (g/min) = peak amplitude concentration ppm / [1 - EXP (-(peak rise time in seconds / 60))] x 60 x 0.656 x 10<sup>-6</sup> g/L. Milk yield and live weight were recorded automatically at each milking. Feed intake included a partial mixed ration measured by electronic feeders (Roughage Intake Control feeders; Fullwood Ltd., Ellesmere, UK) plus concentrate fed at the milking station. Pearson correlation coefficient (r) was used to test the association between methane emission rate estimated from spot measurements and production traits.

**Results** After filtering spot measurements for peaks in emissions during milking, mean (± s.d.) methane emission rate was 0.20 (±0.04) g/min with a range of 0.09 to 0.30 g/min. The results showed that maximum peak amplitude was positively correlated with dry matter intake (P<0.01) and forage dry matter intake (<0.001 (Table 1)). There was no correlation between methane emission rate and concentrate intake, milk yield or liveweight.

**Table 1.** Mean production values and correlation (r) with methane emission rate.

Trait	Mean (s.d)	Range	r	P value
Dry matter intake (kg/d)	19.7 (3.2)	12.4 – 26.1	0.367	<0.01
Forage intake (kg/day)	10.5 (2.2)	6.6 – 16.0	0.568	<0.001
Concentrate intake (kg/day)	9.2 (2.3)	5.1 – 13.0	-0.033	0.785
Milk yield (kg/day)	33.3 (9.4)	14.4 – 55.2	-0.074	0.539
Liveweight (kg)	646 (68)	473 – 805	-0.037	0.758

**Conclusion** Estimating methane emission rate from the amplitude of eructation peaks whilst cows are being milked appears to provide a means of quantifying individual cow emissions on commercial farms. The range in emission rate found in the current study would equate to 130 to 432 g methane/day, which is similar but lower than the range of values observed for cows in a respiration chamber of 208 to 539 g methane/day (Bell et al. 2016). The difference may partly be explained by the large range in forage composition for the diet of chamber cows (27 to 100%) in the study of Bell et al. (2016) compared to cows in the current study (39 to 70%).

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# Post weaning performance of Scottish Blackface male lambs slaughtered at carcass weights between 12-16 kg following ad-lib concentrate feeding

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**Application** The option to finish hill bred male lambs to carcass weights of between 12-16kg offers an alternative to primary hill producers to selling lambs as stores or finishing to carcass weights >18kg.

**Introduction** Previous research has shown the potential to finish hill bred male lambs to carcass weights in excess of 18 kg while meeting market muscle and fat score specifications satisfactorily (Claffey et al., 2018). These authors also demonstrated that hill bred lambs were highly responsive to improved nutrition post-weaning. However, for most primary hill farmers the option to finish lambs to carcass weights >18kg is restricted by limited grass supplies post-weaning (Diskin et al., 2015) meaning they are heavily reliant on store lamb prices. Therefore, the option to finish hill bred lambs to lighter carcass weights of between 12-16 kg offers an alternative when store lamb prices are below cost of production.

**Material and methods** A total 220 Scottish blackface lambs were purchased from commercial hill sheep flocks in August and September over two consecutive years. Lambs were weighed on arrival, treated for internal and external parasites, given a clostridia and pastuerella pneumonia vaccination and foot bathed in a copper sulphate solution. Following this lambs were penned in groups of 5 and randomised by live weight and sex (entire male or castrate male). Lambs were built up to ad-lib concentrates over 14 days and at all times were offered grass silage as a fibre source. Once lambs were consuming concentrates ad-lib they were weighed fortnightly and selected for slaughter. The concentrate ration offered contained 15% crude protein, 8.45% crude fibre, 1.1 UFL/kg DM (one UFL is defined as the energy contained in one kg of air dry standard barley; Jarrige, 1989) and contained 0.5% ammonium chloride per tonne. Entire male lambs were drafted for slaughter at >31kg and castrate lambs were drafted at >30kg, in all instances the loin muscle area of the lamb was assessed by hand to ensure they had sufficient muscle and fat cover to reach a minimum grade of O2 using the EUROP scale (Commission Regulation (EC) No 22/2008). At slaughter carcass weight,

muscle grade and fat score were all collected and from this kill out percentage was calculated. Concentrate intakes were measured, on a pen basis, for a 3 week period once lambs were on full concentrate diet.

**Results** As presented in Table 1 average daily gain, slaughter live weight and carcass kill out percentage differed between rams and castrates ( $P < 0.05$ ). Concentrate intakes for ram lambs were 1.14 kg DM/head/day and 1.18 kg DM/head/day for castrates ( $P > 0.10$ ). From day of purchase weight on the source farm to slaughter, ram lambs were housed for 67 days compared to 74 days for castrate lambs ( $P < 0.01$ ).

**Conclusion** There is potential to finish hill bred male lambs at lighter carcass weights while meeting market specifications for muscle and fat cover. However, markets are somewhat limited for these carcasses so it is important to have a contract for these type lambs and careful selection of lambs for slaughter is important to ensure carcasses meet minimum specification for payment.

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**Table 1.** Effect of lamb sex on lamb performance pre- and post-slaughter

	Rams	Castrates	S.E.M.	P-value
Housing live weight (kg)	25.7	25.5	0.37	0.70
Slaughter live weight (kg)	34.1	33.1	0.16	0.01
ADG from housing to sale (g/day)	233	181	6.4	0.01
Carcass Weight (kg)	14.8	14.6	0.1	0.23
Carcass grade	2.3	2.4	0.04	0.47
Fat score	2.5	2.6	0.04	0.44
KO%	43.3	44.2	0.22	0.03

# Evaluating the Causes of Variation in Performance of Broiler Chicks at first week of life

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The current study provides some preliminary data on the variation in body weight in 1-week old broiler chicks.

The demand for broiler meat has risen high in the recent decade and its production has been estimated to reach 130 million tons in 2020, to meet this increasing demand of poultry meat, overall improved performance becomes imperative. Variation in broiler performance could be closely linked to some factors such as genetics, diet, age and environment (Aggrey, et al., 2010; Engberg, et al., 2004). To achieve optimal performance in broiler production, it is pertinent to maintain a good flock uniformity, uniformity of flock is a clear indicator of production process and tends to increase productivity, revenue and reduce waste. The current trial aims at identifying the possible biological factors that could cause variation in performance of broiler chicks at an early stage of their production life (first 7days) and develop some strategic solutions to improve overall uniformity.

The experiment was conducted at the University of Nottingham, Sutton Bonington campus. All experimental protocols used in the study were approved by University of Nottingham Animal Ethics Committee and conforms to the research code of conduct. A total number of 87-day old chicks of Ross 308 breed (males) were used for the study. The study was conducted in an environmentally controlled house and the chicks were reared in a deep litter system. Chicks were kept for 7 days and fed commercial starter diet that met the nutritional requirement of the Ross 308 breed. Feed and water were given ad libitum. Initial body weights (BW) of chicks were recorded on arrival and final weight was recorded on day 7. On day 7, chicks were grouped based on their day 7 BW. The mean of the group +/- 1 st. dev. was used to group chicks into BW classes. Hence chicks with BW >260g were ranked as super performers (SP), while chicks with body weights of 220-240g were ranked as average performers (AP) and chicks weighing <200g were ranked as under performers (UP). Furthermore, ten chicks from each group SP, AP and UP (n=30) were randomly selected and euthanised. The liver, gizzard and full intestine were excised and weighed appropriately. Data collected were subjected to one-way analysis of variance using SPSS software (version 21) with day 7 BW groups as the factor, means were separated using the Tukey test. Significant differences were observed at (p<0.05).

The mean, sd and CV of the broiler chicks was 224g, 43.43 and 19.39 on day 7 respectively. The organ characteristics of the chicks in the BW groups are presented in table 1. There was a significant increase (p<0.05) in the weight of the liver, gizzard and intestine as well as the intestinal length with increased body weight. There was no significant difference (p>0.05) in the liver weight proportion to body weight (BW), however the weight of the liver of the AP and SP were higher than the UP chicks.

**Table 1** Digestive Organ Weight of Chicks at 7days of Age

Parameters	Body weight groups			SEM	P-value
	UP	AP	SP		
BW of chicks at 7d (g)	174.60	227.80	269.60	-	-
Initial BW (g)	52.2b	55.7ab	60.8a	1.05	0.00
Av. Daily wt gain (g)	17.49c	24.59b	29.8a	1.03	0.00
Liver weight (g)	7.63b	10.51a	11.96a	0.42	0.00
Liver % BW (%)	4.39	4.62	4.42	0.10	0.65
Gizzard weight (g)	10.24b	12.74a	14.08a	0.39	0.00
Gizzard %BW (%)	5.87a	5.59ab	5.24b	0.59	0.05
Intestine weight (g)	14.53b	21.43a	23.14a	0.79	0.00
Intestine %BW (%)	8.33b	9.42a	8.59a	0.16	0.01
Intestinal length (cm)	94.33	107.54a	110.05a	2.03	0.01

It was observed that Chicks ranked as SP had correspondingly larger organs but not proportionately larger. The differences in BW observed in day 7 were reflected in day of hatch weight. Ongoing research will seek to determine differences in intestinal transcriptomics and caecal microbiota

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# Nutritional evaluation and composition of eight commercial adult and senior dog diets available in the UK

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**Application** This study reports some preliminary data on the nutrient composition of commercially available diets tailored for adult or senior dogs in the UK.

**Introduction** The life expectancy of dogs kept as companion animals continues to rise in line with advancements in veterinary science and nutrition (Laflamme, 2012; Larsen et al., 2014). This increase in lifespan is accompanied by a greater incidence of age-related disease conditions including cognitive decline, arthritis and obesity. In response to changing dietary requirements, there are specific nutrient formulations available on the commercial pet food market that are tailored for senior dogs. Depending on body condition status and exercise levels and specific disease conditions such as kidney disease (Pugliese et al., 2005), senior dogs may require increased or reduced nutrient and energy density compared with adult diet formulations. The objective of this study was to conduct detailed nutrient composition and make comparisons for a range of adult and senior dog foods available on the commercial pet food market.

**Material and methods** Eight dry commercial diets were obtained for adult dogs (n=2) and for senior dogs (n=6). Samples were analysed for dry matter (DM), crude ash (Ash), crude protein (CP), crude fibre (CF) and neutral detergent fibre (NDF) using standard laboratory procedures (AOAC). Fatty acid profile was determined by gas chromatography. Fatty acids are reported in groups as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA). The nutrient for each diet was analysed in duplicate or triplicate and repeated if the CV between replicates exceeded 10%. Data were analysed for statistical differences between adult and senior dog diets using Welch's unequal variances t-test.

**Results** Results are presented in Table 1. The concentrations of CP, Ash and PUFA were comparable between senior and adult dog diets. Senior dog diets had decreased SFA and decreased MUFA compared with adult dog diets. The ratio of omega-6/omega-3 was lower for senior dog diets when compared with adult dog diets.

**Table 1** Nutrient composition of dry commercial pet foods formulated for adult and senior dogs.

	Adult diet n=2	Senior diet n=6	P value
DM %	91.89±0.01	92.24±0.01	0.641
Ash %	5.48±0.38	6.19±4.13	0.335
CP %	28.03±2.79	26.79±4.13	0.748
CF%	1.24±0.32	2.26±0.73	0.098
NDF %	10.23±4.57	18.44±5.54	0.281
SFA (g/kg)	15.79±0.05	12.27±0.04	0.011
MUFA (g/kg)	24.58±1.03	18.67±3.33	0.022
PUFA (g/kg)	11.54±1.16	10.38±2.33	0.512
ω-3 (g/kg)	1.35±0.08	2.45±0.99	0.056
ω-6 (g/kg)	10.02±1.05	7.83±1.95	0.220
ω-6/ω-3	7.40±0.35	4.05±2.26	0.021

## Conclusion

This preliminary study reports on some analysed nutrient concentrations for a small number of senior and adult dog diets. Senior and adult dog diets were broadly comparable for the key nutrients typically reported on packaging such as DM, Ash and CP. Differences were observed for nutrients between senior and adult dog diets for various fatty acid groups which may be relevant for dogs in old age.

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# Impact of growth on stomach parts and assessment of their relationships in broiler chickens

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**Application** Understanding the impact of growth rate on stomach parts and assessing their relationships may lead to body composition data for approaches and modelling in poultry production science.

**Introduction** The stomach of poultry demonstrates discrete distinction into two parts; proventriculus and gizzard. Both parts are categorized by great morphological and functional variability, between and within the species (Mobini, 2010). Heavy and light breeds are represented by different growth rates (Plavnik and Hurwitz, 1982). It seems possible that some of the internal organs had undergone modifications to accommodate the different growth rates (Plavnik and Hurwitz, 1982) in meat poultry. A study was conducted to assess the effect of growth rate on stomach parts and relationships between them.

**Materials and methods** A total of 150 a-day-old male broiler chicks (Ross 308) from same parent flock age were used in the study. On day 7, chicks were divided into three groups of 50 each based on their body weights as an indicator of growth rate. Birds with high growth rate had body weight of 160g or above, and with low growth rate had 140g or less at same age of day 7 while birds of medium body weights; 141 to 159g were excluded from the study. Birds with high and low growth rate were reared up to 21 days on commercial diet meeting nutritional requirements for broilers Ross 308. Feed and water were provided ad libitum. At 21 days, 25 out of 50 birds nearest to average weight of each group were euthanized by cervical dislocation. Data was collected on body weight, proventriculus and gizzard weights from these two groups and analysed by t-test taking high and low growth rate as factor variable using Minitab 19 software. Pearson's correlation coefficient was calculated to assess the relationship between the body weights and stomachs' parameters (weights) on all 50 observations; 25 from high growth rate group and 25 from low growth rate birds' group. Organ weight as percent of body weight was calculated as: organ weight  $\times$  100/body weight of bird.

**Results** Gizzard weight, and proventriculus and gizzard weight combined were affected ( $P < 0.05$ ) while proventriculus weight was not affected ( $P > 0.05$ ) by the growth rate of birds being heavy or light. A strong correlation was found between body weight and gizzard weight, and proventriculus and gizzard weight combined while proventriculus and gizzard weights as percent of body weights were found to be inversely and directly related to body weights ( $P > 0.05$ ), respectively.

**Table 1** Effect of growth (as body weight) on stomach parts and their relationships

Parameters	High Growth		Low Growth		P-Value	Pearson Correlation
	Means	SEM	Means	SEM		
Body Weight (g)	904.0a	20.50	641.5b	14.30	0.000	NA
Proventriculus Weight (g)	4.240	0.353	3.600	0.252	0.146	0.345
Gizzard Weight (g)	20.24a	0.876	13.96b	0.485	0.000	0.785
Proventriculus & Gizzard Weight (g)	24.48a	1.020	17.56b	0.523	0.000	0.765
Gizzard Weight as Percent of Body Weight	2.237	0.081	2.181	0.065	0.597	NA
Proventriculus Weight as Percent of Body Weight	0.465	0.033	0.568	0.043	0.061	NA

Means within rows with no common superscripts are statistically different ( $P < 0.05$ ).

**Conclusion** Growth rate of broiler chicks had significant effect ( $P < 0.05$ ) on gizzard weight, and proventriculus and gizzard weight combined. A strong and positive correlation between them suggested better growth rate may have been attributed by well-developed gizzard or proventriculus and gizzard combined (Svihus, 2011). Gizzard weight as percent of body weight directly related to the body weight, suggested the higher gizzard weight may be indicative of its better muscle development, which may have a positive effect on the particle size of digesta and nutrient absorption (Kokoszyński et al. 2017).

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# Influence of maturity stages on nutritive quality of two *Panicum maximum* varieties in South Western Nigeria

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**Application** The quality of grasses decreases from young to mature stages as a result of difference in plant composition between levels of maturity.

**Introduction** The quality of the forage plants are usually affected by maturity stages and some other management factors which determines the efficiency of use of light, water and nutrients by the plants. The nutritive quality of grasses decreases irrespective of species as they matured (Van Soest, 1994). In view of that, the crude protein (CP) decreases leading to low intake and impairment on animal performance. As the plant matures, neutral detergent fiber content (NDF) and its lignification increases. One of the important and productive grasses in the tropics is Guinea grass (*Panicum maximum*). It is valuable for pasture, hay and silage especially palatable in the younger stages, tending to become coarse and less eaten by ruminants as it matures (Reed, 1976). *Panicum maximum* enhances high intake and utilization by animals due to high leaf production, high nutritive value and low fiber content especially at early stage of growth.

**Materials and methods** An experimental land area measuring 1134 m<sup>2</sup> (54 m × 21 m) was mapped out in Federal University of Agriculture, Abeokuta, Nigeria. The land was cleared and prepared for planting and were divided into 4 × 5 m plots with a 1 m boundary between plots. The grasses were harvested at different maturity stages (i.e. vegetative, early reproductive and late reproductive stages). Sub-samples were oven dried at 65 °C until constant weight was attained to obtain the dry matter percentage. The dried samples were milled and allowed to pass through a 1 mm sieve screen following which they were subjected to chemical analysis. The DM, CP, EE and ash contents were determined according to the procedure of AOAC (2000). NDF and ADF contents were determined following the procedure of Van Soest et al. (1991). The study was laid out as a 2 × 3 factorial arrangement, in a split-plot design. This constituted of two *P. maximum* varieties (i.e. var. Local and var. Ntchisi) as the main plot and three maturity stages as the sub-plot (vegetative (harvested at 10 weeks of age), early reproductive (harvested at 13 weeks of age) and late reproductive stages (harvested at 15 weeks of age). The experiment was replicated three times and the experimental land area was kept weed free as much as possible throughout the period of the experiment.

**Result** Crude protein content of the grasses declined at the late reproductive stage when compared with other stages. There were no significant changes in the EE and ash contents as the grasses matured. The NDF of the grasses significantly increased at the early reproductive stage of the grass. The ADF content increased as the plants matured, but declined at the late reproductive stage of the grass.

**Conclusion** There were no differences in the proximate composition and fiber fractions of the two varieties of *Panicum maximum*. The CP content reduced and the NDF content increased as the plant matured.

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**Table 1:** Effect of harvest stage on the proximate composition and fibre fractions of two *Panicum maximum* varieties

	DM	CP	EE	Ash	NDF	ADF
<b>Varieties</b>						
Panicum maximum (Local)	93.46	8.92	17.25	11.41	65.28	37.43
Panicum maximum (Ntchisi)	93.75	8.67	16.86	11.15	66.96	35.05
SEM	0.33	0.66	0.95	0.53	2.08	1.84
<b>Maturity stages</b>						
Vegetative	95.27 <sup>a</sup>	10.41 <sup>a</sup>	17.50	12.00	59.55 <sup>b</sup>	28.61 <sup>c</sup>
Early Reproductive	92.66 <sup>b</sup>	9.35 <sup>a</sup>	16.83	11.34	68.15 <sup>a</sup>	43.03 <sup>a</sup>
Late Reproductive	92.89 <sup>b</sup>	6.62 <sup>b</sup>	16.83	10.50	70.67 <sup>a</sup>	37.07 <sup>b</sup>
SEM	0.20	0.60	1.07	0.59	2.03	1.42

# Relationship between somatic cell counts, milk yield and gross composition of milk at the last stage of lactation in dairy sheep

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**Application** Somatic cells in sheep milk affect its quality and technological properties. Estimating these effects is crucial to justify the cost of interventions to improve milk quality.

**Introduction** Gross chemical composition of sheep milk is of paramount importance for the cheese-manufacturing industry (Albenzio et al. 2004), which has established milk-pricing criteria based on it. For this reason, dairy sheep farmers have intensified their efforts to improve both the quantity and the quality of the produced milk. To facilitate these efforts, the detailed investigation of factors with potentially negative effects on milk yield and quality traits, and the quantification of these effects are critical and justify planning and implementation of evidence-based improvement schemes. The objective of the study was to investigate and quantify the effects of somatic cell counts (SCC) on milk-, fat-, protein-, lactose-, solid-non-fat- and total solids- yield using a half-udder model in dairy sheep.

**Material and methods** A total of 55 purebred ewes of three Greek dairy sheep breeds (Chios, Karagouniko and Boutsko), from one flock, at the last stage of lactation (one month before drying off), were enrolled in the study. Two milk samples (one from each udder half) were collected from each individual ewe during morning milking, following standard milk sampling procedures. Milk samples were tested for fat-, protein-, lactose-, solid-non-fat- (SNF) and total solids- (TS) content using the MilkoScanTM FT+. SCC were estimated using FossomaticTM FC (Foss). Daily milk- (DMY), fat- (DFY), protein- (DPY), lactose- (DLY), SNF- (DSNFY) and TS- (DTSY) yields were calculated for each udder half to increase the statistical reliability of the data set (Leitner et al. 2004), using the morning milking records and adjusting according to ICAR recommendations. Descriptives (mean±standard error) were calculated and data were analysed with SPSS v23. Analysis of covariance was used under a randomized block design, where ewes served as blocks, the two udder halves as the primary factor within each block, and the logarithm of SCC (LogSCC) as covariate, as follows:

$$Y_{ij} = \mu + E_i + U_j + a_1 \times \text{LogSCC} + e_{ij}$$

where,  $Y_{ij}$ = dependent variables (DMY, DFY, DPY, DLY, DSNFY and DTSY),  $\mu$ = overall mean,  $E_i$ = random effect of the ewe  $i$  ( $i$ = 55 levels,

1 to 55 ewes),  $U_j$ = fixed effect of half udder side ( $j$ = 2 levels 1= left half, 2= right half),  $a_1$ = linear regression coefficient of LogSCC,  $e$ = error term.

**Results** The effects of LogSCC were statistically significant in all the studied traits ( $P < 0.001$  for DPY, DLY, DSNFY, DTSY and  $P < 0.01$  for DMY, DFY). A 1-log increase in SCC was associated with a reduction on DMY (217 g, 33.8%), DFY (11 g, 39.3%), DPY (13 g, 40.6%), DLY (14 g, 45.2%), DSNFY (30 g, 44.8%), and DTSY (40 g, 42.6%). Random effect of the ewe was statistically significant in all cases ( $P < 0.001$ ), whereas half udder side effect was not in any case (Table 1).

**Table 1**

	DMY	DFY	DPY	DLY	DSNFY	DTSY
Average value	642±33.0	28±1.6	32±1.5	31±1.6	67±3.4	94±4.8
Half udder side effect†	-58±51.7	-5±2.5	-2±2.4	-3±2.5	-6±5.3	-10±7.6
LogSCC effect	-217±76.7**	-11±3.8**	-13±3.7***	-14±3.8***	-30±8.2***	-40±11.7***

\*\* $P < 0.01$ , \*\*\* $P < 0.001$ , †Right half udder was used as reference

**Conclusion** Increased SCC in sheep milk adversely affect milk yield and all of its gross chemical composition traits. Regular assessment of SCC can be achieved either on a point-of-care basis (e.g. using California Mastitis Test) or via advanced laboratory equipment. Updated information may facilitate timely decisions for evidence-based modifications on factors determining SCC in sheep milk.

**Acknowledgements** The authors acknowledge dairy company Delta for analyzing the milk samples.

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# Effects of lactation number and calving season on milk yield and quality traits in dairy cattle in Greece

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**Application** Recently, dairy cattle industry has been reshaped in Greece. For its sustainability, the study of factors affecting milk production and suggestion of targeted modifications, are essential.

**Introduction** Consumer demands, environmental factors and socio-economic aspects have challenged the development of dairy cattle industry in Greece and still reshape its structure and evolution schemes, accelerating the integration of improved breeding stocks, state-of-the-art knowledge and modern technologies. To assess the efficiency of the fore mentioned interventions and suggest evidence-based modifications, the determination of factors affecting productivity is of paramount importance (von Keyserlingk et al.,2013). The aim of the study was to investigate the effects of lactation number and calving season on milk yield (MY) and quality traits (MQT), in Greek dairy cattle farms the last 17 years.

**Material and methods** A total of 63 dairy cattle farms from six counties in Greece were enrolled in the study. Data regarding physiological and production traits from 20,203 cows were initially

retrieved from the data base of Holstein Association of Greece (years 2003 to 2019). Finally, a total of 12,687 cows with a complete set of records were retained for the purposes of the study. Records included farm ID, ear tag, calving date, age at calving, lactation number, total milk yield in 305-days of lactation (305d MY), as well as daily milk- (DMY), fat- (DFY), protein- (DPY) and lactose yield (DLY) in the first stage of lactation (15 to 40 days post-calving). SPSS v23 was used for data analyses, which included descriptives (mean±standard error) and analytical statistics. Namely, univariate analysis of variance was used to estimate the fixed effects of lactation number (5 levels; 1st, 2nd, 3rd, 4th and >4th lactation), calving season (12 levels; January to December) and year of observation (17 levels; from 2003 to 2019) and the random effect of farm, on 305d MY and DMY, DFY, DPY and DLY.

**Results** The average 305d-MY was 10,757±22.78 kg and DMY was 34.5±0.09 kg. DFY, DPY and DLY were 1.42±0.005, 1.09±0.003 and 1.69±0.005 kg, respectively. Figures 1 and 2 present the relationship between 305d MY, MQT and lactation number, calving month and year. Effects of lactation number, calving season, year and farm on all the studied traits were significant at the 0.001 level.

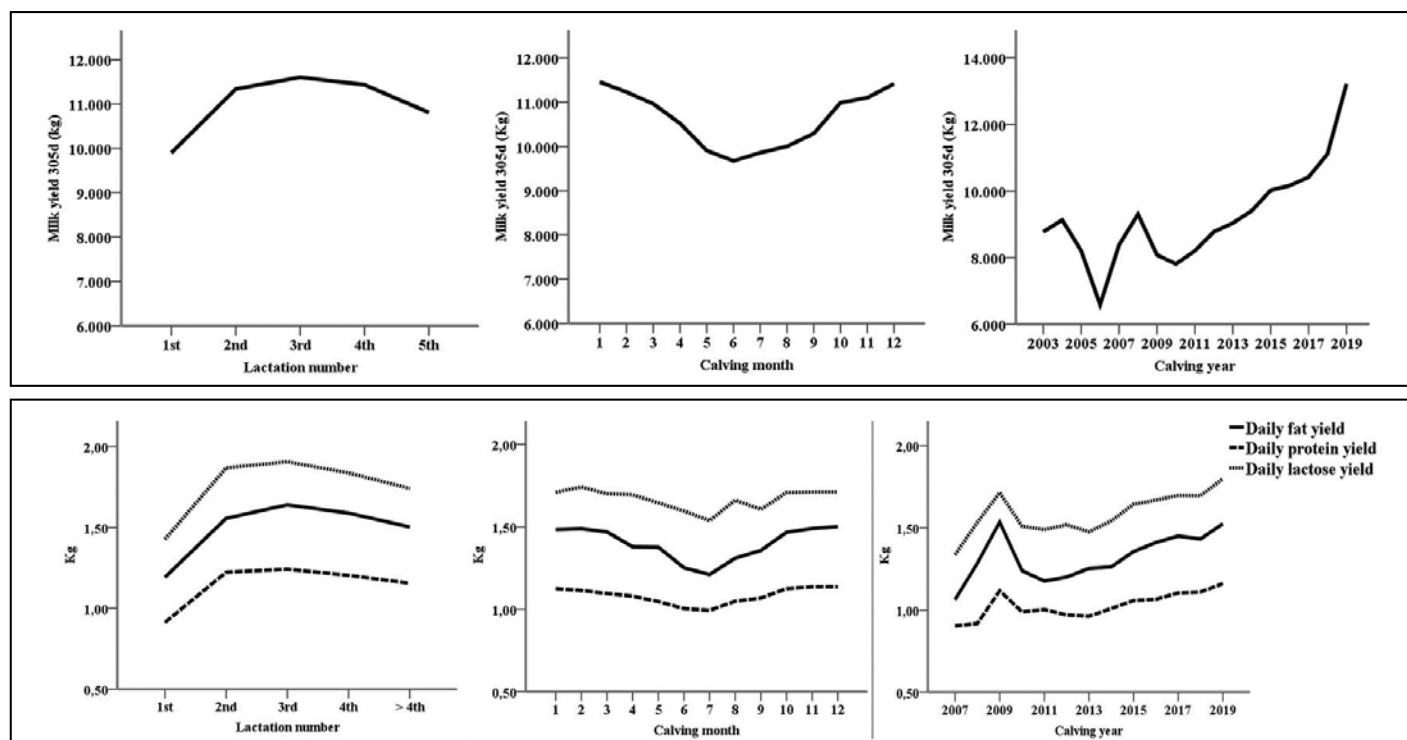
**Conclusion** MY and MQT are improving in dairy cattle in Greece the last ten years. Calving season significantly affects these traits and summer calving is associated with a reduced productive potential, unlike winter that favours it.

**Acknowledgements** The authors acknowledge Holstein Association of Greece for its collaboration.

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**Figure 1.** Average milk yield in 305 days of lactation (kg) according to lactation number, calving month and year.



**Figure 2.** Average daily fat-, protein- and lactose yield (kg) according to lactation number, calving month and year.

# A case-study with increased prevalence of white line disease in a dairy sheep flock

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**Application** White line disease is a non-infectious cause of foot-related lameness in dairy sheep. It is considered a welfare issue, but its aetiology and epizootiology remain unclear due to data scarcity.

**Introduction** Recently, increased demands for sheep milk have led to intensification of dairy sheep farming systems in Greece. Intensive farms enjoy the benefit of increased productivity, however, they are challenged by health and welfare issues, such as foot-related lameness, associated primarily with inadequate management, permanent housing conditions and increased metabolic stress. A significant cause of foot-related lameness is white line disease (WLD). In sheep, its aetiology is unknown, the epizootiological data are scarce and mainly refer to meat breeds, whereas, production losses associated with WLD have not been estimated, although the adverse effect of WLD in cows' milk yield is known. The objective of the present cross-sectional study was to assess the epizootiological traits of WLD in an intensively reared flock of dairy sheep in Greece, that reported increased occurrence of WLD.

**Material and methods** Eighty purebred ewes of three Greek dairy sheep breeds (38 Chios, 22 Karagouniko and 20 Boutsiko ewes), from one flock, at the last stage of lactation, were enrolled in the study (15, 13, 13 and 39 ewes of 1st, 2nd, 3rd and >4th parity, respectively). Exploratory foot-trimming was performed at each individual ewe by trained personnel who assessed hoof overgrowth and lesion severity and topography. Data regarding animal ID, breed, number of lactation, body condition score (BCS), daily milk yield (DMY) and WLD lesions were recorded. Descriptive epidemiology was used to estimate the prevalence of WLD, whereas, the likelihood of WLD occurrence according to age and BCS was assessed using binary regression analysis. Possible effect of WLD status on DMY was investigated using univariate analysis of variance, where breed (3 levels, Chios, Karagouniko and Boutsiko), lactation number (4 levels, 1st, 2nd, 3rd and > 4th lactation) and WLD status (2 levels, presence and absence of WLD at least in one hoof) were used as fixed effects and BCS (five-degree scale with increments of 0.25; 1=emaciated, 5=obese) as covariate. All statistical analyses were performed with SPSS v23.

**Results** Overall prevalence of WLD in the studied sheep population was 50.0% (40/80). The highest prevalence was observed in Boutsiko (55.0%, 11/20), followed by Chios (50.0%, 19/38) and Karagouniko ewes (45.5%, 10/22). WLD lesions, were more prevalent in fore limbs (67.5%, 27/40) compared to hind limbs (52.5%, 21/40) and in internal hooves (19.4%, 31/160) compared to external (10.6%, 17/160). Moreover, they were observed in one, two, three and four limbs in 67.5% (27/40), 30.0% (12/40), 0.0% (0/40) and 2.5% (1/40), respectively. Ewes at > 4th lactation were ca. 5 times ( $P<0.05$ ) and 3.7 times ( $P=0.059$ ) more likely to develop WLD lesions (at least in one limb) compared to 3rd and 2nd lactation ewes. Average BCS was 2.4 and varied from 1.5 to 3.5, whereas, it did not have a significant effect on WLD occurrence (Table 1). Prevalence of WLD-associated lameness was 1.25% (1/80), whereas no significant effect of WLD on DMY was found.

**Table 1.** Effects of age and body condition score on the occurrence of white line disease.

	B1	S.E. <sup>2</sup>	Wald	P	Odds ratio	95% C.I. <sup>3</sup> for Odds ratio	
						Lower	Upper
1st lactation	-0.037	0.624	0.003	0.953	0.964	0.284	3.276
2nd lactation	-1.298	0.687	3.567	0.059	0.273	0.071	1.050
3rd lactation	-1.605	0.747	4.613	0.032	0.201	0.046	0.869
>4th lactation BCS	-0.258	0.504	0.262	Ref. <sup>4</sup> 0.609	0.773	0.288	2.074

1B: Regression coefficient, 2 S.E.: Standard Error, C.I.3: Confidence Interval, 4 Ref.: Reference category, BCS: body condition score

**Conclusion** WLD is misdiagnosed due to the inability of the farmer to recognize characteristic lesions during hoof trimming (Winter and Arsenos, 2009). Thus, its prevalence in dairy sheep may be underestimated. An increased likelihood of developing WLD lesions in old ewes compared to young adult is evidenced. Adverse effect of WLD on DMY has not been observed in the present study, however, further research needs to be done to assess the effects of WLD on other production, health and welfare traits.

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# How are lamb performance and ewe milk yield influenced by ewe genetic merit?

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**Application** Ewes of high genetic merit, regardless of origin, will have increased milk yield in early lactation and produce lambs of greater live weight by six weeks of age resulting in a reduced number of days to slaughter.

**Introduction** Within the sheep industry, it has been demonstrated that milk yield is a driver of superior lamb growth in early lactation (McGovern et al., 2015). Increased availability of milk will improve weaning weight, which in turn, as shown by Santos et al. (2015) reduces days to slaughter and therefore, improves overall efficiency of the ewe. The aim of the abstract is to evaluate whether ewes of high genetic merit for maternal traits have increased milk yield in early lactation and in turn result in superior lamb performance up to six weeks of age and to slaughter.

**Material and methods** A four year study was carried out at Teagasc Athenry, Co. Galway, Ireland from 2015 to 2019. Three groups of animals (n=60), comprising of two main breeds (equal split of Texel and Suffolk), were assembled; representing high genetic merit New Zealand ewes (High NZ), high genetic merit Irish ewes (High Irish), and low genetic merit Irish ewes (Low Irish) as selected from New Zealand's Maternal Worth Index and Ireland's Euro-star Replacement Index, respectively. Ewes of high genetic merit were ranked within the top 20% of animals on the replacement index while ewes of low genetic merit were within the bottom 20%. In early October, ewes were artificially inseminated to a synchronised oestrus within genetic strain. In January, ewes were pregnancy scanned. The mean lambing date was March 8th. Ewe milk yield was measured at six weeks post-lambing using the weigh-suckle-weigh technique, and average daily milk yield was calculated. The procedure is displayed in Table 1. Lamb live-weight was measured at six weeks and fortnightly thereafter (kg). Lambs were drafted from the treatment group at a target weight of 43 kg in June (i.e. weaning), the target draft weight increased by an additional kilogram live-weight each month thereafter. The number of days from birth until drafting was calculated and reported as days to slaughter. Data was analysed using a linear mixed model in PROC MIXED (SAS 9.4) where genetic strain (breed and genetic merit), year and either number of lambs born or reared were included in all models. Additional fixed effects used included sex of the lamb, date of birth of the lamb and the ewe's age. Ewe age and lamb's sire within genetic strain were included as repeated and random effects, respectively.

**Results** Milk yield was highest for High NZ ewes compared to High Irish and Low Irish where High NZ ewes produced 18% more milk than the Low Irish (P<0.05; Table 2). Lamb six week weights for High Irish and Low Irish were similar, however, High NZ were 1.4 kg heavier than Low Irish (P<0.01). Days to slaughter for High NZ ewes were five and fifteen days lower than High Irish and Low Irish, respectively (P<0.05).

**Table 1.** Weigh-suckle-weigh procedure

Timeline	Procedure
8am	Ewes and lambs are brought in from grazing and penned separately for three hours. Lambs are placed within sight and smell of the ewes but where they are unable to feed.
11am	1ml oxytocin is administered intramuscularly per ewe to induce milk let down. Ewes are suckled by their lambs or hand-milked until all accumulated milk is removed and the ewe is dry. Lambs and ewes are penned separately again once fed/milked.
2pm	Three hours after being milked dry, another 1ml oxytocin is administered per ewe. The lambs are weighed and then reunited with their mothers where they can feed. Lambs are reweighed immediately after feeding to calculate milk intake. Surplus milk is milked out by hand and measured.
	The difference in the lamb weight plus the amount of surplus milk is totalled and multiplied by 8 as a predictor for daily milk yield (kg/day).

**Table 2.** Results

	High NZ	High Irish	Low Irish	S.E.	P-Value
Milk Yield (kg/day)	2.11 <sup>a</sup>	1.88 <sup>ab</sup>	1.74 <sup>b</sup>	0.146	P<0.05
Six Week Weight (kg)	18.22 <sup>a</sup>	17.50 <sup>ab</sup>	16.82 <sup>b</sup>	0.548	P<0.01
Days to slaughter	160 <sup>a</sup>	165 <sup>a</sup>	175 <sup>b</sup>	4.6	P<0.05

**Conclusion** Milk yield, six week weights and days to slaughter are similar for both High and Low Irish ewes. However, NZ ewes outperform Low Irish ewes for milk yield and six week weights. Ultimately, ewes with increased milk yield produce lambs that reach their target slaughter weight earlier.

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# Discrimination of contagious and environmental strains of *Streptococcus uberis* by mass spectrometry and machine-learning to combat mastitis in dairy herds

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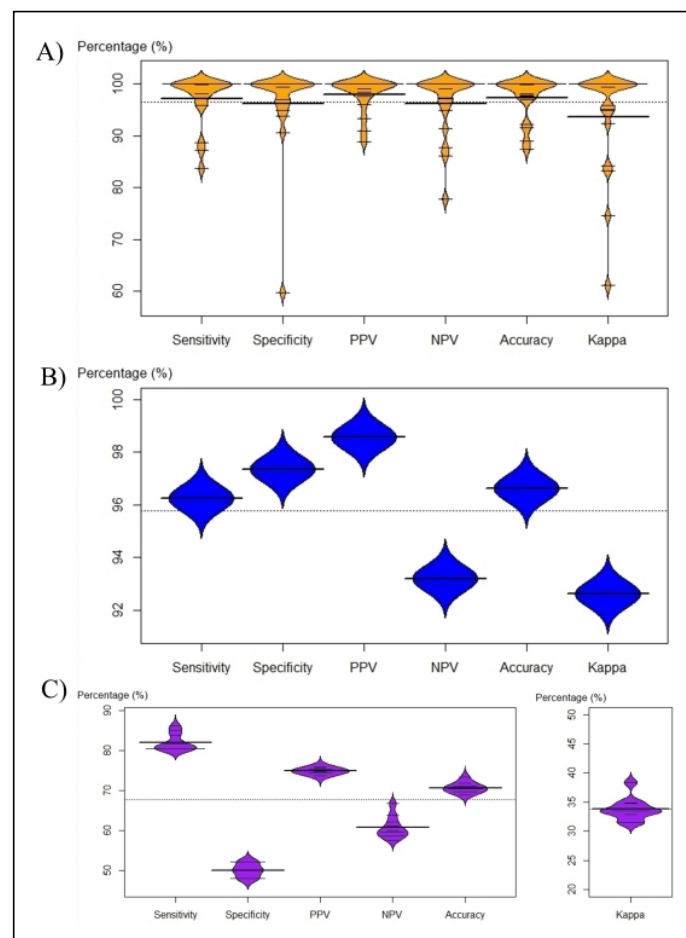
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**Introduction** Clinical mastitis is one of the most important challenges faced by the dairy industry and *Streptococcus uberis* is one of the most common causal pathogens. Mastitis can be classified as environmental or contagious, depending on the transmission route adopted by the pathogen involved. Knowledge of transmission route is essential for the selection of the most suitable intervention. The identification of diagnostic biomarkers to discriminate between environmental and contagious strains of *S. uberis* is, therefore, a fundamental milestone towards the development of tools for disease control. Here we show that the use of spectral profiles acquired from clinical isolates using the matrix-assisted laser desorption ionization/time of flight (MALDI-TOF) technique, combined with the development of classifiers/predictors powered by machine learning technologies, can lead to the successful discrimination of environmental and contagious *S. uberis* strains.

**Methods** Clinical mastitis isolates of known origin (environmental or contagious, determined by MLST) were acquired from fifty-two dairy farms around the UK and processed via MALDI-TOF to obtain spectra. Several classifier models were developed based on machine learning technologies such as genetic algorithms (GAs) and supervised neural networks (SNNs). Classifiers dedicated to individual farms were first developed, targeting nineteen farms featuring the co-presence of both forms of mastitis.

**Results** The best classifiers were found to be those powered by genetic algorithms, achieving 97.81% accuracy during cross-validation, with Cohen's kappa coefficient of 0.94 (means of the values obtained for the nineteen individual farms). A global classifier was also developed by merging all the data available from the same farms. The accuracy was 95.88% at cross-validation (kappa 0.93) but dropped to 70.67% during external validation, using data from another 10 farms left as holdout (kappa 0.34). MALDI-TOF spectral peaks deemed significant for the discrimination were extracted from the trained classifiers and used to identify proteins in the NCBI databases via bioinformatics.

**Figure 1:** Distribution of the performance indicators for the classifiers/predictors. (a) intra-farm cross-validation; (b) inter-farm cross-validation; (c) inter-farm external validation.



**Conclusion** This study demonstrates that matrix-assisted laser desorption ionization/time of flight (MALDI-TOF) mass spectrometry coupled with machine learning can be used to develop diagnostics solutions that can discriminate between environmental and contagious strains of *S. uberis*. The contagious and environmental *S. uberis* isolates were found to be related to bacteriocin and ribosomal activities suggesting that functions correlated to immunity, growth and competition over nutrients may be related to the different transmission routes.

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# A cost, impact and efficiency investigation into the effect of two different farrowing systems on sow and piglet welfare and production

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**Application** A study demonstrating the comparative similarity in piglet mortality rates and weight gain between Freedom Farrowing (FF) and Traditional Farrowing (TF) cohorts with identified differences in behavioural welfare indicator outcomes.

**Introduction** Pork production has increased by 1.5% per annum over the last 10 years; one potential consequence of increased demand at a low price point is the risk of declining welfare standards (ADHB, 2019). Farrowing crates have traditionally been used to reduce occurrence of piglet mortality as a result of sow crushing however the utilisation of TF crates is suggested to contribute to negative welfare for both the sow and piglets (Jarvis et al., 2005). Alternatives to traditional farrowing crates exist in the forms of farrowing pens, loose-housing systems and outdoor systems and in recent years development of modified free-farrowing crates has provided practical alternatives to TF crates (FAWC, 2015).

**Methods** Mortality rates, weight gain and behaviour of two cohorts, FF and TF, of sows were compared. In total 24 sows, consisting of Large White cross Landrace in two batches of 12, were housed in two different farrowing systems (6 TF and 6 FF per batch) within a purpose-built farrowing barn. Both sets of farrowing pens measured 2.8mx2m giving a total area of 5.6m<sup>2</sup>. Sows in TF crates were confined by conventional steel farrowing bars whilst the FF crate was non-confined. Data were recorded on six cameras (Sannce 1080p HD Night Vision) via continuous sampling during a 12-hour period daily (8am-8pm). Behavioural observations were recorded for seven days, following a similar method to Chidgey et al.,(2016). Behaviours recorded included maintenance behaviours, nursing behaviours, social interactions between sows and piglets and

**Table 1:** Sow behaviour Chi-squared analysis

(a) Piglet behaviour Chi-squared analysis  
 (b) (significant results in bold).

**Table 1a**

Sow behaviour	Chi-squared	p-value
<b>Lying</b>	<b>5.69</b>	<b>0.017</b>
Sitting	0.222	0.637
Standing	0.047	0.827
Feeding	0.105	0.746
<b>Nursing</b>	<b>6.66</b>	<b>0.01</b>
<b>Socialising with piglets</b>	<b>12</b>	<b>0.001</b>
<b>Exploring pen</b>	<b>6</b>	<b>0.014</b>
Sham chewing	1	0.317
Bar biting	0.333	0.564

TF piglets spent more time lying away from the sow (lying elsewhere)  $X^2(2, N=24) = 4.78, p=0.029$  and engaging in agonistic behaviours with other piglets  $X^2(2, N=24) = 4.76, p=0.029$  whilst FF piglets spent more time feeding from the sow  $X^2(1, N=24) = 63.18, p<0.001$  and playing with other piglets  $X^2(2, N=24) = 4.37, p=0.036$  (Table 1b).

**Table 1b**

Piglet behaviour	Chi-squared	p-value
By creep	1.40	0.236
By sow	0.891	0.345
<b>Lying elsewhere</b>	<b>4.78</b>	<b>0.029</b>
Walking	1.65	0.198
<b>Playing</b>	<b>4.37</b>	<b>0.036</b>
<b>Feeding from sow</b>	<b>63.1</b>	<b>&lt;0.001</b>
Massaging sow	3.64	0.056
Climbing sow	0.142	0.705
Nosing sow	3.2	0.074
<b>Agonistic with piglets</b>	<b>4.76</b>	<b>0.029</b>
Biting pen	2.66	0.102
Biting sow	1.28	0.257

between piglets, explorative behaviours, consummatory behaviours and stereotypic behaviours. The weight of each piglet was recorded weekly from birth to weaning using digital weighing scales. Piglet mortality data was also collected to compare prevalence in these cohorts.

**Statistical analysis** Statistical analysis was performed using Minitab 17 Statistical Software (2010). Data was tested using Chi squared test for goodness of fit to analyse behavioural data and 2-sample t-tests to analyse the piglet weight data. Piglet mortality was calculated as a percentage of the cohorts and analysed via 2-sample t-test.

**Results** Results indicated no significant difference in mortality rates (2-sample t-test,  $t_{24} = -0.08, p=0.761$ ) between systems (Figure 1) or in weight gain of piglets (batch 1: 2-sample t-test,  $t_{12} = -0.01, p=0.993$ ; batch 2: 2-sample t-test,  $t_{12} = -0.12, p=0.904$ ).

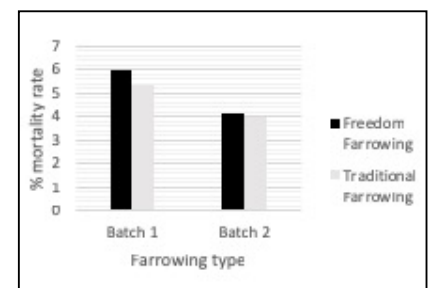
**Conclusion** As farmed production animals it is important that management changes to improve welfare consider economic impacts; this study demonstrates that both mortality rates and weight gain of piglets are comparable between the two systems giving an overall advantage to the implementation of FF crates in a pig production environment due to the demonstrated increase in positive behaviours and decrease in undesirable behaviours in both sows and piglets.

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**Figure 1:** Comparison of percentage mortality rates per batch in Freedom versus Traditional Farrowing crates with standard error



Behaviour of sows indicated a number of significant differences between FF and TF cohorts including TF sows spending more time lying down,  $X^2(2, N=24) = 5.69, p=0.017$  and FF sows spending more time nursing their piglets  $X^2(1, N=24) = 6.66, p=0.01$ , socialising with their piglets  $X^2(2, N=24) = 12, p=0.001$  and exploring the pen  $X^2(2, N=24) = 6, p=0.014$  (Table 1a).

# Investigating the relationship between temperament and social role in dairy cattle

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**Application** Manipulating group composition in dairy cattle based on individual cow temperament may reduce the social instability, increased aggression and reduced welfare frequently seen in dynamic groups.

**Introduction** Social dynamics in dairy cattle are frequently disrupted by regrouping which leads to increased levels of aggression, reduced lying and feeding times (von Keyserlingk et al, 2008) and elevated plasma cortisol levels (Mench et al, 1990). Social network analysis can be used to quantify interconnected social relationships in groups of cattle allowing group members with different social roles to be identified. Social roles in other species have been shown to depend on individual temperament type (Aplin et al, 2013). Manipulating group composition based on individual social roles has been shown to alter social stability in primates (Flack et al, 2006) but has not been investigated to date in cattle. This study investigated the relationship between individual cow temperament and social role in groups of commercial dairy cattle.

**Materials and Methods** Direct observation of displacements, allogrooming and non-displacement aggressive behaviour (pushing, butting and threats) at the feed face was used to create social networks in a dynamic group of 90 lactating Holstein cows. Ad libitum behavioural sampling was carried out on 15 days from 30 minutes before to 120 minutes after delivery of a TMR ration. Two tests were used to quantify temperament: Novel arena test (NA): each cow was placed in an unfamiliar visually isolated arena 6m x 7m: distance travelled, steps taken, time spent moving, sniffing, vocalising, defaecation and urination were recorded over 5 minutes; Novel object test (NO): immediately following the novel arena test, a multi-coloured umbrella was introduced through the arena wall and repeatedly opened and closed for 30 seconds: distance travelled, time spent moving, sniffing, vocalising, defaecation and urination and time in contact with the umbrella were recorded over 5 minutes. Social networks were constructed for displacement, allogrooming and non-displacement aggressive interactions and data analysed using RStudio 1.1.456. Univariate regression analysis was used to identify relationships between network degree centrality and outcome variables in the temperament tests, cow age, bodyweight and days in group (DG). Variables showing associations at the level of  $P \leq 0.1$  were selected for inclusion in multiple regression models based on degree centrality in each network. Models were then simplified by stepwise elimination based on AIC.

**Results** On univariate regression, allogrooming centrality was significantly positively correlated with days in group ( $P < 0.001$ ) and number of vocalisations during the NA test ( $P < 0.05$ ). Allogrooming centrality tended to be positively correlated with movement time during the NA test ( $P < 0.1$ ). These three variables were included in the allogrooming model (Table 1). Non-displacement aggression centrality was significantly positively correlated with days in group ( $P < 0.05$ ) and age ( $P < 0.001$ ). Non-displacement aggression centrality tended to be negatively correlated with distance travelled in the NO test ( $P < 0.1$ ). These variables were included in the non-displacement aggression model (Table 2). Displacement centrality had no variables meeting the criteria for inclusion in a model.

**Table 1:** Allogrooming centrality model

Variable	Coefficient	P Value
DG	-0.00978	0.361
NA movement time	-0.00691	0.016*
NA vocalisation	-0.316	0.00958*
DG: NA movement time	0.000338	0.00089*
DG: NA vocalisation	0.00438	0.0224*

**Table 2:** Non-displacement aggression centrality model

Variable	Coefficient	P Value
DG	0.0358	0.07
Age	1.962	0.209
NO distance travelled	0.0388	0.0633
DG: age	-0.00983	0.0382*
DG: NO distance travelled	-0.000583	0.053

**Conclusions** Cows with a more active temperament (as quantified in the NA and NO tests) had a higher allogrooming network centrality and a lower non-displacement aggression network centrality indicating that these individuals are likely to be key in maintaining social stability in dynamic groups. Manipulating group composition based on individual temperaments is therefore likely to improve social stability and reduce welfare issues seen in dynamically grouped dairy cattle.

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# Effect of early life nutrition on the Subcutaneous Adipose proteome of Holstein Friesian bull calves

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**Application** This study highlights the key proteins underlying biological response in subcutaneous adipose tissue as a consequence of an enhanced dietary intake for the first 12 weeks of life in bull calves. Results from this study may be further utilised for the potential optimisation of enhanced early-life nutrition programs for high genetic merit Holstein Friesian bull calves.

**Introduction** Enhanced dietary intake during the critical early life period has been shown to have a positive effect on subsequent lifelong growth and production potential of cattle. However the effect of enhanced nutrition during the early life period on the biochemical regulation of many metabolically important tissues including the adipose depots is currently unknown. Adipose tissue represents an endocrine organ with important functions affecting feed intake, immune function, growth and reproductive development. The objective of this study was to evaluate the effect of enhanced dietary intake during the first 12 weeks of life on the subcutaneous adipose proteome in Holstein-Friesian bull calves.

**Material and methods** Holstein Friesian bull calves with a mean (SEM) age and bodyweight of 17.5 (2.8) days and 48.8 (5.3) kg, respectively, were assigned to either a high (H; n=10) or moderate (M; n=10) plane of nutrition, to achieve an average target growth rate of 1.0 and 0.5 kg/day, respectively. Calves on H and M received 1.5 and 0.5 kg of milk replacer (MR) per day, reconstituted at 15 and 12.5% (w/v), respectively. Calves on H were offered concentrate ad libitum, while M received, 500g day<sup>-1</sup>. At 87 days ( $\pm 2.141$ ) of age, all calves were euthanized and tissue samples were harvested from the subcutaneous adipose fraction for subsequent proteomic analyses. Proteins were extracted from the tissue and subjected to trypsin digestion followed by liquid chromatography and subsequent mass spectrometry. The acquired raw MS data were processed by MaxQuant v1.6.2.3 followed by protein identification using the integrated Andromeda search engine. Spectra were searched against a Uniprot Bos Taurus reference database.

**Results** As planned, calves offered a higher plane of nutrition were heavier at slaughter (112 v 88 kg,  $P < 0.001$ ), reflecting their higher average daily gain (0.88 v 0.58 kg, for H and M groups respectively,  $P < 0.001$ ). 185 differentially abundant proteins (DAP) were identified between the H and M groups ( $P_{adj} < 0.1$ ; fold change  $> 1.5$ ). Significantly enriched pathways are described in Table 1. These proteins were involved in fatty acid metabolism, glycolysis, fatty acid biosynthesis and steroid biosynthesis.

**Table 1** significantly enriched pathways identified as between H M groups

Term description	Observed protein count	Background protein count	False discovery rate
Lipid biosynthesis	12	90	0.000000298
Mitochondrion	27	671	0.00000173
Lipid metabolism	15	235	0.00000938
Oxidoreductase	19	393	0.00000938
Steroid biosynthesis	4	17	0.002
Fatty acid metabolism	6	64	0.003
Fatty acid biosynthesis	5	41	0.0032
Mitochondrion inner membrane	9	205	0.0108
Sterol biosynthesis	3	14	0.0126

**Conclusion** 48 DAP identified in this study were consistent with differential expressed genes previously identified at the transcript level between calves fed differentially during the first 16 weeks of life (English, 2018). These results undoubtedly indicate a greater functionality at a phenotype level of the genes coding for proteins identified as differentially abundant. This data aids in the identification of potential biomarkers which can add to genomically assisted breeding programmes in selecting genetically elite bulls with enhanced pubertal development.

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# Incidence and impact of abnormal reproductive cycles identified in modern UK dairy cows through milk progesterone analysis

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**Application** Poor reproductive function in dairy cows continues to present a challenge to efficient production in the modern high yielding dairy cow.

**Introduction** Studies have previously reported an incidence of reproductive cycle problems, measured by milk progesterone analysis, of around 39 to 46% (Opsomer et al., 1988; Royal et al., 2000; Mann et al., 2005). More recent European studies have reported an incidence of 31% (Nyman et al 2014). The aim of the present study was to reassess this issue in the modern UK dairy cow.

**Material and Methods** Data from a number of experimental studies were collated into a single dataset of 228 cows. All animals were lactating Holstein Friesian cows maintained within a commercial herd environment and fed various diets comprising a total mixed ration based on grass and maize silage with additional feedstuffs as well as concentrates fed according to yield. In all studies milk samples were collected twice or three times per week from around day 10 post calving until around day 120. All samples were analysed for progesterone by ELISA (Ridgeway Scientific). Milk progesterone data were used to define three types of cycle abnormality (Mann et al. 2005) namely delayed onset of luteal activity (progesterone < 3ng/ml until > 50 days post-partum), cessation of luteal activity (progesterone < 3ng/ml for > 2 weeks following a period of > 3 ng/ml) and prolonged luteal activity (progesterone > 3ng/ml for >3 weeks). Data were analysed by analysis of variance with study as a factor. Conception to first service data were analysed by chi squared analysis.

**Results** Of 228 cows studied, 157 (0.69) had normal cycles while 71 (0.31) exhibited reproductive cycle problems with delayed onset (0.066), cessation (0.044) or prolonged (0.202) luteal activity. Overall problem cycles were more prevalent in older cows while condition score at calving showed no association. Problem cycles were associated with a significantly later days to first progesterone rise and days to first AI as well as a significantly lower conception rate to first service and a longer time to conception.

**Table 1.** Parameter in cows exhibiting normal and problem reproductive cycles

	Normal (n=157)	Problem (n=71)	
Parity	2.72 ± 0.12	3.24 ± 0.25	P < 0.05
BCS at calving	2.64 ± 0.04	2.72 ± 0.07	ns
Days to first P4 rise	27.6 ± 0.8	37.7 ± 2.6	P < 0.001
Days to 1st AI	66.2 ± 1.7	77.7 ± 3.1	P < 0.001
Conception to 1st AI	0.382	0.238	P < 0.05
Days to conception	91.3 ± 5.4	129.4 ± 14.0	P < 0.001

**Conclusion** Abnormal reproductive cycles continue to be a problem in modern UK dairy cows and are associated with a substantial reduction in reproductive performance.

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# In-vitro gas production and methane concentrations when using horse cecal or rumen fluid as inoculum

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**Application** A better understanding of feedstuff degradation and methane production from different species can provide new insight and a better understanding of the factors affecting methane emission from animals.

**Introduction** In recent years, methane emission from ruminants has received numerous focus whereas little is known about methane emission from horses (Knapp et al. 2014; Dansen et al. 2015). The aim of the present study was to investigate differences and similarities between horses and cows when measuring in-vitro gas production and methane concentrations.

**Materials and methods** In-vitro gas production was measured in two experiments using either rumen or cecal fluid as inoculum. Three non-working cecum cannulated horses and three non-lactating rumen cannulated dairy cows fed a daily ration (as fed) of 7.5 kg of hay, 1 kg of straw and 0.3 kg of pelleted barley were used in the study. The ANKOM RF wireless gas production system (ANKOM Technology, Macedon, New York, USA) was used and procedures described by the company were followed. In brief, bottles were incubated for 48 h with 1 g of ground feed sample (hay, straw or barley) weighed into 250 mL incubation bottles and mixed with 34 ml of inoculum (rumen or cecal fluid) and 66 ml of buffer. In each experiment, a total of six bottles per feed were used together with three blank bottles without any test feed and two bottles with an internal standard. In-vitro gas production was measured every ten minutes by the system, and after 48 h of incubation gas samples were collected from the head space of half of the bottles and analysed in duplicate for methane concentrations. Gas production data (mL gas/g DM) was fitted to the model by Groot et al. (1996) and the parameters asymptotic gas production (A), time after incubation at which half of the maximum gas production is reached (B) and a constant related to the shape of the curve (C) were estimated. Experiments were analysed independently using SAS® in a model where the response variables depended on feedstuff.

**Results** Total gas production (A) was higher when using rumen fluid as inoculum than cecal fluid, but the ranking of the three feedstuffs was the same (barley was highest, then hay and lowest for straw). It took longer time for straw to reach half of the total gas production (B) than for hay and barley. The methane concentration was not affected by feed for horses but

using rumen fluid as inoculum resulted in hay having the highest concentration followed by barley and then straw. The methane concentration was considerably higher for rumen inoculum than for cecal inoculum.

**Table 1** The asymptotic gas production (A), time after incubation at which half of the maximum gas production is reached (B), a constant related to the shape of the curve (C) and methane concentration (ppm/mL) after 48 h of incubation using either cecal or rumen fluid as inoculum in an in-vitro gas production system.

Parameter	Inoculum	Straw	Hay	Barley	SEM	P*
A	Horse	136 <sup>c</sup>	153 <sup>b</sup>	280 <sup>a</sup>	3.3	<0.001
	Cow	206 <sup>c</sup>	235 <sup>b</sup>	312 <sup>a</sup>	5.7	<0.001
B	Horse	19.1 <sup>a</sup>	7.7 <sup>c</sup>	12.7 <sup>b</sup>	0.3	<0.001
	Cow	17.4 <sup>a</sup>	12.2 <sup>b</sup>	10.5 <sup>c</sup>	0.3	<0.001
C	Horse	1.841 <sup>a</sup>	1.352 <sup>b</sup>	1.945 <sup>a</sup>	0.058	<0.001
	Cow	2.128 <sup>b</sup>	1.632 <sup>c</sup>	2.535 <sup>a</sup>	0.056	<0.001
CH <sub>4</sub>	Horse	18912	20469	21063	801	NS
	Cow	72081 <sup>c</sup>	98106 <sup>a</sup>	87514 <sup>b</sup>	3325	<0.001

\* Values in a row with different superscripts are significantly different.

**Conclusion** In-vitro gas production and methane concentrations were lower when using cecal fluid from horses than rumen fluid from cows but ranking of feedstuffs based on total gas production was similar when using the two sources of inoculum. Interestingly, the methane concentration was considerably lower when using cecal than rumen fluid as inoculum, and further analysis (microbiota and fermentation products) might identify what caused these differences.

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