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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Fourth Meeting of the Nutrition Society was held in the Biochemistry Department of the University of Liverpool (Lecture Theatre 2, Life Sciences Building), Crown Street, Liverpool on Friday, 25 *March,* 1977, *when the following papers were read:*

https://doi.org/10.1079/PNS19770038 Published online by Cambridge University Press

The energy value to sheep of three mixed grass silages. By J. S. SMITH, F. W. WAINMAN and P. J. S. DEWEY, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Little is known about the efficiency with which the metabolizable energy (ME) content of grass silages can be used to support maintenance (k_m) and promote fattening (k_f). The few values available for k_f in particular are very variable probably because of difficulties incurred in getting sheep to eat silage in amounts significantly above maintenance (Ekern & Sundstøl, 1973, Sundstøl & Ekern, 1976; Thomas, Kelly, Chamberlain & MacDonald, 1976).

Measurements have been made of the energy values of three silages, two of which were prepared from first harvest grass and one from third harvest. A preliminary report on one of these silages has already appeared (Smith, Wainman & Dewey, 1975).

The dry matter (DM) intakes as multiples of maintenance and the mean daily energy balances of the sheep at the high level of intake for the three silages are shown below. All results have been expressed as kg metabolic body size ($W^{0.75}$).

	Silage		
	1	2	3
Harvest	1st	1st	3rd
DM intake (×maintenance)/d	1·60	1·56	1·39
ME intake (kJ)/d	604·9	549·4	538·6
Metabolizability (2)	0·57	0·63	0·56
Retention (kJ)/d	+107·4	+117·8	+47·7
Efficiency of utilization of ME above maintenance (k_f)	0·49	0·54	0·31
Net energy (above maintenance) (kJ/g DM)	5·29	7·10	3·51

To obtain energy balances at the high level of intake, sheep were offered each silage *ad lib.* and then this amount was reduced by 10% so as to minimize refusals. Values for k_f are those calculated at the observed intakes and have not been corrected to a theoretical intake of twice maintenance.

The Agricultural Research Council (1965) and Blaxter (1973) give equations to predict k_m and k_f from ME content. The findings which formed the basis for these equations did not include silages, nevertheless both equations predict k_m and k_f well for the first harvest silages. For the third harvest silages the equation of Blaxter (1973) is much better than that of ARC (1965).

These results suggest that the relationship between the ME content of grass silage and its efficiency of utilization for maintenance and fattening in sheep is very similar to that which exists for hay and dried grass.

Agricultural Research Council. (1965). The Nutrient Requirements of Farm Livestock No. 2, Ruminants. London: Agricultural Research Council.
Blaxter, K. L. (1973). Nutrition Conference for Feed Manufacturers, 7. University of Nottingham.
Ekern, A. & Sundstøl, F. (1974). *Publs Eur. Ass. Anim. Prod.* No. 14, p. 221.
Smith, J. S., Wainman, F. W. & Dewey, P. J. S. (1975). *Proc. Nutr. Soc.* 34, 101A.
Sundstøl, F. & Ekern, A. (1976). *Publs. Eur. Ass. Anim. Prod.* No. 19, p. 241.
Thomas, P. C., Kelly, N. C., Chamberlain, D. G. & MacDonald, L. (1976). *Publs. Eur. Ass. Anim. Prod.* No. 19, p. 245.

Evaluation of dried coffee residue as a component of diets for ruminants.

By M. M. Ali, J. H. Topps and the late T. B. Miller, *Division of Agricultural Chemistry and Biochemistry, School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Dried coffee residue (DCR), a by-product from the manufacture of 'instant' coffee, has been included in certain concentrate mixtures recommended for cattle. Evidence, derived mainly from chemical analysis and digestibility trials and reviewed by Ali (1976), has shown that it has a high content of energy, fat and fibre but doubts exist as to the extent of its digestibility in the ruminant. The nutritional characteristics of a large sample of DCR have been studied by the use of numerous laboratory procedures and in eight animal trials.

The DCR contained 60 g water/kg and the composition of the dry matter (g/kg) was acid detergent fibre 728, cellulose 493, lignin 233, fat 225, crude protein 102, ash 7, tannin 1·2 and caffeine 0·5. The fat was predominantly triglycerides with a high content of unsaturated acids which were principally linoleic and oleic acids.

A laboratory method (Tilley & Terry, 1963) of measuring digestibility gave very low values (approximately 0·05) but various delignification processes raised the values to between 0·07 and 0·12. Removal of fat resulted in a very small improvement in digestibility. When contained in a nylon bag and suspended in the rumen of fistulated cattle, DCR was very slowly fermented; after 72 h 15% of the dry matter had disappeared.

Inclusion of DCR at a level of 110 g/kg in a diet of hay and barley given to steers depressed the digestibility of all the dietary constituents, the greatest depressions were in digestibility of fat and fibre, 0·19 and 0·13 units respectively. Similar but smaller decreases in digestibility were found for diets fed to sheep which contained either 45 or 75 g DCR/kg. For both species the digestibility of the constituents in DCR, obtained by difference, was in the range 0·096–0·516.

The acceptability of concentrate diets containing DCR, whether pelleted or unpelleted, by either cattle or sheep was poor and variable. Frequently a cyclic pattern of food and water intake was evident with animals consuming high and low amounts on alternate days. When given through a rumen fistula to cattle, larger amounts, as a fraction of the diet, were required to depress food intake. This difference indicated that palatability was an important characteristic affecting intake. Animals receiving relatively large amounts of DCR, e.g. mature cattle given 1·2 or 1·5 kg/d, drank large quantities of water and excreted large volumes of urine. A diuretic effect of DCR was established, the cause of which is yet to be ascertained.

Ali, M. M. (1963). Studies of dried coffee residue as a component of diets for cattle and sheep. PhD thesis, University of Aberdeen.

Tilley, J. M. A. & Terry, R. A. (1963). *J. Br. Grassld. Soc.* 18, 104.

Disruption of feeding behaviour in sheep by portal vein infusions of mixed volatile fatty acids or propionate. By M. H. ANIL and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

The observation that propionate, infused into the ruminal vein of sheep during spontaneous meals, was a potent depressor of daily food intake (Baile, 1971) has been extended by continuous portal infusions of volatile fatty acid (VFA) mixtures or propionate alone at rates similar to the rates of absorption after feeding.

Sheep were prepared under general anaesthesia with portal vein catheters by the method of Harrison (1969). Polyvinyl catheters (NT2) were introduced through a branch of a mesenteric vein with their tips lying at the porta hepatis. Jugular vein catheters were fitted under local anaesthesia at least 24 h before infusion into that vein. When the animals had returned to normal *ad lib.* intake of the complete pelleted ration, infusions were made, at least 48 h apart, from 30 to 240 min after offering fresh food in the morning. Meal patterns were recorded by continuous weighing of the feed buckets which were suspended from strain gauge beams. Solutions of VFA salts (55% Na acetate, 30% Na propionate, 15% Na butyrate on a molar basis; pH 7·3) were infused at 1 ml/min.

4 mmol/min of VFA salts, intraportally, completely prevented feeding during all but the first 30 min of infusion ($n=5$). 184±68 (SE) g was eaten between 60 and 240 min after feeding on intervening control days. Jugular vein infusions at the same rate depressed but did not prevent feeding compared to control days (53±22 *v.* 170±60; $n=4$). 2 mmol/min VFA inhibited feeding when given intraportally (127±75 *v.* 204±95; $n=6$). Lower levels of infusion into the portal vein tended to stimulate intake, compared with jugular infusions.

When sodium propionate alone was infused into the portal vein at the same rates as its previous levels of infusion in the mixtures, feeding behaviour was affected to a lesser extent (74±65 g) while jugular infusions had little effect when compared with controls (184±87 *v.* 226±92; $n=4$). Total daily intakes were not significantly affected by any treatment.

The depression in food intake during VFA infusion which seems to be mediated by the liver is therefore largely due to the propionate content.

Baile, C. A. (1971). *Physiol. Behav.* **7**, 819.
Harrison, F. A. (1969). *J. Physiol., Lond.* **200**, 28P.

Dietary lipid supplementation and the flow of fatty acids to the duodenum of sheep. By R. KNIGHT, J. D. SUTTON and J. E. STORRY, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The flow of fatty acids to the duodenum of the sheep may exceed dietary intake by 100% (Sutton, Storry & Nicholson, 1970) suggesting that microbial synthesis of fatty acids may occur in the rumen.

To examine this further five sheep fitted with rumen and re-entrant duodenal cannulas, anterior to the entry of the bile and pancreatic duct, were given a basal ration of 200 g hay and 400 g concentrate mix/d alone or with supplements of 40 g/d of linseed oil (LSO) or coconut oil (CCO) either protected or unprotected in a 5×5 latin square design. Flow of fatty acids at the duodenum was estimated by taking spot samples over a 5 d period, chromic oxide being used as the marker. Fatty acids were measured by chloroform–methanol extraction followed by gas–liquid chromatography.

The increase in total fatty acids (TFA) in the stomach on the basal ration (Table 1) consisted mostly of C18:0 with smaller amounts of C16:0. The increase in C18:0 was greater than could be accounted for by hydrogenation of C18 unsaturates alone. A smaller increase in TFA occurred when free LSO was fed, due mainly to a decrease in C18 acids, particularly C18:0. When free CCO was fed, large decreases in total fatty acids occurred. In terms of individual fatty acids, however, C16 and C18 acids increased whereas medium-chain acids, notably C12:0, were markedly reduced. Protection had only small effects on flow of the main groups of fatty acids and only reduced hydrogenation by about 20%.

It is suggested that the large amount of C18 acids from LSO may have inhibited the de novo synthesis of C18:0 that occurred on the basal ration. The decrease in C12:0 when CCO was fed may be due to absorption from the rumen or catabolism within it.

Table 1. *Mean intake (I) and flow to the duodenum (D) of three groups of fatty acids (g/d)*

	C6–14		C16		C18		Total	
Fatty acid group	I	D	I	D	I	D	I	D
Basal	0·06	0·35	1·66	2·49	4·15	7·64	6·06	11·37
LSO	0·05	0·25	3·82	6·57	40·78	38·64	45·22	47·83
Protected LSO	0·16	0·21	4·01	4·75	39·66	37·78	44·12	44·45
CCO	29·37	6·26	4·67	6·34	8·11	11·67	42·31	24·96
Protected CCO	30·47	11·36	4·75	5·86	8·19	10·68	43·59	28·59

R.K. acknowledges receipt of an Agricultural Research Council studentship.

Sutton, J. D., Storry, J. E. & Nicholson, J. W. G. (1970). *J. Dairy Res.* 37, 97.

The relative importance of bile salts and phospholipids in fat absorption in the sheep. By W. M. F. LEAT and F. A. HARRISON, *ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

In the sheep bile is essential for fat absorption into the lymphatics (Heath & Morris, 1963; Harrison & Leat, 1972). In ruminants, as in non-ruminants, bile salts are important for solubilizing lipids in the lumen of the intestine (Lough, 1970), but the relative importance of the other major component of bile, namely phospholipid, in absorption is ill defined. Ovine thoracic duct lymph contains 20–25% phospholipid which is mainly of endogenous origin, and it has been calculated that the flow of biliary lecithin could account for a major part of lymph phospholipid (Leat & Harrison, 1974).

To determine the relative importance of biliary constituents in fat absorption, and the extent to which biliary lecithin could contribute to lymph lecithin, a sheep was prepared with a biliary re-entrant cannula and with fistulation of the thoracic lymph duct. Diversion of bile resulted in a decrease in the secretion of lymph total fatty acids from 960 mg/h to 120 mg/h within 3 h. At this stage the infusion of 7% bile salts (Oxoid) for 50 min at 1 ml/min resulted in a net secretion into the lymphatics of 288 mg fatty acid in a 3 h period, over and above baseline secretion. A similar infusion of bile salts containing 500 mg purified biliary lecithin resulted in a net secretion of 483 mg fatty acid; and with bile salts plus 350 mg purified lysolecithin of common bile duct origin the corresponding value was 295 mg fatty acid.

In a second experiment bile was diverted and replaced immediately by a continuous infusion into the intestine of 5% bile salts at 1 ml/min. Secretion rates of lymph triglyceride decreased from a mean value of 812 mg/h to 350 mg/h after 3 h, and lymph phospholipids from 244 mg/h to 126 mg/h. In the absence of the bile salt infusion the secretion rates of triglyceride and phospholipid were 45 mg/h and 30 mg/h respectively. Other results suggest that the lymph phospholipids secreted during the infusion of bile salts alone were mainly of endogenous origin.

It is concluded that under short term conditions (a) bile salts alone maintain fat absorption at about 50% of control values but the response appears to decrease with time, and (b) part of the lymph phospholipids are derived from endogenous sources other than bile e.g. mucosa or plasma.

Harrison, F. A. & Leat, W. M. F. (1972). *J. Physiol.* **225**, 565.
Heath, T. J. & Morris, B. (1963). *Br. J. Nutr.* **17**, 465.
Leat, W. M. F. & Harrison, F. A. (1974). *Quart. J. exp. Physiol.* **59**, 131.
Lough, A. K. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 519 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Energy status and body-weight in underfed dairy cows. By C. J. ROBERTS, SALLY M. DEW, G. D. BAIRD and I. M. REID, *ARC, Institute for Research on Animal Diseases, Compton, Newbury, Berks RG16 0NN*

The shortage and high cost of feedstuffs in recent winters led to many cases of debility in dairy cows, and the present study was undertaken to measure the biochemical and pathological effects of undernutritional debility.

Twenty Friesian×Ayrshire cows were allocated to four treatment groups. Three groups were fed at 100, 60 and 40% of requirement for energy (ARC, 1965) for 13 weeks before and 13 weeks after calving, and the fourth group was fed at the basic maintenance requirement during the same period. All groups were then fed at 100% of control requirement for a further 26 weeks. The feeding regime was based on a daily allowance of 4·5 kg barley straw for each animal, with requirements above this being supplied by a cubed concentrate feed.

The mean daily metabolizable energy (ME, MJ/d) surpluses (+) or deficits (−) have been estimated (MAFF, 1975) for each of the groups of cows during the underfeeding and recovery periods, and are shown below, together with mean daily live weight changes (LW kg/d). The results from the 40% and maintenance groups have also been combined in two further groups on the basis of duration of lactation; cows in the Milk group continued to lactate to the end of the experiment whilst those in the Dry group stopped lactating 19–23 weeks after calving.

Group		100%	60%	40%	Maintenance	Milk	Dry
No. of cows		3	6	6	5	4	7
Underfeeding	ME	+9·15	−22·1	−32·3	−28·5	−34·0	−29·6
period (6 months)	LW	+0·28	−0·16	+0·09	+0·02	+0·07	+0·06
Recovery period	ME	+3·4	+19·4	+36·1	+24·5	+11·5	+41·7
(6 months)	LW	+0·04	+0·14	+0·09	+0·18	+0·01	+0·21

The results, particularly from the 40% and Dry groups, suggest that undernutrition in late pregnancy and early lactation has a pathological effect on cows such that full use cannot be made subsequently of adequate energy supplies. For example, the mean ME surplus in the 40% group in the first 3 months of adequate feeding was 41·1 MJ/d, which should have resulted in weight gains of about 1·2 kg/d, rather than the 0·4 kg/d observed. Blood and tissue samples from these animals are being studied at present to determine the nature of this pathological effect.

ARC. (1965). The nutrient requirements of farm Livestock. No. 2. Ruminants. London: Agricultural Research Council.

MAFF. (1975). Energy allowances and feeding systems for ruminants. Technical Bulletin 33. London: Her Majesty's Stationery Office.

Rumen ciliates of sheep given cellulose, lactose, sucrose or starch diets.

By J. P. JOUANY, J. SENAUD, J. GRAIN, P. DE PUYTORAC and P. THIVEND, *INRA, Centre de Recherches Zootechniques et Vétérinaires de Theix,* 63110 *Beaumont, France.*

High readily fermentable carbohydrate diets can greatly alter rumen protozoal populations (Fauconneau & Gausseres, 1961). The present experiments were designed to study the interactions between diets rich in various carbohydrates and ciliates in sheep rumen contaminated with one or more species.

Ten adult sheep fitted with a permanent rumen cannula were used. Their rumen ciliate populations were composed of *Polyplastron multivesiculatum* (two sheep), or *Entodinium sp.* (four sheep), or these two ciliates together (two sheep), or a normal protozoa population (two sheep). Each sheep was given a control diet composed of hay and groundnut meal, then three experimental diets in which one part of the control diet's hay was replaced by sugar beets, deproteinized whey or barley. The amounts of cellulose, sucrose, lactose and starch ingested were respectively 20·8, 21·0, 22·1, 21·4 g/kg live weight$^{0.75}$. Diets were complemented with groundnut meal to be isonitrogenous. The diets were given in two equal meals daily. Every day, the rumen juice was sampled 1 h after food intake; ciliates were counted during 10 d at least for each animal.

Numbers of protozoa (10^3/*ml*) *in rumen fluid of sheep fed rations containing different carbohydrates*

(Mean values with their standard errors)

Rumen contaminated with:	Diets: Cellulose	Sucrose	Lactose	Starch
Entodinium sp.	61·0±1·9^{a}	183·0± 9·9^{b}	269·0±31·9^{c}	2050·0±29·5^{d}
P. multivesiculatum	3·5±0·2^{aA}	4·2± 0·2^{aB}	6.4± 0·2^{b}	2·6±0·1^{c}
Entodinium sp. +	54·6±1·8^{a}	421·2±24·9^{b}	430·7± 7·7^{b}	692·8±18·6^{c}
P. multivesiculatum	6·5±0·4^{a}	16·2± 1·0^{b}	6·8± 0·4^{a}	23·7± 0·4^{c}
Entodinium sp. +	180·3±9·1^{a}	464·5±13·0^{b}	877·2±28·9^{c}	2463·5±79·9^{d}
P. multivesiculatum +	6·3±0·5^{a}	6·3± 0·2^{a}	0·7± 0·1^{b}	33·1± 1·5^{c}
Isotricha sp. +	1·3±0·1^{a}	5·9± 0·3^{b}	4·4± 0·3^{c}	6·1± 0·4^{b}
Dasytricha r.	15·5±1·6^{a}	52·4± 2·0^{b}	5·4± 0·6^{c}	18·6± 1·4^{a}

Means followed by different superscripts are significantly different (a, b, c, d, $P<0.01$; A, B, $P<0.05$)

The *Entodinium sp.* population became larger and larger with cellulose, sucrose, lactose and starch, independently from the presence of other ciliates. The development of *P. multivesiculatum* depends on the ingested carbohydrates and on the presence of other ciliates: when *P. multivesiculatum* was the only species in the rumen, it developed more with soluble sugars than with starch. With *Entodinium sp.* or with other ciliates, its development was limited or reduced on the lactose diet, but it increased on the starch diet. *Isotricha sp.* developed with lactose and chiefly with starch and sucrose. The population of *Dasytricha* was considerable with the sucrose diet but not with the lactose diet: this is believed to be due to changes in the extent of the rumen pH drop (5·51 versus 6·16).

Fauconneau, G. & Gausseres, B. (1961). Festchrift zum VIII intern. Tierzuchtkongreb Hamburg. p. 32. Stuttgart: Verlag Eugen Ulmer.

Digestion of lactose in the rumen of sheep. By P. THIVEND and M. A. EHOUINSOU, *INRA Centre de Recherches Zootechniques et Vétérinaires de Theix, 63110 Beaumont, France*

By-products from the manufacture of cheese represent a source of lactose which could advantageously be used by ruminants (Schingoethe, 1976). However, the digestion of lactose in the rumen has not been clearly studied, especially when lactose is given in large quantities with non-protein nitrogen.

To provide information on this aspect, five adult sheep, fitted with a rumen cannula were given 1·250 kg lucerne hay/d; then they received four diets consisting of dry deproteinized whey supplemented with urea–hay (20:80; 40:60: 70:30; 90:10; w/w). Diets containing either 0, 165, 340, 580 or 720 g lactose/kg dry matter (DM) and 0, 13, 23, 41 or 56 g urea/kg DM were given twice daily at a feeding level of 45 g/kg live weight$^{0.75}$. Animals were accustomed to the experimental diets by progressively increasing the lactose intake (100 g/d); they received each diet for a period of 21 d. During each period, rumen liquor was sampled before and 0·5, 1, 1·5, 2, 4, 6, 8, 10 and 12 h after feeding. The main results are given below:

	Control	Experimental diets			
Proportion of lactose (g/kg DM)	0	165	340	580	720
Dry matter intake (g/d)	1118	1126	1160	1223	1238
pH	6·65^{aA} ±0·12	6·55^{aAB} ±0·11	6·49^{aB} ± 0·10	6·32^{b} ± 0·80	6·24^{b} ± 0·34
Volatile fatty acids (mM/l)	91·1^{A} ±7·4	77·6^{B} ±1·8	81·6^{AB} ±28·8	84·4^{AB} ± 7·7	83·5^{AB} ±14·3
Molar percentage of VFA:					
acetic acid	69·0^{a} ±0·5	58·4^{bA} ±5·0	54.0^{bB} ± 3·1	53·7^{bB} ± 9·5	57·2^{bA} ± 8·2
propionic acid	19·7^{a} ±0·8	15·1^{b} ±2·2	19·4^{a} ± 2·2	15·4^{b} ± 5·0	7·8^{c} ± 1·2
butyric acid	6·7^{a} ±0·6	23·4^{bA} 7·6	23·7^{bA} ± 2·7	27·0^{bB} ±11·7	30·1^{bc} ± 8·4
Ammonia (mg/100 ml)	28·8 ±3·9	26·1 ±1·5	21·5 ± 2·0	24·2 ± 2·1	23·2 ± 4·5
Lactic acid (g/l)	0·04^{A} ±0·03	0·18^{AC} ±0·02	0·24^{BC} ± 0·05	0·32^{BC} ± 0·14	0·14^{AC} ± 0.06
Soluble carbohydrates (g/l)	0·26^{a} ±0·04	0·70^{a} ±0·06	1·65^{b} ± 0·77	0·53^{a} ± 0·29	0·34^{a} ± 0·13

Mean values with their standard deviations.

Means followed by different superscripts are significantly different ($P<0·01$ for small letters; $P<0·05$ for capital letters).

The increase of the lactose and urea contents in the diet did not affect the dry matter intake and did not induce digestive disorders but rumen fermentations were modified. PH values in the rumen were significantly lower when sheep were given 580 g lactose/kg DM intake. The proportion of acetic and propionic acids decreased and the proportion of butyric acid increased, as soon as lactose was given. The concentration of lactic acid was low but significantly higher with diets containing 340 and 580 g lactose/kg DM. In spite of the large amount of urea intake, NH_3 concentration was low and not significantly different between diets. This probably means a high bacterial activity when sheep are given lactose. Soluble carbohydrate concentrations considerably increased when sheep were given 165 and 340 g lactose/kg DM, but decreased with higher lactose diets. The evolution of the rumen fermentations according to the lactose content of the feed can be related to the feeding behaviour of animals. Thus, the number of lactose meals daily was, respectively, 6±3 and 13±5 (means and SD) when sheep were given 340 and 580 g lactose/kg DM.

Schingoethe, D. J., (1976). *J. Dairy Sci.* **59**, 556.

The effect of glucose infusion on liver metabolism in the dairy cow in vivo. By M. A. LOMAX, G. D. BAIRD, H. W. SYMONDS & C. B. MALLINSON, *Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berks RG16 0NN*

The rate of production of metabolites by the liver following intravenous glucose infusion was measured in dairy cows that had been catheterised by a combination of the procedures of Symonds & Baird (1973) and Baird *et al.* (1975).

Three mature Friesian×Ayrshire cows, two lactating and one non-lactating, were infused with a 50% aqueous solution of glucose at a rate of 4·2 mmol/min for 48 h via a jugular vein catheter. Portal and hepatic blood flow rates together with metabolic production rates were determined using the procedures described by Baird *et al.* (1975). The effects of the glucose infusion on the hepatic metabolite production rates were sufficiently similar in the three cows to allow these values to be pooled.

	Hepatic production rates (mmol/min)†				
		Period of glucose infusion (h)			
Time (hours)	Control (0)	4	24	48	72
Metabolite					
Glucose	6·20	2·20••	3·08•••	4·37•	6·23
Lactate	−3·38	−2·25	−1·32•	−1·15••	−2·02•
Propionate	−7·19	—	−5·85	−6·17	−6·66
D-(−)-3-hydroxybutyrate	3·41	2·89	1·61••	2·01•	3·28
Butyrate	−2·72	—	−0·63••	−1·07•	−1·04•

•$P<0.05$; ••$P<0.01$; •••$P<0.001$ (as compared with control value).
†A positive value indicates net production by the liver and a negative value net utilization by the liver.

The infusion of glucose decreased hepatic glucose output, in confirmation of the results of Thompson *et al.* (1975), and decreased the uptake of the gluconeogenic precursor lactate. Perhaps surprisingly, the infusion did not significantly affect the hepatic uptake of propionate. The hepatic output of D-(−)-3-hydroxybutyrate was decreased (c.f. Treacher *et al.* 1976), and a corresponding decrease in hepatic uptake of the ketogenic precursor, butyrate, was also noted. The output of acetate from the gut decreased during glucose infusion and this was accompanied by a decrease in blood acetate concentrations. The infusion elicited a 2-fold increase in the plasma insulin concentrations, measured by radioimmunoassay, in each of the lactating cows, and a 6-fold increase in the non-lactating cow. Hepatic metabolite production rates were not affected appreciably during a control infusion of saline.

Baird, G. D., Symonds, H. W. & Ash, R. (1975). *J. Agric. Sci.* **85**, 281.
Symonds, H. W. & Baird, G. D. (1973). *Res. Vet. Sci.* **14**, 267.
Thompson, J. R., Weiser, G., Seto, K. & Black, A. L. (1975). *J. Dairy Sci.* **58**, 362.
Treacher, R. J., Baird, G. D. & Young, J. L. (1976). *Biochem. J.* **158**, 127.

Feed protein degradation and microbial protein synthesis in the rumen of sheep fed lucerne and barley. By J. C. MATHERS and E. L. MILLER, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX*

There have been suggestions that the energetic efficiency of microbial protein synthesis in the rumen may vary with the substrate being fermented. We have investigated this possibility in sheep fed diets varying from all roughage to all concentrate.

In a randomized block design, four wethers fitted with rumen and duodenal cannulas were offered, at 2 hourly intervals, 760 g/d of diets consisting of chopped lucerne (L), 2:1 lucerne:barley (LB), 2:1 barley:lucerne (BL) and rolled barley (B). In addition, each animal received 40 g/d of supplements containing minerals, vitamins and chromic oxide plus urea so that each diet supplied 19·5 g N/d. Non-ammonia-nitrogen (NAN) flow to the duodenum was measured and microbial protein synthesized in the rumen determined by the ^{35}S method (Mathers & Miller, 1977). Feed protein escaping ruminal degradation was calculated by difference after allowing 1·5 g NAN/d as endogenous secretions. Organic matter fermented (FOM) in the rumen was estimated as OM apparently fermented plus microbial OM synthesized therein. Some of the results are shown in the table.

Diet	Non-urea-N intake (g/d)	Flow to duodenum (g NAN/d)			Degradability of feed N*	Microbial yield (g NAN/kg FOM)
		Total	Microbial	Feed		
L	19·6	17·3	10·3	5·5	0·72	29·6
LB	17·9	17·7	12·3	3·9	0·78	33·2
BL	16·1	15·7	12·2	2·0	0·87	26·0
B	14·3	15·1	12·0	1·6	0·89	22·9
SE of mean		0·95	0·64	0·65	0·034	2·06

*Excluding dietary urea.

Flow of NAN to the duodenum increased with increasing inclusion of lucerne (up to 66%) in the diet due to increased protein intake, reduced ruminal degradation of dietary protein and increased efficiency of microbial protein synthesis. There was no interaction between these feeds in the extent of ruminal protein degradation when fed in mixed diets since deviations from linearity in degradability with change in diet were very small. However, in agreement with the work of Offer, Evans & Axford (1974), there was a suggestion of a synergistic effect of dietary constituents on the energetic efficiency of microbial protein yield since there was a large quadratic component.

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The digestibility of rumen microbial protein in the small intestines of sheep. By M. V. TAS, R. F. E. AXFORD and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor*

Three Welsh Mountain×Suffolk sheep were fitted with re-entrant cannulas at the proximal duodenum and terminal ileum. They were restrained in metabolism cages and fed at 2 h intervals. Samples of digesta representative of the daily flow were taken by an automatic procedure (Axford, Evans & Offer, 1971). The amino acid composition of the samples was determined and the daily passage of amino acids in and out of the small intestine was calculated. Sampling was continued for 40 d for sheep 1, 25 d for sheep 2 and 26 d for sheep 3. Sheep 1 was fed dried grass and sugar beet pulp at a constant rate of 600 g/d but of various proportions ranging from 100% dried grass to 25% dried grass giving a nitrogen intake ranging from 24 to 12 g/d.

Sheep 2 and 3 were fed 400 g hay and 400 g concentrates daily throughout the experiment. They also received a slow infusion into the duodenal digesta samplers of graded amounts of microbial dry matter (DM) isolated from the rumens of sheep, providing additional microbial DM ranging from 0–100 g/d.

The quantities of microbial dietary and endogenous amino acids entering the small intestine daily were calculated by the method of Evans, Axford & Offer, 1975. The treatments applied provided a range of daily passage of microbial amino acids into the duodenum. The digestibilities of dietary and microbial amino acids were estimated by multiple regression using the equation:

Absorbed amino acids $= c + a \times$dietary amino acids $+ b \times$microbial amino acids $+ d \times$endogenous amino acids and the values below were obtained.

Sheep	c	a	b	d
1	−8·6	0·88±0·04	0·89±0·02	0·84±0·13
2	−15·7	0·76±0·10	0·89±0·07	0·54±0·43
3	−15·7	0·86±0·09	0·84±0·09	0·78±0·23

The mean digestibility of microbial amino acids in the small intestine was found to be 0·87±0·02.

The negative value of the constant c represents the amino acids (g/d) passing into the small intestine as a postabomasal endogenous secretion.

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Urea-nitrogen recycling in sheep given low quality hill herbages.

By J. C. MACRAE, S. WILSON, J. A. MILNE and ANGELA M. SPENCE,
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In sheep fed diets of low nitrogen content greater amounts of non-ammonia-nitrogen (NAN) enter the small intestine than are ingested (Clarke, Ellinger & Phillipson, 1966; Hogan & Weston, 1969). It has been argued that the additional NAN comes mainly from the incorporation of recycled urea-N into rumen microbial protein. Recent work in this laboratory suggests that when sheep consume low quality hill herbage recycled urea-N contributes only a small part of the additional NAN.

Eight Scottish Blackface wethers, each prepared with rumen, duodenal and ileal cannulas and continuously fed a range of intakes of freeze-stored hill grass (*Agrostis/Festuca* (A/F)) or heather (*Calluna vulgaris*), were used to obtain relationships between N intake and the amounts of NAN entering and leaving the small intestine. The same diets were then fed to four sheep each prepared with a rumen and two caecal cannulas and chronic jugular catheters. Single injections or continuous infusions of [^{14}C]urea (intravenous) and [^{14}C]bicarbonate (intraruminal) were used to estimate the amounts of plasma urea-C entering the rumen fermentation pool. Plasma urea concentrations (A/F<14 mg/100 ml; heather <9 mg/100 ml) and urea entry rates (A/F<11 mg/min; heather<6 mg/min) were very low on both diets and at an intake of 450 g/d OM only 0·38 g/d (A/F) and 0·46 g/d (heather) of urea-C was degraded to rumen bicarbonate-C.

The amounts of urea-N entering the rumen fermentation pool (calculated from the urea-C results) together with the amounts of N consumed and NAN entering the small intestine of sheep given similar intakes (450 g/d OM) of each diet are given in Table 1. Only 23% (A/F) and 32% (heather) of the additional NAN arriving at the duodenum could be accounted for as recycled urea-N. This would suggest that substantial secretions of endogenous protein-N (3·0 g/d A/F; 2·3 g/d heather) occurred in sheep given each of these poor quality hill herbages.

Table 1. *Estimated N balance anterior to the duodenum in sheep given diets of* Agrostis/Festuca *or heather* (Calluna vulgaris) (450 *g* OM/*d*)

	g/24 h	
	Agrostis-festuca	Heather
N intake	7·1	5·2
Duodenal NAN	11·0	8·5
Net addition of NAN	3·9	3·4
Urea-N entering rumen fermentation pool	0·9	1·1
Other endogenous NAN secretions	3·0	2·3

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Estimation of extent of protein degradation from basal feeds in the rumen of sheep. By E. R. ØRSKOV and A. Z. MEHREZ, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The importance of the extent of rumen degradation of protein in determining both the supply of nitrogen for the rumen microbes and the protein available for digestion in the small intestine has been discussed in several recent reviews (Miller, 1973; Ørskov, 1974). We have adapted a technique involving incubation of feed in synthetic fibre bags in the rumen to provide a rapid and accurate method of determining rate and extent of degradation of protein. The technique is described in detail by Mehrez & Ørskov (1977).

The sheep fitted with large rumen cannulas (35 mm diameter) are fed on hay (dry matter digestibility >0·7) containing at least 25 g N/kg dry matter (DM), at about 50 g DM/kg $W^{0.75}$ per d. Dry roughages, cereals and other concentrates are coarsely ground through a hammer mill (2·5 mm) while silages and succulent materials are minced through a 5 mm screen. Samples of about 5 g are incubated in relatively large bags (10×17 cm). Four or five bags incubated simultaneously are withdrawn at predetermined intervals to determine the rate of disappearance of protein and DM, the last being removed at a time when the proportion of DM lost from the bag is estimated to be greater than the known DM digestibility of the feed in vivo. For straw and hay of low digestibility we have used incubation times of 12, 24, 30 and 36 h; for high quality hay, dried grass and silages 3, 6, 12, 18 and 24 h; for cereals, seeds and protein concentrates 3, 6, 12 and 20 h; and for roots, kale etc., 2, 5, 8 and 12 h.

The extent of protein degradation occurring under normal feeding conditions is assumed to be that reached when 90% of digestible DM of the feed sample has disappeared from the bag; this assumption is supported by the extent of rumen digestion of cellulose (Weston & Hogan, 1973), soluble carbohydrate and starch (Armstrong & Beever, 1969).

By this procedure the extent of protein degradation in the following basal feeds has been estimated to be: barley (containing 16·0 g N/kg DM), 0·80; barley (20·0 g N/kg), 0·73; wheat (17·9 g N/kg), 0·72; maize (18·7 g N/kg), 0·55; dried grass (20·7 g N/kg), 0·70; timothy silage (19·9 g N/kg), 0·82; timothy/ryegrass silage (21·2 g N/kg), 0·78; maize silage (10·3 g N/kg), 0·60; swede roots (21·3 g N/kg), 0·89.

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Amino acid composition of beef carcass meat and amino acid requirements of growing cattle. By T. W. GRIFFITHS, *The Agricultural Institute, Dunsinea, Castleknock, Co. Dublin, Eire*

Amino acid (AA) requirements of ruminants cannot be estimated directly from feeding experiments because of the modifying effects of the rumen microflora and indirect methods have been used. Factorial requirements for AA for growth in cattle have been estimated by Hutton & Annison (1972) and require findings on the composition of deposited tissue and the efficiency with which individual absorbed AA are utilized. There is, however, little recent information on the AA composition of beef carcass protein or on efficiency of utilization of individual AA.

Results were obtained from a comparative slaughter trial using Friesian castrate cattle (Griffiths & Spillane, 1976) on the AA composition of carcass meat and fatty tissues (CMF) at 150 kg and 400 kg live weight (LW). The AA composition of the CMF was similar at each LW, but protein from lean meat differed markedly from the protein associated with the fatty tissues. The table gives the net deposition (g/d) of some essential AA in CMF over the LW range 150–400 kg for an estimated LW gain of 1 kg/d, obtained by regression analysis. The table also shows (a) the estimated total body (TB) requirements using the relationship of Fox *et al.* (1976) and further assuming that the AA composition of carcass and non-carcass tissues were similar, and (b) the levels required at the duodenum assuming the digestibility coefficients of Tamminga (1975).

	CMF	TB	At duodenum (estimated)
Methionine + cystine	2·6±0·8	4	8
Threonine	3·8±0·6	7	11
Lysine	7·5±1·6	14	19

Net tissue requirements were similar to those calculated by Hutton & Annison (1972) whilst Fenderson & Bergen (1976) have estimated the absorbable AA requirements (g/d) in similar animals to be: methionine + cystine 18·6, threonine $\leqslant$15·1, and lysine $\leqslant$22·5.

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Metabolism of alanine in sheep muscle. By D. B. LINDSAY, J. W. STEEL* and P. J. BARKER, *Department of Biochemistry, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

It has been suggested (Felig, 1975) that much alanine-carbon released from muscle is derived from glucose, through the transamination of pyruvate. Alanine thereby acts as a nitrogen carrier from muscle to liver. In a study of the output of amino acids by sheep muscle (Lindsay, Steel & Buttery, 1977) it was shown that, of the amino acids estimated the largest output was that of alanine. Of the α-amino-N released from muscle, 24% was carried in this form. This is substantially larger than would be expected from the proportion of alanine in sheep muscle (10%).

In some of these experiments, constant intravenous infusions of [U-^{14}C]alanine were also made for 2–3 h, and towards the end of the infusions estimations were made of the radioactivity in alanine in arterial blood and in venous blood draining predominantly from muscle. Radioactivity measurements showed that although there was a net production of alanine, it was simultaneously taken up. The uptake (as a fraction of arterial input) was $0{\cdot}26\pm0{\cdot}10$ (3) in hourly-fed sheep, and $0{\cdot}15\pm0{\cdot}03$ (6) in sheep starved 48–96 h. From these values and the arterial concentrations, together with blood flow and the net exchange of alanine the gross output and uptake could be calculated. Although the net output of alanine in fed animals ($0{\cdot}32\pm2{\cdot}14$ μmol/kg muscle per min) was insignificant and much less than in fasted animals, ($5{\cdot}12\pm0{\cdot}66$), the gross output in fed animals ($4{\cdot}42\pm0{\cdot}92$) was significantly greater than zero and appreciable even in comparison with the gross output of fasted sheep ($7{\cdot}43\pm1{\cdot}48$).

The mean estimate of glucose uptake by muscle ($9{\cdot}3\pm8{\cdot}4$ μmol/kg per min) was much lower than we have previously observed, and had a large standard error because of two estimates showing an apparent production of glucose. Even so, the net available glucose $\left(\text{glucose} - \frac{\text{lactate}+\text{pyruvate}}{2}\right) - 3{\cdot}1$ μmol/kg per min – was sufficient to provide carbon for a substantial fraction of the gross alanine released from muscle.

Alanine entry rate was quite similar in fed ($11{\cdot}8\pm1{\cdot}9$ mmol/h) and fasted ($9{\cdot}7\pm1{\cdot}3$ mmol/h) sheep. Only 50–60% of this flow can however be accounted for as alanine output from muscle even in fasted sheep.

Felig, P. (1975). *Ann. Rev. Biochem.* **44**, 933.
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Eventual plateaux of body-weights of sheep and cattle predicted by simulation models not incorporating set points for body fat content.

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Although it has been acknowledged that regulation of energy balance in farm ruminants may have been changed by centuries of selection for fast growth, it has usually been assumed that fat deposition is limited by lipostatic mechanisms similar to those postulated for simple-stomached species (see Baile & Forbes, 1974). Such control mechanisms have not been quantified however, and in the construction of simulation models of the control of food intake and energy balance of sheep (Forbes, 1977) and cattle it was necessary to adopt the nul hypothesis that there is no direct feedback from fat depots to those centres which control food intake.

Voluntary food intake was assumed to be that weight of food necessary to supply the metabolizable energy requirements of the animal with a constant daily rate of fat deposition, unless physical limitation (see Baile & Forbes, 1974) intervened. Physical limitation, which was related to the digestibility of the diet and gut capacity as influenced by the deposition of abdominal fat, resulted in a decrease in rate of fat deposition until eventually an equilibrium was reached with constant body-weight and food intake. With the sheep model, for example, animals with a fat-free empty body-weight of 40 kg were, after 700 d of iteration, approaching asymptotic empty body-weights of 49, 60 and 68 kg for feeds of 55, 65 and 75% DM digestibility, respectively.

Voluntary intake was predicted to decline more steeply for the higher digestibility feeds, but eventually to reach asymptotic levels of 830 to 900 g DM/d for all feeds, a similar situation to that observed by Osbourn (1970) which showed large differences in the level of intake of feeds of different digestibilities initially, these differences becoming smaller as the sheep fattened. The cow model predicted plateaux in body-weight and food intake similar to those observed by Monteiro (1972).

It is possible, therefore, to explain the eventual plateau in body-weight in sheep and cattle by physical limitations of food intake without recourse to postulation of an inherent set point.

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Fluorine retention in growing sheep: a comparison between two phosphorus supplements as sources of fluorine. By R. G. HEMINGWAY, *Glasgow University Veterinary School, Bearsden, Glasgow*

Soluble fluorides lead to a greater deposition of F in bone than F present in rock phosphates or limestones (Phillips *et al.* 1960). It is expensive to reduce by acid and/or heat treatment the F content of rock phosphates to below about 2 g F/kg which is generally considered advisable for animal feeding. F present in dicalcium phosphate (DCP) may be more soluble than F contained in the complex structure of defluorinated phosphate (DFP).

Two groups each of four sheep (22 kg live weight) were group-fed (allowing about 1 kg/sheep per d) a basal diet consisting of (%) 45 barley, 30 barley husk siftings, 15 fish meal and 10 extra-molassed sugar beet pulp plus trace elements and vitamins (15·6 mg F/kg DM). An additional 50 mg F/sheep per d was provided as either (a) 37·2 g DCP (Croda Agricultural Ltd, London, with 1·34 g F and 175 g P/kg prepared by acid treatment of bone mineral) or (b) 30·8 g DFP (Negev Phosphates Ltd, Israel, with 1·62 g F and 178 g P/kg prepared by reacting phosphoric acid and soda ash with rock phosphate followed by heating to 1500°). Over an 8-week period both groups of sheep grew to about 36 kg live weight.

The sheep were then placed in cages and given 1100 g/d of the diet (14·9 mg F/d) supplemented with 50 mg F/d as described for 7 d followed by a 7 d balance period. The tail bones were then removed for analysis. Highly significant differences ($P<0·01$) were recorded. Sheep given DCP retained more F than those given DFP during both the 7 d balance and the 10 week growth period.

	Intake (mg F/d)	Faeces (mg F/d)	Urine (mg F/d)	Retention (mg F/d)	Bone Ash (g F/kg)
DCP	64·9	18·3	12·6	34·0	2·61
DFP	64·9	38·2	6·3	20·4	1·59
SED	—	1·22	1·25	2·29	0·176

In contrast, four comparable lambs given the unsupplemented diet (14·9 mg F/d) had 5·0 mg F/d in the faeces, 8·3 mg F/d in the urine and retained 1·6 mg F/d. Calculations showed that the availability of the F (i.e. not present in the faeces) was 34% for DFP and 73% for DCP.

It is concluded that the F content alone of a mineral supplement may not usefully indicate the extent to which it may be stored in the bone of growing sheep.

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The effect of phenothiazine on the toxicity of bracken to sheep. By A. Z. IDRUS, H. F. WALKER, D. C. MACDONALD and J. H. TOPPS, *Division of Agricultural Chemistry and Biochemistry, School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Liver is considered to detoxify foreign compounds, including certain polycyclic aromatic hydrocarbons through the action of benz(α)pyrene (BP) hydroxylase. Wattenberg & Leong (1965) found that BP hydroxylase activity in rat liver was greatly increased by adding phenothiazine to the diet: our experiments confirmed their results. Pamukcu, Wattenberg, Price & Bryan (1971) reported that feeding phenothiazine to rats fed on bracken decreased the incidence of intestinal and urinary bladder tumours by 60% compared with the number found in untreated rats. If the same toxin in bracken induces both tumours in rats and bone marrow damage in calves as suggested by Evans, Jones & Mainwaring-Burton (1972), then treatment with phenothiazine of sheep fed on bracken might protect them from bone marrow damage and other adverse effects by increasing the production of BP hydroxylase.

Two groups each of four wether sheep, age 8 months, were fed on barley and hay; group I received the diet only while group II was given phenothiazine at 4 g/kg feed for 5 d. Thereafter group I received a diet containing 20% ground dried bracken and 80% ground barley pelleted together with certain minerals and vitamins, while group II received the same diet containing 4 g phenothiazine/kg food. During the 69 d experimental period which followed sheep either maintained or increased their live weights: there was no appreciable difference in rate of growth between the two groups. Both groups of sheep seemed to be in normal health as judged by their appearance and rectal temperature. Pre-experimental mean leucocyte counts in blood were close to 8000 per cu mm, after 69 d on the bracken diet values for group I were 2163 (control) and for group II were 2869 (phenothiazine treated): the difference between these final values was not significant. After slaughter estimations of BP hydroxylase activity in liver showed that the sheep treated with phenothiazine had on average levels of enzyme activity six times greater than those of untreated sheep ($P<0.05$).

Thus the phenothiazine treatment which diminished the incidence of cancer among rats fed on bracken did not prevent sheep from suffering a decrease in blood leucocytes.

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Seasonal variations in the nutritive value of the mussel protein *(Mytilus edulis)*. By G. VARELA, M. JOYANES and A. TORRALBA, *Institute of Nutrition (C.S.I.C.) and Department of Physiology, Veterinary Faculty, Madrid – 3*

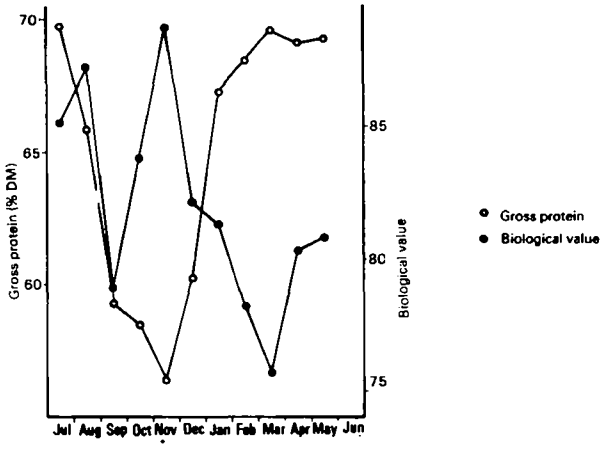
The production of mussels in Spain has been increasing and we studied the possibility of using this product in the form of protein concentrate, which offers a high protein content and excellent quality. It is possible to use mussels contaminated by organochlorated pesticides which decrease considerably during the process of extraction with organic solvents (Joyanes *et al.* 1976).

In the present work we study seasonal variations in the quality of mussel protein, which may be attributable either to circumstantial changes in the molluscs themselves or to variations in the environment (Bayne, 1973; Dare & Edwards, 1975; Widdows, 1973). We have studied the influence of these variations as a whole, as they appear in the ecological medium.

We used mussels from Lorbé on the 'ria' of Sada (La Coruña) collected on the same dates of each month from July 1975 to May 1976. We were unable to obtain mussels in June due to contamination in all that area.

We have worked with whole mussels (mechanically separated from the valves) dried by heat not exceeding 80° and then pulverised. Once analysed, this served as a source of protein for the preparation of synthetic diets for rats with 12% of protein according to the technique of Mitchell (1923).

We obtained a minimum value for the concentration of protein in November (56·4% on DM) with progressive increases until reaching maximum value in May (69·3% on DM), the levels of fat remaining relatively constant.



The figure summarizes the seasonal variations of gross protein (% dry matter) and the nutritive quality of mussel protein. It shows that when the protein concentration decreases (November) the quality of the protein increases.

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Seasonal changes in plasma retinol-binding holoprotein concentration of Japanese quail. By J. GLOVER and SUZANNE LARGE, *Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX*

Plasma retinol-binding holoprotein (holoRBP) concentration of sheep was found to increase 4-fold in the autumn above the summer minimum value (Glover, Jay, Kershaw & Reilly, 1976). This surge in RBP is associated with gonadal development in the annual cycle and is related to the shortening of the daylight period. Parallel experiments have now been completed in following the seasonal changes in plasma holoRBP of Japanese quail, which become sexually active in the spring as the daylength increases.

Groups of four male and four female adult birds obtained from a local breeder were maintained on a diet of Superlayer's pellets (Rank, Hovis and McDougall) in cages within an animal room at 20° and illuminated by daylight. Blood samples were taken every 2 weeks in the spring and early summer and monthly thereafter over the year. Blood was taken from a toe vein directly into a 100 μl EDTA-treated capillary tube and the plasma analysed for holoRBP as previously described (Glover, Moxley, Muhilal & Weston, 1974).

Commencing mid-January the mean holoRBP concentrations of both groups increased from 140 (♂) and 110 μg/ml (♀) to peak values of 250 (♂) and 270 μg/ml (♂) respectively at the end of March. The values then underwent a slight dip in early April before rising to second peak values of 230 (♂) and 280 μg/ml (♀) at the end of April. Afterwards the concentrations in both sexes declined to minimum levels around 150–160 μg/ml for the male in June and July and 80 μg/ml for the female in September to October. Thus the surge in holoRBP concentration in the female starting from a lower level than that of the male is much greater than that of the male.

In the case of the sheep, the male values of plasma RBP were generally higher than the female throughout the annual cycle. However, the surges in plasma holoRBP of both species show double peaks.

These results confirm the observations on sheep that plasma holoRBP concentration also increases in association with the development of the reproductive tissues in Japanese quail. In these birds, however, the gonadal changes are known to be initiated by increasing daylength in the spring and are also associated with changes in the gonadotropins (Follett, 1976).

It will be of interest to determine the temporal sequence of the RBP changes in relation to those of other hormones known to be involved in gonadal development and function.

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Diet-induced hyperuricaemia and platelet aggregation in rats. By P. D. WINOCOUR, K. A. MUNDAY, T. G. TAYLOR and M. R. TURNER, *Department of Physiology and Biochemistry, The University, Southampton SO9 3TU*

An increase in the rate of platelet aggregation may increase the risk of arterial thrombosis. In rats made hyperuricaemic by feeding on a nucleic acid-rich diet together with oxonate, a uricase inhibitor, we have demonstrated a relationship between plasma uric acid concentration and platelet aggregation induced both by ADP and by thrombin (Winocour, Munday, Taylor & Turner, 1976).

The time course of the effect of nucleic acid-rich diets containing oxonate on platelet aggregation and plasma uric acid concentration has been examined in rats given a *Fusarium* mould (Lord Rank Research Centre, High Wycombe) as a source of protein, fibre and nucleic acid for 0, 4, 14 or 21 d before investigation. The plasma and kidney uric acid concentrations had increased significantly after 4 d of feeding, but it was only after 21 d that there was a significant change in the maximum rate of aggregation (VA_{max}) induced either by ADP or by thrombin as is shown in the Table. Thus there was a time-lag between diet-induced hyperuricaemia and change in VA_{max}.

Days on experiment			Uric acid		VA_{max} (cm/min per 10^8 platelets)	
Total	With oxonate	Without oxonate	Kidney (mg/g tissue per (100 g body wt)	Plasma (mg/l)	ADP-induced	Thrombin-induced
0	0	0	0·24±0·01 (30)	19·3±1·1 (29)	4·51±0·19 (22)	5·58±0·19 (22)
4	4	0	0·34±0·03 (20)•••	34·6±3·2 (20)•••	4·40±0·12 (16)	5·63±0·20 (16)
14	14	0	0·34±0·04 (20)•	39·2±3·8 (20)•••	5·02±0·24 (15)	6·06±0·21 (15)
21	21	0	0·79±0·06 (40)•••	44·0±2·4 (38)•••	5·49±0·27 (30)••	6·31±0·22 (30)•
26	21	5	0·31±0·01 (10)†††	21·2±1·5 (9)†††	5·06±0·32 (7)	6·28±0·42 (7)
31	21	10	0·28±0·01 (20)†††	25·2±1·0 (20)†††	4·35±0·21 (13)†	4·98±0·31 (13)††
43	21	22	0·23±0·02 (10)†††	21·6±2·5 (10)†††	4·30±0·26 (7)†	4·80±0·31 (7)††

Values represent means ±SEM for the number of observations in parenthesis.
Significance of difference from day 0 •$P<0.02$; ••$P<0.01$; •••$P<0.001$
Significance of difference from day 21 †$P<0.05$; ††$P<0.01$; †††$P<0.001$

When the oxonate was removed from the diet after 21 d, there was a significant fall in both plasma and kidney uric acid concentration within 5 d. However, the fall in VA_{max} for ADP-induced and thrombin-induced aggregation did not parallel the fall in uric acid concentration and it was only after 10 d on an oxonate-free diet that a significant reduction was seen (see table). The time-lag thus is confirmed.

The time-lag between the increased plasma uric acid concentration and the increased platelet aggregation considerably exceeds the rat platelet life-span, which is about 4 d (Maupin, 1969). This suggests that uric acid may not exert its effect directly on circulating platelets, but more probably may act at a site of platelet production, the major site of which is from the megakaryocytes in the bone marrow. However, in preliminary histological studies no deposits of uric acid were detectable in the bone marrow or in the spleen, the main storage site for platelets, of rats given nucleic acid-rich diets supplemented with oxonate for 21 d. Further studies are needed to elucidate the possible mechanisms involved.

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Calcium appetite in growing pigs. By D. W. PICKARD, W. G. HEDLEY and SUSAN SKILBECK, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Four Large White pigs weighing approximately 20 kg at 8 weeks of age were fed on a diet containing 0·12% Ca. Calcium lactate solution (2·4% w/v) (Ritcher & Eckert, 1937) and tap water were offered after the morning and evening feeds of 500 g pelleted ration. The volumes of calcium lactate solution and tap water consumed were recorded. Negligible volumes of calcium lactate were drunk during the first 14 d. The concentration of lactate was reduced to 1·2% for 2 d and then to 0·6% for 12 d. The pigs continued to avoid the calcium lactate solution.

Two of the pigs were then parathyroidectomised. Their plasma calcium levels fell from 2·57 mM to 1·4 mM within 30 min of parathyroidectomy. One of the pigs died during the following night, the other was saved by intravenous injections of calcium borogluconate. Despite plasma calcium levels as low as 0·9 mM this pig refused to drink calcium lactate solution unless this was the only fluid offered. The pig died after 10 d, apparently from tetany.

The intact pigs also refused to drink calcium lactate solution, even though saccharin was added to both the lactate and water containers. When calcium lactate was offered in concentrations of 2·4, 1·2 and 0·6%, the lowest concentration was always selected, though grudgingly.

After this time (6 weeks) of feeding the low calcium diet it was clear that the pigs found the flavour of calcium lactate solution to be very objectionable. They were offered calcium carbonate in powder form and immediately found it to their liking. One pig ate 16 g at its first meal. Both pigs consumed significantly more calcium carbonate during days 1–8 than during days 9–16 ($P<0.05$). The consumption of the carbonate again increased during days 17–23, when the experiment ended.

The aversion which these pigs showed towards calcium lactate solution was quite conclusive. Wood-Gush & Hughes (1971) noted a similar aversion in their work on calcium appetite in chickens. It is evident that calcium carbonate is palatable to pigs and that their appetite for it is spontaneous. The cyclical pattern of consumption of the calcium carbonate may indicate the pigs' attempts to regulate intake according to their requirements for calcium.

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