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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Forty-ninth Meeting of the Nutrition Society was held at the University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD on Tuesday and Wednesday, 9–10 *September* 1980 *when the following papers were read:*

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The digestibility of legumin (11S globulin) isolated from field bean (*V. faba var* Throws M.S.). By R. JONES, R. J. NEALE and G. NORTON, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

The possible reasons for indigestibility of field-bean meal are numerous. A major protein component of the meal was isolated, purified and its nutritive value investigated.

The crude protein content (nitrogen × 6·25) of the Throws M.S. bean is 26±1·2% of the dry weight and approximately 70% of this protein is legumin. The legumin was isolated from the dehulled meal by salt extraction and repeated isoelectric precipitation, until a fraction was obtained that gave a single band on a non-dissociating polyacrylamide gel and on dissociation gave three bands on an SDS polyacrylamide gel. In these aspects and in amino acid composition, the legumin resembled that isolated by Bailey & Boulter (1970). It does not, however, so closely agree with the properties of legumin reported by Wright & Boulter (1974). Repeated isoelectric precipitation is preferred to zonal isoelectric precipitation in this case because of the large amount of purified sample required for subsequent feeding trials.

The chemical score for legumin was 48·5 due to the low content of sulphur amino acids: methionine 0·38 g/16 g N and cystine/2, 1·32 g/16 g N. This purified and freeze dried legumin was incorporated into the following test diets and in each case fed to a group of six male weanling Wistar rats for 14 d.

Diet 1 consisted of legumin (120 g/kg) in a diet based on corn starch, peanut oil and glucose. Diet 2 had the same composition as Diet 1 but the legumin was autoclaved at 121° at 15 p.s.i. for 15 min. Diet 3 was a protein-free diet.

After 14 d the NPU of Diet 1 was 42·5 with a digestibility of 86%; for Diet 2 the NPU was 46 and the digestibility 92%. Autoclaving significantly increased the digestibility ($P<0·01$) and NPU ($P<0·001$).

The non-availability of protein-N for growth in field-bean meal, of which legumin is the major protein, appears to be overcome when the legumin is extracted and incorporated into a diet of such composition as Diet 1. The high digestibility of the legumin suggests that sulphur amino acid supplementation would make it a very high quality protein source indeed.

The support of MAFF is acknowledged in carrying out this work.

Bailey, C. J. & Boulter, D. (1970). *Eur. J. Biochem.* **17**, 460.
Wright, D. J. & Boulter, D. (1974). *Biochem. J.* **141**, 413.

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Manipulation of dietary carbohydrate in a carbohydrate-loading regime.

By S. A. WOOTTON, M. SHORTEN and C. WILLIAMS, *Department of Physical Education and Sports Science, Loughborough University of Technology, Loughborough LE11 3TU*

The glycogen concentration in human skeletal muscle can be increased (i.e. supercompensation) by a combination of exercise and a carbohydrate(CHO)-rich diet (Bergström & Hultman, 1966). This CHO-loading regime has been shown, in laboratory studies, to increase the capacity for prolonged heavy exercise (Bergström *et al.* 1967). Many athletes, competing in endurance events, have used the CHO-loading procedure in their preparation for competition. Failure to improve endurance performance after CHO-loading is commonly attributed to the inadequacy of the dietary manipulation and or the inapplicability of this technique to athletic competition.

In an attempt to reinvestigate the efficacy of the CHO-loading regime, for the improvement of distance running performance, we have begun by considering the ability of inexperienced individuals to significantly alter their CHO-intake.

The study involved monitoring the weighed food intake of thirteen active individuals who were following the CHO-loading regime in an attempt to enhance their endurance capacity. Dietary analysis of the food intake records for the three phases of the regime, i.e. normal, low-CHO and high-CHO diets, were performed using computer-based food composition tables (Paul & Southgate, 1978).

(Values are expressed as percentages of normal diet)

	Low-CHO diet			High-CHO diet		
	Range	Mean	SEM	Range	Mean	SEM
Total energy intake	39·6–102·6	76·1	5·3	79·8–181·6	115·2	8·2
CHO-intake	6·2– 38·3	17·9	2·8	106·2–264·6	150·1	12·5

All the subjects were able to restrict their CHO intake on the low-CHO phase of the procedure, but not all the subjects were subsequently able to increase their CHO intake above that of their normal diet. Furthermore the changes in CHO intake were accompanied by large differences in total energy intake during the different phases of the dietary procedure. Thus the results of this study suggest that the variable effect of the CHO-loading regime on performance may be related to the failure of the individual to consume sufficient CHO to achieve supercompensation of muscle glycogen.

This study is part of a larger project supported by The Sports Council.

Bergström, J. & Hultman, E. (1966). *Nature, Lond.* **210,** 309.
Bergström, J., Hermansen, L., Hultman, E. & Saltin, B. (1967). *Acta physiol. Scand.* **71,** 140.
Paul, A. A. & Southgate, D. A. T. (1978). *McCance & Widdowson's: The Composition of Foods.* London: HM Stationery Office.

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Synthesis of alanine from ammonia by rumen bacteria. By J. S. BLAKE, D. N. SALTER and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Glutamate dehydrogenase (*EC* 1.4.1. 2 & 4) and glutamine synthetase (*EC* 6.3.1.2) are generally believed to be mainly responsible for ammonia assimilation by rumen bacteria although recent work has indicated that alanine may also play a part (Wallace & Henderson, 1978).

Three Friesian steers were given, twice a day, diets containing tapioca (0·48–0·56 kg/kg dietary dry matter (DM)), alkali treated straw pellets (0·40 kg/kg DM), vitamins and minerals (including sulphate) and either decorticated groundnut meal (DCGM) or urea as the nitrogen source to give about 37 g N/d and 11·3 MJ metabolizable energy/kg DM.

After 3 weeks on a diet, a dose of ^{15}N-labelled ammonium chloride was added to the rumen 60 min after a morning feed, rapidly mixed by a high capacity recirculating pump, and rumen samples taken at intervals thereafter. Mixed bacteria were separated by centrifugation and disrupted by ultrasonic treatment. Protein was precipitated with sulpho-salicylic acid (36 g/l), the soluble fraction ('cell sap') separated by centrifugation and subjected to amino acid analysis. For all three calves, each with both diets, alanine was generally the most highly labelled of all the amino acids although the difference between alanine and glutamate was not significant at $P<0·05$. Even at 2 min, alanine was appreciably labelled usually to a greater extent than glutamate, and always more than any other amino acid.

^{15}N abundance (% ^{15}N excess) and concentration (μmol/g bacterial dry matter) in 'cell sap' fractions

(Mean values for three experiments)

Diet	Time after dose (min)	Ammonia		Glutamate		Alanine	
		^{15}N abundance	Con-centration	^{15}N abundance	Con-centration	^{15}N abundance	Con-centration
Urea	10	1·29	15·44	0·72	12·38	0·79	16·36
	30	1·08	14·26	0·42	8·95	0·80	12·28
	90	0·33	12·20	0·52	12·30	0·84	17·21
DCGM	10	2·47	34·11	1·17	20·17	1·41	26·40
	30	3·24	31·23	1·44	17·27	1·95	24·00
	90	2·32	25·46	1·78	14·18	1·69	19·78

Alanine synthesis may simply be a means of ammonia fixation but reports of alanine excretion from bacteria (Stevenson, 1978) suggest that it may provide a means of removing excess pyruvate when readily available energy is supplied. Whatever its purpose, alanine dehydrogenase (*EC* 1.4.1.1) may be of importance in rumen bacteria when soluble carbohydrate and ammonia levels are high.

Stevenson, I. L. (1978). *Can. J. Microbiol.* **24**, 1236.
Wallace, R. J. & Henderson, C. (1978). *Proc. Soc. Gen. Microbiol.* **5**, 102.

0029–6651/81/4001–349A $00.35

Investigation into the role of insulin in diet-induced thermogenesis in the rat. By NANCY J. ROTHWELL, M. J. STOCK and B. P. WARWICK, *Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17 0RE*

In view of the close association between diabetes and obesity and the role of diet-induced thermogenesis (DIT) in the prevention of obesity (Rothwell & Stock, 1979) it was decided to investigate the influence of insulin on the development of DIT in the rat.

Forty-eight male, Sprague-Dawley rats were divided into six groups of equal body-weight (200 g) and four of these groups were injected with streptozotocin (8 mg/100 g body-weight) to induce diabetes. During the experiment, two of these groups (high replacement, HR) were injected with four units of protamine-zinc insulin (PZI) and the other two (low replacement, LR) with two units PZI every other day. All groups were fed on a pelleted stock diet (PRD) but three groups (normal, HR and LR) were additionally allowed access to a varied and palatable cafeteria diet. Metabolizable energy intake was elevated by approximately 100% in all cafeteria-fed rats but in normal rats this had no effect on rate of body-weight gain whereas in the diabetic groups it resulted in a relatively greater weight gain.

Resting oxygen consumption (VO_2) was measured on day 7, 36 h after the last dose of PZI. In normal animals, cafeteria feeding resulted in a significantly (22%) greater VO_2 and a greater response to noradrenaline (25 μg/100 g body-weight; increase VO_2 (%): control 66±6, cafeteria 84±5, $P<0.01$). In diabetic animals, resting VO_2 was normal with no observable thermogenesis in the cafeteria rats. The HR cafeteria group did exhibit an enhanced response to noradrenaline but this was not seen in the LR cafeteria group. On day 9 all groups received eight units PZI and VO_2 was measured 12 h later. On this occasion all three cafeteria groups (normal, HR and LR) showed an increased resting VO_2 and an enhanced response to noradrenaline compared to their respective stock-fed controls, which were unaffected by insulin. Diabetic rats also had a significantly lower rectal temperature (37.5±0.1°) than normal control (38.1±0.1) and cafeteria animals (38.9±0.1), and showed a larger fall in temperature on exposure to 5°, indicating that diabetic rats have both impaired cold- and diet-induced thermogenesis. After 15 d all rats were killed and the interscapular brown adipose tissue (IBAT) removed and weighed. Although diabetic animals had less IBAT than normal rats, there was a relative hypertrophy in the diabetic cafeteria groups. These results suggest that insulin is required for DIT but may not be required for the hypertrophy of BAT associated with increased food intake.

Rothwell, N. J. & Stock, M. J. (1979). *Nature, Lond.* **281**, 31.

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Energy and nitrogen exchanges during growth in the kestrel (*Falco tinnunculus*). By J. K. KIRKWOOD (Introduced by A. J. F. WEBSTER), *Department of Animal Husbandry, Langford House, Langford, Bristol BS18 7DU*

The kestrel (*Falco tinnunculus*), like other altricial birds (those reared in the nest), grows extremely rapidly (Ricklefs, 1968). Body-weight reaches a peak of about 260 g at 24 d after hatching, then falls slightly.

According to Taylor's (1965) interspecies comparison, mammals reach 98% of mature body-weight (A) in an average of 440 metabolic d ($t.A^{-0.27}$; where t is age in days from conception). Kestrels reach 98% of peak weight in 76 metabolic d.

Measurements have been made of energy and nitrogen exchanges from hatching to maturity in thirteen hand-reared kestrels. The birds were reared in brooders at temperatures comparable to those experienced in the nest, and fed on diets of minced, whole laboratory mouse and day-old domestic chicks. Food intake was measured by weighing the birds before and after each meal. Excreta, regurgitated pellets, down and feather sheath dust were collected for the energy and N balance measurements. The energy content of the food, excreta and pellets were measured in an adiabatic oxygen bomb calorimeter and the N content of these materials was determined by the Kjeldahl technique.

Maturation of energy and nitrogen exchanges

	Stage of maturity		
	Maximum growth rate	Peak weight	Maturity
Body weight (g)	154	266	248
Age from hatching (d)	13	24	35
Metabolic age ($t.A^{-0.27}$)	60	76	92
Metabolizable energy (kJ/d)	380	330	240
Heat production (kJ/d)	220	265	190
Energy retention (kJ/d)	160	65	50
Nitrogen retention (g/d)	0·50	0·22	0·0

At peak growth rate metabolizable energy (ME) was 380 kJ/d (1560 kJ/$kg^{0.75}$ per d) and energy retention (RE) was 43% of ME. At peak weight RE was still positive and N balance was not achieved until about 35 d post partum, by which time plumage development was almost complete. Values for energy and N retention were also obtained for a few birds from carcass analysis. These agreed well with the balance experiments.

It is concluded that body-weight does not provide a good index of maturity in this species. Maturity in terms of N balance was achieved in about 92 metabolic d from conception. This is still considerably faster than the interspecies mean value proposed by Taylor (1965).

I am grateful to the Wellcome Trust for supporting this study.

Ricklefs, R. E. (1968). *Ibis* **110**, 419.
Taylor, St. C. S. (1965). *Anim. Prod.* **7**, 203.

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The effect of dietary protein quantity and quality on rat milk composition.

By R. GRIMBLE, *Department of Nutrition, University of Southampton, Southampton SO9 5NH*

Reports from countries where malnutrition is common show women producing milks of normal protein and total N content (Lonnerdal *et al.* 1976). However, amino acid analysis of total milk proteins from Pakistani mothers demonstrated reduced amounts of methionine and lysine, suggesting a reduction in the proportion of lactalbumin (Lindblad & Rahimtoola, 1974). The present study examines the effect of dietary protein quantity and quality on milk composition and protein proportions. Wistar rats of three previous pregnancies were mated and fed RPD pellets (Rank Hovis McDougall). After birth, litters were adjusted to eight pups. Each dam was fed on one of four diets. Two protein sources were used, casein + 0·3% methionine, and cereal (maize gluten feed RHM). 100 g/kg and 200 g/kg protein diets were prepared from each. The 200 g/kg cereal protein diet was achieved by adding Zein (BDH) to the maize gluten feed. Similar fibre, fat and energy contents were achieved by addition of solcofloc, sucrose, cornstarch and corn oil. The dams were milked under nembutal anaesthesia after overnight separation from their litters. Milk was analysed for protein, total amino acids, lactose and fat. Proteins were separated by SDS polyacrylamide gel electrophoresis on 12·5% gels and stained with Coomassie blue (Methods in Enzymology, 1972). Proteins were quantified with a gel scanner by relating each band density to that of α casein.

	Casein		Cereal protein			Analysis of variance: Diet		
Milk composition	200 g/kg A mean	100 g/kg B mean	200 g/kg C mean	100 g/kg D mean	SEM	Protein content	Protein source	*F* test
Total protein (g/l)	131	115	104	105	5·9	NS	••	CD<A
Amino acids (g/l)	0·75	0·91	0·44	0·67	0·14	NS	NS	C<B
Lactalbumin (% α casein area)	40·5	41·6	40·4	19·0	3·7	•	••	D<ABC
Lactose (g/l)	26·2	25·9	24·3	24·5	0·59	NS	NS	NS
Fat (g/l)	106	102	102	116	12	NS	NS	NS

NS, not significant.
*$P<0.05$, **$P<0.01$.

Analysis of variance showed that the poorer quality of cereal protein caused reduced total protein and lactose concentrations, whereas a combination of lower protein quantity and quality caused a reduction in lactalbumin without affecting other milk protein proportions. As lactalbumin is richer than casein in methionine and lysine, the content of these amino acids in total milk proteins would be lower in milk from the 100 g/kg cereal protein group. These results support the observations from Pakistan, where a significant proportion of dietary protein comes from cereals.

Lindblad, B. S. & Rahimtoola, R. J. (1974). *Acta Paediat. Scand.* **63,** 125.
Lönnerdal, B., Forsum, E. & Gebre-Medhin, M. (1976). *Am. J. clin. Nutr.* **29,** 1134.
Methods in Enzymology (1972). 26C, 3. London: Academic Press.

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The effect of dietary protein quantity and quality on hormonal status, serum albumin concentration and milk production of rats. By R. GRIMBLE, *Department of Nutrition, University of Southampton, Southampton SO9 5NH*

The animals used were described previously (Grimble, 1981). Insulin and corticosterone, which play a major role in milk production and protein metabolism were measured. The level of the hepatic export protein, serum albumin was determined and an index of total milk protein produced, obtained by pup growth measurement. Maternal and pup weights were recorded between days 3 and 13 of lactation. Post-milking cardiac blood samples were taken from mothers and non-lactating multiparous rats.

	Casein		Cereal protein			Analysis of variance (n 20)	
	200 g/kg A mean	100 g/kg B mean	200 g/kg C mean	100 g/kg D mean	SEM	Protein source	F test
Change in maternal weight (g)	−3	−13	−34	−29	3·3	$P<0·01$	CD>AB
Food intake (g/100 g body-weight per d)	12·4	11·0	11·6	11·0	1·5	NS	NS
Pup growth (g)	22·0	18·0	7·3	9·0	1·8	$P<0·01$	CD<AB
Serum albumin (g/l)	34·6**	32·4**	26·8**	27·6**	1·5	$P<0·01$	CD<AB
Insulin (mU/l)	152*	156*	71	50	36	$P<0·05$	NS
Corticosterone (μg/l)	60·6	55·2	51·5	47·8	3·7	$P<0·05$	D<A

Non-lactating insulin 71·0±10 Corticosterone 52·5±3·2 Serum albumin 43·8±2·2
NS, not significant.
Significantly different from non-lactating values: *$P<0·05$, **$P<0·01$.

Analysis of variance showed no effect from protein concentration but poorer quality cereal protein caused a decrease in maternal albumin, corticosterone and insulin concentrations. The latter in these groups fell to non-lactating values. The reduced proportion of insulin to corticosterone could account for the greater weight loss in the cereal protein groups and be an attempt to supply substrate for milk synthesis from maternal sources. Pup growth was severely reduced in both cereal protein groups. These diets caused small changes in milk composition thus milk quantity was probably severely affected by protein quality. Whether reduced pup growth was due to reduced milk protein or energy production is debatable. Total milk protein production was reduced. A significant positive correlation between pup growth and maternal serum albumin concentration occurred ($P<0·01$). Most milk proteins and albumin are export proteins, produced on membrane bound polysomes. In liver, export protein synthesis is depressed by protein deficiency, while that of proteins made by non-membrane bound polysomes is not (Waterlow *et al.* 1978). The present study suggests a sensitivity of export protein synthesis to protein deficiency in mammary gland also.

Grimble R. (1981). *Proc. Nutr. Soc.* **40**, 7A.
Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978). In *Protein turnover in mammalian tissues and in the whole body*. North Holland Publishing Co.

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Interconversions of the carbon in volatile fatty acids and carbon dioxide in the rumen of sheep. By R. W. MAYES, J. A. MILNE, C. S. LAMB and ANGELA M. SPENCE, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY*

In order that valid estimates of volatile fatty acid (VFA) absorption from the rumen can be obtained by isotope dilution methods it is necessary to know the extent of ruminal interconversion of VFAs and other fermentation products. From the irreversible loss (IL) rates and transfers of ^{14}C when $NaH^{14}CO_3$, [U-^{14}C]acetate, [1-^{14}C]propionate and [2-^{14}C]propionate were infused into the rumen and $NaH^{14}CO_3$ was infused into the jugular vein of sheep, a four-pool model was constructed such that carbon transfers between acetate, propionate and carbon dioxide in the rumen could be evaluated. Six mature wether sheep received by continuous feeder 1 kg OM/d of chopped dried perennial ryegrass either alone or replaced with 32 or 61% of a cereal-based concentrate. The three treatments were compared using an incomplete Latin Square design. A comparison was also made between [1-^{14}C]propionate and [2-^{14}C]propionate with four sheep given the dried grass diet.

The IL rates of acetate from the rumen were similar on the dried grass and 32% concentrate diets (135 and 139 g C/d respectively) and higher for the 61% concentrate diet (179 g C/d). Using [1-^{14}C]propionate, the mean IL rates of the dried grass, 32 and 61% concentrate diets were 99, 80 and 110 g C/d respectively. However, the use of [2-^{14}C]propionate gave a lower estimate of propionate IL rate (47 g C/d) for the dried grass diet. Although estimates of ruminal IL rate of CO_2 were variable, the model revealed that interconversions of acetate-C and propionate-C were small (0–11 g/d). Similarly, interconversions of acetate-C and ruminal CO_2-C were also small (1–11 g/d). However, the nature of the interconversions of propionate-C and CO_2-C depended upon the position of the ^{14}C label. When the intra-ruminal infusion of [1-^{14}C]propionate was carried out, 64 g of propionate-C were apparently converted to CO_2 in the rumen whilst with the [2-^{14}C]propionate negligible amounts of ruminal CO_2 were derived from propionate. Since both estimates depend on the assumption that all the C atoms of propionate are metabolized in the same manner within the rumen, the values obtained from the [1-^{14}C]- and [2-^{14}C]propionate infusions must be respectively overestimates and underestimates. However, when propionate-C absorption is represented as the sum of the C flows from propionate to plasma CO_2 and the propionate-C lost from the four-pool model, both isotopes gave the same estimate (45 g/d) of propionate-C absorbed from the rumen.

It is concluded that the interchanges of C between acetate and propionate and between acetate and ruminal CO_2 have little effect upon estimates of VFA production in the rumen. Because of the interconversions of propionate and CO_2, [1-^{14}C]propionate is less useful than [2-^{14}C]propionate for measuring propionate absorption from the rumen.

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The effects of feed restriction on hepatic enzyme activities in the immature pullet. By J. PEARCE and A. H. JOHNSON, *Agricultural and Food Chemistry Research Division, Department of Agriculture for Northern Ireland, Newforge Lane, Belfast BT9 5PX*

The effects of feed restriction on metabolism in the domestic fowl have received little attention despite the practical interest in restricting the energy intake of laying hens. It has been shown that restricted feeding significantly reduced hepatic lipogenesis in laying hens but had no significant effects on glycolytic enzyme activity (Pearce, 1980). The present study was prompted by the unexpected observations of Balnave *et al.* (1979) who reported that restricted feeding increased liver weight, liver lipid content and lipogenic enzyme activity in immature pullets.

Three groups of twenty 7-week-old pullets were allocated at random to three feeding regimes. The diet used was a cereal-based rearing diet; one group had *ad lib.* access to the diet and the other groups received 75 and 60% of the *ad lib.* intake. At 8 weeks of age and at subsequent fortnightly intervals (to 16 weeks of age) birds were killed and liver cell-free extracts prepared as previously outlined (Pearce, 1980). The extracts were assayed for the activities of acetyl CoA carboxylase, malate dehydrogenase (decarboxylating) ($NADP^+$), phosphoglucose isomerase, phosphofructokinase, fructose bisphosphate aldolase, pyruvate kinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

Liver weight (expressed as percentage body-weight) and liver total lipid contents were significantly reduced ($P<0.05$) after 1 week of restricted feeding and the effects were greater at the higher level of feed restriction. These effects were maintained throughout the experiment. The changes in liver total lipid contents were reflected in the specific activities of the lipogenic enzymes acetyl CoA carboxylase and malate dehydrogenase (decarboxylating) ($NADP^+$) which were also significantly reduced ($P<0.001$ and $P<0.01$, respectively) after 1 week of restricted feeding; again this pattern was maintained for the duration of the experiment. None of the glycolytic or pentose phosphate pathway enzymes were significantly affected by feed restriction.

These results agree with previous work (Pearce, 1980) where feed restriction significantly reduced liver weight liver lipid content and lipogenic enzyme activities but are contrary to the observations of Balnave *et al.* (1979) who reported that, in 13-week-old pullets, which had been subjected to 7 weeks of restricted feeding (to 60 and 75% of *ad lib.* intake), these indices increased as a consequence of feed restriction.

Balnave, D., Farrell, D. J., Cumming, R. B. & Wolfenden, J. (1979). *Aust. J. agric. Res.* **30**, 377.
Pearce, J. (1980). *Br. J. Nutr.* **44**, 81.

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Sympathetic responsiveness in relation to fatness in pigs. By N. G. GREGORY and D. LISTER, *ARC, Meat Research Institute, Langford, Bristol BS18 7DY*

Lean Pietrain pigs have a higher lipolytic response to noradrenaline (NA) than fatter genotypes (Wood *et al.* 1977). This could explain their greater leanness provided their adipose tissue is exposed to equal or raised amounts of NA. In this experiment, therefore, the potential output of NA was assessed in Pietrain and fatter Gloucester Old Spot (GOS) pigs by comparing their cardiovascular responses to various sympathetic stimuli.

Thirteen Pietrain and thirteen GOS pigs were subjected to the Valsalva like manoeuvre (VLM) at 140 d age using a raised airway pressure of 40 cm H_2O for 90 s, whilst under thiopentone plus N_2O/O_2 anaesthesia. The heart rate response to the VLM was greater in the Pietrains. It is unlikely that this breed difference was caused by any difference in parasympathetic response, since a sample of seven pigs from each breed showed identical heart rate responses per unit blood pressure rise ($\Delta HR/\Delta BP$) during an intravenous phenylephrine infusion maintained to give an elevation in diastolic pressure of 20 mm Hg. The greater response during the VLM in the Pietrains could not be explained by a greater heart rate response to a 5 s injection of 30 ng NA/kg. However, after hexamethonium pretreatment, the primary chronotropic response to a 12 s tyramine injection (0·1 mg/kg) was greater in the Pietrains, suggesting that they had a larger or more readily releasable NA pool in their sympathetic nerve endings.

	Pietrain		GOS		
	Mean	SE	Mean	SE	Significance
140 d live weight (kg)	52	2	75	4	$P<0.001$
Fat in empty body (%)	14·3	0·8	23·4	0·9	$P<0.001$
Peak heart rate rise during VLM (beats per min)	33	5	15	5	$P<0.001$
$\Delta HR/\Delta BP$ during phenylephrine (beats per min/mm Hg)	−0·3	0·1	−0·3	0·1	NS
Change in heart rate 20 s after NA (beats per min)	−0·3	0·5	1·7	0·7	$P<0.05$
Incremental area under blood pressure curve after NA (mm Hg × s)	355	79	614	44	$P<0.05$
Incremental area under heat rate curve after Tyramine (beats per min × s)	424	51	244	35	$P<0.05$

If the greater pre-adrenoreceptor sympathetic responsiveness of the Pietrains occurred throughout their lifetime, then the combination of enhanced release of NA and enhanced lipolytic sensitivity to NA could have lead to greater fat mobilization and hence a leaner body.

Wood, J. D., Gregory, N. G., Hall, G. M. & Lister, D. (1977). *Br. J. Nutr.* **37**, 167.

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An attempt to explain differences in the nutritive value of spring and autumn harvested dried grass. By J. M. C. R. RIBEIRO, J. C. MACRAE and A. J. F. WEBSTER, *The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

Sheep given spring harvested dried grass (S) utilize their metabolizable energy intake (MEI) more efficiently than those given autumn harvested grass (A) (k_f, S 0·43–0·48; A, 0·30–0·34; see Corbett *et al.* 1966; Blaxter *et al.* 1971; Lonsdale & Taylor, 1971; Ribeiro, 1979); such differences should be reflected in the supply of metabolites to the animals. Rates of production of rumen VFAs, amounts of organic matter (OM) and nitrogen entering the small intestine and uptake of free amino-N by the portal drained viscera were measured in mature wether sheep given each grass at approximately 1·5 × maintenance.

Observations	Number of sheep	1st cut grass	3rd cut grass
Efficiency of utilization of ME above maintenance (k_f)	(4)	0·48	0·30
Ruminal production of acetate (mol/MJ ME intake)	(4)	0·41	0·46
propionate (mol/MJ ME intake)	(4)	0·13	0·13
butyrate (mol/MJ ME intake)	(4)	0·06	0·08
OM entering small intestine (g/MJ ME intake)	(2)	47·8	44·1
N in feed (g/MJ ME intake)		2·21	2·69
N entering small intestine (g/MJ ME intake)	(2)	1·80	1·44
Free amino-N uptake by portal vein (g/MJ ME intake)	(5)	0·81	0·69
N excreted in urine (g/MJ ME intake)	(4)	0·99	1·34

VFA production rates were similar between grasses, but there was a considerable difference in the availability of the nitrogenous component. Although the N intake per unit of MEI was higher with A, a large proportion of this N disappeared anterior to the duodenum (A, 46% of N intake, compared with 18% of N intake for S); 25% more N/unit MEI entered the small intestines of sheep given S. There was also an increased (17%) flow of free amino-N in the portal vein of these sheep.

The much greater disappearance of N, anterior to the duodenum, probably as ammonia, in sheep given A (0·85 g N/MJ MEI greater than in sheep given S) was partly reflected in a higher urinary excretion of N (0·35 g N/MJ MEI) in these sheep. The increased OM flow at the duodenum of sheep given S could be accounted for if the extra N was of microbial origin; the slightly higher water soluble carbohydrate (WSC) content of S (S 192 mg/g DM; A 176 mg/g DM) would tend to improve the efficiency of microbial utilization of dietary N, indeed Blaxter *et al.* (1971) put foward the difference in WSC as one possible reason for differences in k_f.

The extra amino-N available to sheep given S may have improved the utilization of their energy yielding substrate, perhaps by providing extra glycogenic precursors required to support fat synthesis; circulating glucose concentrations were some 10% higher in sheep given S (S, 500±18 mg/l; A, 456±19 mg/l).

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The effect of the growth promoter trenbolone acetate, dexamethasone and thyroxine on skeletal muscle cathepsin D (*EC* 3.4.4.23) activity.

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The relationship between proteolytic activity in muscle and growth rate is not well understood. In this study we have monitored the response in muscle cathepsin D activity assayed using H^3 acetyl haemoglobin (see Barrett, 1972) following administration of thyroxine (T_4), dexamethasone (dex) and trenbolone acetate (3-oxo-17β-hydroxy-4,9,11-oestratriene acetate (TBA)) to the female rat. Rats were individually housed in metabolism cages and fed a powdered synthetic diet containing 160 g/kg casein. Rats were injected daily via the neck skinfold. Two separate experiments were conducted.

Rats (six/group) were injected for 3 d with either placebo, T_4 (600 μg/kg body-weight) or dex (5 mg/kg body-weight). The weight gains (g/3 d; mean ± SEM) were $2{\cdot}4\pm0{\cdot}79$, $-1{\cdot}2\pm0{\cdot}31$ ($P<0{\cdot}05$) and $-15{\cdot}5\pm0{\cdot}57$ ($P<0{\cdot}001$) for the control, T_4 and dex treatments respectively. Corresponding free cathepsin D activities (disintegrations/min per mg protein) were $19{\cdot}8\pm1{\cdot}33$, $18{\cdot}3\pm0{\cdot}95$, and $28{\cdot}1\pm1{\cdot}56$ ($P<0{\cdot}001$) and for total enzyme activity were $103{\cdot}3\pm7{\cdot}23$, $93{\cdot}1\pm3{\cdot}97$ and $112{\cdot}1\pm5{\cdot}08$.

With TBA (800 μg/kg body-weight) the experiments lasted 7 d (six rats/treatment). Weight gain for controls was $5{\cdot}9\pm3{\cdot}6$ g and for the TBA $13{\cdot}7\pm1{\cdot}36$ g ($P<0{\cdot}001$). Free cathepsin D activities (disintegrations/min per mg protein per min) were $17{\cdot}7\pm1{\cdot}31$ and $12{\cdot}9\pm0{\cdot}89$ ($P<0{\cdot}05$) respectively with the corresponding values for the total activity of $82{\cdot}8\pm4{\cdot}9$ and $72{\cdot}3\pm1{\cdot}45$ respectively.

3-methylhistidine:creatinine excretion values indicated marked myofibrillar protein breakdown in the dex treated rats. Molar ratios were $0{\cdot}019\pm0{\cdot}0017$, $0{\cdot}021\pm0{\cdot}001$ and $0{\cdot}037\pm0{\cdot}0037$ ($P<0{\cdot}001$) respectively for the control, T_4 and dex treatments. The TBA treated rats had molar excretion ratios of $0{\cdot}022\pm0{\cdot}0018$ and the control animals of $0{\cdot}024\pm0{\cdot}006$. Other results indicate decreased muscle protein breakdown in TBA treated animals (Vernon & Buttery, 1976).

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Lactosyl urea as an NPN source for ruminants. By R. J. MERRY, R. H. SMITH and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

In developing new dietary NPN sources for ruminants to replace urea, attempts are usually made to achieve a reduction in the rate of release of ammonia in the rumen. This reduces the chance of toxicity, but has the possible disadvantage that, if the compound is too slowly attacked, a proportion may be swept from the rumen with the digesta flow and lost.

Lactosyl urea (LU) is virtually unattacked in the unadapted animal (Merry *et al.* 1979) and even after adaptation, degradation rates of both the N and sugar components of the molecule are considerably slower than for a corresponding mixture of lactose and urea.

Two sheep with rumen and abomasal cannulas were given a basal diet of barley and alkali-treated straw cubes in equal weights, with LU added during an adaptation period. At intervals, doses of polyethylene glycol (PEG) and LU were added to the rumen 30 min after a basal feed, and a series of rumen and abomasal samples taken and analysed. Disappearance curves of PEG and bound urea (in the form of ureide) were used to calculate rates (proportions/h) of fluid turnover and LU degradation (Maeng & Baldwin, 1976). Cumulative losses of bound urea to the abomasum, as proportions of the dose, were calculated from these measurements and the composition of abomasal contents. Values were:

	Sheep 164				Sheep 165			
Adaptation time (d)	0	7	14	21	0	7	14	21
Fluid turnover rate	0·059	0·069	0·048	0·028	0·085	0·043	0·039	0·052
Bound urea degradation rate	0·16	1·54	—	—	0·14	1·24	—	—
Cumulative losses of bound urea to the abomasum, up to:								
1h	0·057	0·059	0·033	0·020	0·079	0·023	0·028	0·034
3h	0·161	0·098	0·056	0·046	0·216	0·048	0·041	0·047
6h	0·286	0·103	0·061	0·059	0·366	0·054	0·041	0·047

In the adapted animals cumulative losses of bound urea up to 1, 3 and 6 h respectively, were directly related to fluid turnover rates in the rumen, with correlation coefficients of 0·94, 0·84 and 0·72 and levels of significance of $P<0·01$, $P<0·05$ and $P<0·10$.

Under the present conditions little bound urea was lost with the digesta flow, except in the unadapted animals. However, it is likely that diets inducing higher turnover rates would lead to greater losses of bound urea.

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The effects of the gut microflora on protein synthesis in chick liver and jejunum. By T. MURAMATSU, M. E. COATES, D. HEWITT and D. N. SALTER, *National Institute for Research in Dairying, Shinfield, Reading* and P. J. GARLICK, *London School of Hygiene and Tropical Medicine, Keppel Street, London SC1E 7HT*

Because earlier work had indicated that the presence of a gut microflora may influence nitrogen metabolism in the chick (Salter *et al.* 1974) protein synthesis was compared in jejunal mucosa and livers of germ-free (GF) and conventional (CV) chicks. The birds were reared to 19 d on a purified diet based on casein and starch, then the fractional synthesis rate (FSR) was measured by the large dose injection method of Garlick *et al.* (1980) using ^{14}C-labelled phenylalanine. Similar birds were maintained for a further 9 d on either a N-free diet (NF) or one supplemented with 5 g methionine and 2 g arginine/kg (MA) and the FSR determined.

(Values are means with their standard errors for four observations)

			Day 19		Day 28			
			Normal diet		NF diet		MA diet	
			Mean	SEM	Mean	SEM	Mean	SEM
Jejunal mucosa	FSR (%/d)	GF	70	7	60	4	51	4
		CV	78		56		63	
	Protein synthesized (μg/mm per d)	GF	158	25	120	13	152	13
		CV	191		112		168	
Liver	FSR (%/d)	GF	95	15	65	8	73	8
		CV	103		70		82	
	Protein synthesized (mg/g body-weight per d)	GF	4·8	1·1	2·6	0·4	2·8	0·4
		CV	5·1		3·2		3·6	

Although there were no statistically significant effects of environment, on all but one occasion the FSR, and therefore the amount of protein synthesized, was greater in the tissues from CV birds. The higher FSR in gut mucosa might be related to the more rapid turnover of epithelial cells in the CV state (Rolls *et al.* 1978). In the liver it may reflect increased activity due to the need to metabolize microbial products.

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The effect of dietary leucine excess on nicotinamide nucleotides in the rat. By B. I. MAGBOUL and D. A. BENDER, *Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN*

It has been suggested that the relative excess of leucine in the protein of jowar (*Sorghum vulgare*) may be a causative factor in pellagra among people eating jowar as a dietary staple (Gopalan & Srikantia, 1960). However, Manson & Carpenter (1978*a*,*b*) were unable to demonstrate any pellagragenic effect of excess leucine in dogs, chicks or rats.

In an attempt to resolve this discrepancy, tissue nicotinamide nucleotide concentrations have been measured in male rats maintained since weaning on diets providing marginally adequate intakes of tryptophan and niacin, with or without the addition of L-leucine (15 g/kg diet). The diet was based on that described by Carter *et al.* (1977), containing maize meal, sucrose and gelatin with casein, corn oil, amino acid, vitamin and mineral salt mixtures. It contained (mg/kg) 98 tryptophan, 1·5 nicotinamide and 1·2 pyridoxine hydrochloride; preliminary studies showed that these amounts of tryptophan and nicotinamide were marginally adequate to permit normal growth.

Addition of leucine to the diet depressed growth, but had no effect on the excretion of N^1-methyl nicotinamide, the principal metabolite of nicotinamide nucleotides, as noted by Manson & Carpenter (1978*b*). After 7 weeks, but not earlier, the animals receiving excess leucine had lower blood and liver concentrations of nicotinamide nucleotides than those receiving the control diet.

(Values are means with standard deviations for ten rats)

			Nicotinamide nucleotides		
			Blood	Liver	
Diet	Growth (%)	N^1-methyl nicotinamide (mmol/mol creatinine)	Total (μmol/l)	oxidized (μmol/g)	reduced (μmol/g)
Control	68±31	6·5±3·3	59±17	0·49±0·09	0·18±0·08
+15 g/kg leucine:					
for gelatin	30±18*	6·9±2·0	35±8*	0·38±0·09*	0·14±0·07
for sucrose	49±28	8·4±2·8	30±5*	0·37±0·09*	0·12±0·04*

Significance of difference from control by Student's *t* test.
*$P<0.025$.

The finding of lowered tissue nicotinamide nucleotides confirms the pellagragenic effect of excess leucine, and therefore supports the view of Gopalan & Srikantia (1960) that dietary leucine may be an important factor in the development of pellagra.

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Absorption of acetate and butyrate from the caecum of the rabbit. By G. WOODNUTT and D. S. PARKER, *Department of Physiology & Biochemistry, The University, Whiteknights, Reading RG6 2AJ*

Fermentation in the caecum of the rabbit has been shown to produce significant quantities of volatile fatty acids (VFA). Absorption of acetate from the caecum would account for the high entry rate of this acid into portal blood in the fed animal (Woodnutt & Parker, 1979). In order to study the uptake of VFA in the conscious New Zealand White rabbit [U-^{14}C]acetate was infused into the caecum at a constant rate of 0·5 μCi/min for 3 h. This procedure was undertaken in eight fed animals and five animals deprived of food for 18 h. Steady state was achieved after 1 h of infusion and during the final hour samples of portal blood and caecal dialysate were obtained and the VFA concentration and radioactivity determined by radio-gas–liquid chromatography (Parker, 1976).

$$\text{The transfer quotient} = \frac{\text{specific radioactivity of metabolite in portal vein}}{\text{specific radioactivity of metabolite in caecum}}$$ of

acetate and butyrate was calculated from the specific radioactivity of each acid in the caecum and the blood. This value is a measure of the extent to which the acid in the caecum is the source of the same acid in the portal blood. In our experiments these results show that 64% (TQ; 0·64±0·05) of the acetate entering the portal circulation of the fed animal originates in the caecum and that this is significantly reduced ($P<0·02$) to 45% (TQ; 0·45±0·05) in the 18 h starved animal. These results are in agreement with previous results which showed that entry of acetate from the intestine was not completely abolished by a period of 18 h starvation. The results for butyrate show that all of the butyrate in the portal blood is derived from the caecum in both the fed and starved animal (TQ; 0·98–0·03 and 1·00±0·08 respectively). It has been suggested that butyrate absorbed from the caecum may be converted to ketone bodies in the caecal wall (Henning & Hird, 1972). Our analysis of concentrations of 3-hydroxybutyrate and acetoacetate in carotid and portal blood samples show no significant increase in these metabolites across the caecum in either the fed or starved animal indicating that this is unlikely to be the case in the conscious rabbit.

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The effect of abomasal casein infusion on acetate and palmitate kinetics in cows during early lactation. By B. A. KÖNIG and D. S. PARKER, *Department of Physiology & Biochemistry, The University of Reading, Whiteknights, Reading RG6 2AJ* and J. D. OLDHAM, *National Institute for Research in Dairying, Reading, Berks RG2 9AT*

It has been suggested that increases in protein supply to the abomasum can increase tissue mobilization in cows early in lactation (Ørskov *et al.* 1977). This work was designed to quantify the effects of abomasal supplements of casein on the flux rates of acetate and palmitate in jugular blood and on milk production in early lactation. Measurements were made in four potentially high yielding Friesian cows fitted with abomasal catheters. They were fed 4 kg hay in four portions each day and 3 kg alkali-straw cubes and 9 kg concentrates/d dispensed at hourly intervals. The diet contained 110 g crude protein (N × 6·25)/kg DM. The cows were supplemented per abomasum with 0, 240 or 460 g/d sodium caseinate in a 3×3 Latin Square experimental design modified for an extra cow. Treatment periods lasted 21 d beginning one week post-partum. In the last week of each experimental period the following measurements were made: milk yield and composition, and acetate and palmitate flux rates in jugular blood (König *et al.* 1979).

Treatment (g sodium caseinate/d)	Milk fat (g/d)	Milk protein (g/d)	Plasma acetate (mmol/l)	Acetate flux rate (mmol/min)	Plasma palmitate (mmol/l)	Palmitate flux rate (mmol/min)
0	906	607^{a}	1·52	$29·46^{a}$	0·14	0·70
240	909	697^{b}	2·13	$93·78^{b}$	0·09	0·94
460	960	683^{b}	1·44	$81·16^{ab}$	0·11	0·85
SE of difference between means (4 df)	51·0	24·9	0·39	26·69	0·03	0·17

a,b Values in the same column which do not share a common superscript differ significantly.

Abomasal casein infusion resulted in a significant increase in milk protein yield ($P<0·05$) and also in acetate flux rate ($P<0·1$). If it is assumed that casein infusion had no effect on rumen acetate production these results suggest that the production of endogenous acetate by the tissues has increased as a result of the treatment. Mean palmitate flux also increased with casein infusion but the change was not statistically significant. Acetate and palmitate concentrations did not change.

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