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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Seventy-sixth Meeting of the Nutrition Society was held in the Curtis Auditorium of the Physics Building, University of Newcastle upon Tyne, on Tuesday and Wednesday, 21/22 *September* 1982, *when the following papers were read:*

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Measurement of gastric emptying rate in growing pigs. By A. G. Low and ANNA L. RAINBIRD, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Gastric emptying can be measured directly and quantitatively either by excision of the stomach under anaesthesia or by cannulation of the stomach or small intestine, allowing continuous monitoring in conscious animals during several weeks or more. The former method requires a large number of animals for meaningful results and assumes that anaesthesia does not affect gastric emptying; the latter method involves severing nerves and blood vessels and in the case of re-entrant cannulation usually requires complete division of the duodenum, which disturbs a region known to have an important role in the control of gastric emptying. Furthermore, it is usually impossible to place a re-entrant cannula proximal to the bile duct, so measurements include the contribution of bile to the gastric effluent. Indirect methods of measuring gastric emptying involve the use of markers and are not strictly quantitative for specific components of the digesta.

Because we required to make long-term continuous studies of gastric emptying in growing pigs with minimal interference of neural and humoral function, direct simple cannulation of the greater curvature of the stomach was used. The acetal co-polymer cannula had a flange of 70 mm diameter, a barrel height of 50 mm and an internal diameter of 29 mm. The barrel contained a replaceable PVC non-return valve. Most of the digesta were evacuated by introducing a tube, connected to a large flask and a vacuum pump, through the non-return valve into the stomach. Residual digesta were removed with three or four rinses of water, the total volume of which did not exceed half the volume of the gastric contents. After weighing and sampling, the digesta were returned to the pig. One sample was taken per day from each pig.

The growth rate and health of the pigs with gastric cannulas was normal; no problems were experienced with the cannulas except that the moulded PVC valves needed to be replaced every 1–2 weeks.

The method was shown to allow complete evacuation of gastric digesta in preliminary tests using two pigs with gastric and duodenal re-entrant cannulas. In these tests 98–102% of ingested dry matter was recovered from the two cannulas following a meal of either cereal-based or semi-purified diets. The amounts of the diets given were 30 or 42·5 g/kg body-weight per day in two equal meals.

A.L.R. acknowledges receipt of an ARC research studentship.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Lack of effect of guar gum on gastric emptying in pigs. By ANNA L. RAINBIRD, A. G. LOW and I. E. SAMBROOK, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Although the beneficial effects of guar gum on glucose tolerance in normal and diabetic man are well established, its mode of action remains unclear.

In normal humans Holt *et al.* (1979) found that the addition of 16 g guar gum and 10 g pectin to 400 ml orange juice significantly increased the gastric emptying half time from 23 to 50 min. However, the proportion of fibre to glucose in the drink was much higher than in normal meals. By administration of 500 mg glucose with 80 mg of one of three guar gum preparations of differing viscosity in 4 ml water by orogastric intubation to rats, Leeds *et al.* (1979) found the gastric emptying half time increased from 16 to 41 min as viscosity rose.

Both those experiments used test meals of much lower dry matter (DM) content than usually eaten by man. However, Wilmshurst & Crawley (1980) used test meals of somewhat higher DM content in obese humans and found that the addition of 2 g guar gum to a 200 g low-fat milky drink significantly increased mean gastric transit time from 69 to 112 min. Again, only liquid emptying was measured although emptying of solids is of primary importance (Heading *et al.* 1976).

We have investigated the hypothesis that guar gum delays the emptying of a meal from the stomach in four conscious growing pigs (shown to be a suitable model of man; Leeds *et al.* 1980), surgically prepared with a simple gastric cannula (Low & Rainbird, 1982).

The pigs were given a semi-purified diet with 0, 20, 40 or 60 g guar gum/kg air-dry diet which when mixed with water gave meals of increasing viscosity. The stomach was evacuated at 0, 0·5, 1, 2 and 4 h after feeding. The weight of stomach contents was measured and a sample taken for DM analysis.

Gastric emptying of DM was not significantly delayed by guar gum at any level except for 1 h after feeding the 60 g guar gum/kg air-dry diet ($P \leqslant 0{\cdot}01$).

We thank R. M. W. Hopkins of the Meyhall Chemical Company, Wirral, Merseyside, for the gift of the guar gum. A.L.R. acknowledges receipt of an ARC research studentship.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The utilization of a supposedly ideal protein for growing pigs. By M. F. FULLER, A. CADENHEAD and K. H. CHEN, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

According to the Agricultural Research Council (ARC) (1981) an ideal protein for growing pigs contains (g/16 g N) lysine 7·0, threonine 4·2, valine 4·9, methionine + cystine 3·5, isoleucine 3·8, leucine 7·0, phenylalanine + tyrosine 6·7, histidine 2·3 and tryptophan 1·0. These estimates were based on a review of a number of disparate experiments, and the present experiment was an attempt to validate them. Thirteen female pigs of approximately 38 kg were given, at a rate of 110 g/kg $W^{0.75}$, a diet based on casein, maize starch, sucrose and glucose with vitamins and minerals and supplying 18 g N/kg dry matter (DM). Two additional pigs were given protein-free diets to estimate endogenous N losses. The basal diet supplied more of each amino acid, per g of protein, than specified in the ARC ideal protein, except for threonine and methionine + cystine which were marginal. The biological value (BV) of the casein alone was 0·86, in agreement with our earlier estimate (Fuller *et al.* 1982). Addition of threonine (1·5 g/kg diet) was without effect, but with a supplement of methionine (1·2 g/kg diet) BV was increased to 0·90 ($P<0·05$). The combination of the two supplements gave a BV of 0·91, not significantly higher than methionine alone, whilst the triple addition of threonine, methionine and arginine (3·0 g/kg diet) resulted in the same BV as the basal diet. The results suggest that the ARC estimate of ideal protein is less than ideal, at least in its methionine content, and that some further additions of other amino acids are required to perfect it.

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Fuller, M. F., Gorst, C. G. & Cadenhead, A. (1982). *Anim. Prod.* **34**, 363.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Paradoxical changes in 3-methylhistidine production and muscle protein degradation in fasted rats. By L. COTELLESSA, P. W. EMERY and M. J. RENNIE, *Department of Medicine, The Rayne Institute, University Street, London WC1E 6JJ*

In the mammalian body 3-methylhistidine (3MH) is commonly thought to originate mainly from the myofibrillar proteins of skeletal muscle. Since 3MH is not re-utilized for protein synthesis the 3MH production rate is taken to be an index of the rate of muscle protein degradation. However, reported measurements of muscle protein degradation rate (Waterlow & Millward, 1978) and urinary 3MH excretion (Wassner *et al.* 1977) in fasted rats show opposite trends. We have, therefore, measured 3MH production and muscle protein degradation during starvation in young rats, when large changes in the rate of skeletal muscle protein breakdown are known to occur.

Male Wistar rats (100 g) were fasted for periods of up to 4 d. Muscle protein synthesis rates were determined using a large intravenous dose of ^{3}H-phenylalanine (Garlick *et al.* 1980). Muscle weights and protein contents were measured, and protein degradation was calculated as the difference between synthesis and change in muscle protein mass. Free 3MH was measured in blood and muscle samples, and in urine after hydrolysis (Emery & Rennie, 1982). Total 3MH production was calculated as the sum of urinary 3MH excretion plus the accumulation of 3MH in extracellular fluid and muscle (see Table).

	Post-absorptive		Days fasted: 1		2		3		4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Muscle protein synthesis (%/d)	13·7	3·7	9·4	1·7	7·7	1·2	6·9	0·8	1·9	0·4
Muscle protein degradation (%/d)	9·4		8·5		8·1		8·0		19·8	
Total 3MH production (μmol/d)	1·01	0·07	1·42	0·12	1·79	0·08	1·93	0·18	1·76	0·23

During the first 3 d of starvation muscle protein degradation rate fell, but 3MH production rose. On the fourth day of starvation muscle protein degradation rate increased but 3MH production did not change. These results suggest that tissues other than skeletal muscle make a substantial contribution to 3MH production in the rat. Smooth muscle in the gastrointestinal tract is probably the most important of these sources, and we have preliminary evidence that increases in 3MH release from the gut may more than match decreases in 3MH release from skeletal muscle.

This work was supported by The Cancer Research Campaign, The Wellcome Trust, and The Medical Research Council. L.C. is a Fellow of The Advanced Training Programme of the Italian Labour Department and the EEC. We thank Professor R. H. T. Edwards for encouragement.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The effect of dimethylolurea treatment on the metabolism of casein in the rumen of steers. By A. P. WILLIAMS and J. E. COCKBURN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Formaldehyde (HCHO) is widely used for the protection of dietary protein from degradation in the rumen. However, the reaction of HCHO with proteins is difficult to control and, since it is also hazardous to use, HCHO donors such as dimethylolurea (DMU) have been proposed as alternatives (Sutton, 1962). Preliminary studies (Williams, 1979) showed that the optimum level of DMU treatment was about 50 g/kg protein. Lactic acid-precipitated casein treated at this level showed greatly reduced ammonia production on in vitro incubation with rumen contents from steers, whereas its available lysine content, determined by dye-binding assay (Hurrell & Carpenter, 1976) was reduced by approximately the same small amount as casein treated with HCHO (10 g/kg protein).

Four steers, with rumen and simple abomasal cannulas, were given a basal diet of straw and tapioca supplemented with either untreated casein or DMU-treated casein (50 g/kg protein) to give about 19 g N/kg dry matter. The animals were allowed at least 2 weeks to adapt to each diet. Chromic oxide and polyethylene glycol were used as digesta flow markers. Six hours after feeding, concentrations of ammonia-N (g/l, mean values ± SE) in rumen fluid were 0·048±0·004 and 0·014±0·001 for the diets supplemented with untreated and DMU-treated casein respectively. The amounts of total-N and non-ammonia-N entering the duodenum were higher when DMU-treated rather than untreated casein was given. Direct measurement of casein entering the duodenum, essentially by the method of McDonald & Hall (1957) indicated that giving DMU-treated rather than untreated casein led to much greater amounts of dietary casein escaping degradation in the rumen and a higher proportion of casein-N in abomasal non-ammonia-N was found (0·40 *v.* 0·10). These results suggest that DMU is a potentially useful alternative to HCHO for the protection of dietary protein from degradation in the rumen confirming recent in vitro studies by Friedman *et al.* (1982).

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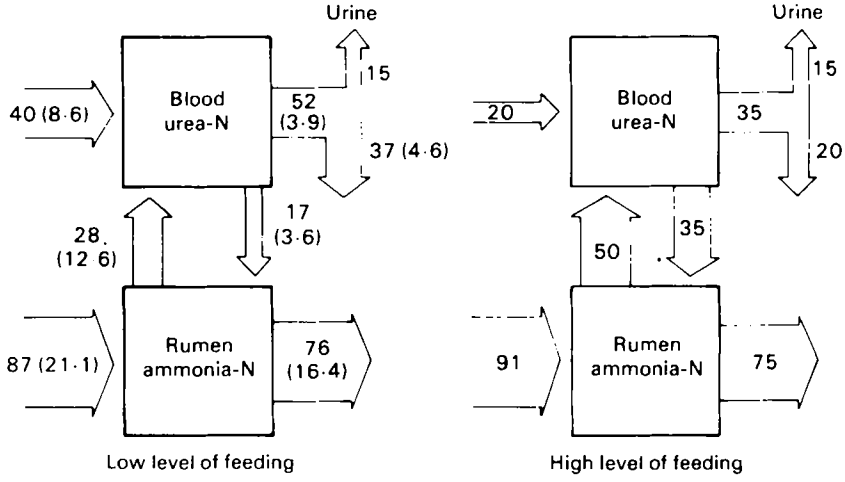
https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Nitrogen cycling in growing cattle fed maize silage. By M. P. GRANTLEY-SMITH and J. D. OLDHAM, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The aim of this experiment was to measure N cycling in young growing cattle offered different amounts of food. Four rumen fistulated steers (250 kg mean initial live weight) were offered a diet of maize silage and concentrates in the ratio 4:1 in dry matter (DM). Two levels of feeding were used, 0·57 (L) and 0·89 (H) MJ metabolizable energy/kg $W^{0.75}$ in a changeover design experiment. Rations contained 86 g crude protein/kg DM.

Transfers between blood urea-N and rumen ammonia-N were calculated from the dilution of ^{15}N ammonium chloride infused into the rumen (72 h infusion) and $^{15}N_2$-urea infused into the jugular vein (48 h infusion), together with measurements of the transfer of ^{15}N between labelled pools. The infusions were separated by 5 d. N balance was measured over 7 d in each period.

Growth rates were 0·33 (L) and 0·59 (H) kg/d. N movements in the rumen ammonia-N/blood urea-N system are shown as percentage of N intake with standard errors of difference between levels of feeding in parentheses.



At both levels of feeding there was a net loss of rumen ammonia-N to blood urea-N, equivalent to 11–15% of N intake, even though by calculation rumen-degradable N intake was only just adequate (Agricultural Research Council, 1980).

The level of feeding had no effect on total urea recycling (% N intake) to the gut, but a substantial effect on the site of re-entry. Urea recycling was predominantly to the rumen at the high level of feeding and to the rest of the gut at the low level of feeding.

These results have implications for extrapolation of results on urea recycling for animals at low levels of feeding to animals at high levels of feeding.

M.P.G.S. is an ARC postgraduate scholar.

Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock*, p. 128. Farnham Royal: Commonwealth Agricultural Bureaux.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Effect of Synperonic upon bulk viscosity, dilution rate and other rumen parameters. By S. JAMES, F. J. T. FILDES, A. W. J. BROOME and A. DAVIES, *ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG*

Ruminal flow plays a key role in ruminant digestion. Fibre-rich diets and osmotically active agents favour high dilution rates resulting in improved microbial protein synthesis (Owens & Isaacson, 1977), more nutrient 'by-pass' but lower total fibre digestion and a shift in fermentation towards acetate. The depression of rumen dilution rate by monensin has been documented (e.g. Bull *et al.* 1979). Using a pelleted ration of 850 g dried grass/kg diet continuously given to fistulated sheep, we recorded a 23% reduction in dilution rate ($P<0.01$) using monensin at 120 mg/d. This was accompanied by a significant increase in bulk viscosity of rumen contents determined using apparatus based upon the Stormer viscometer which is particularly suited to non-Newtonian flow (Stearns, 1970).

Flow in simple slurries can be improved by adding surfactants and we proposed to do the same with rumen contents using the Synperonic-NP series. Tested in vitro this series depressed acetate:propionate (Ac:Pr), methane production and digestibility but NP8 gave the most significant reduction in viscosity. Initial in vivo studies were conducted at graded levels of surfactant in the pelleted feed of non-fistulated sheep. Above 10 g/kg diet food intake was depressed with a highly significant ($P<0.01$) reduction in Ac:Pr, e.g. at 25 g/kg diet the ratio changed from 2·7 to 1·45.

In a more detailed study 10 g Synperonic NP8/kg diet was given to two groups of fistulated sheep, with and without monensin. There was no decrease in food intake. 2, 8 and 10 d after commencing treatment, 24 h liquid dilution rate estimates were made using Cr-EDTA. The surfactant caused an 18% reduction in dilution rate in the basal diet, but no change was observed in other groups. After 8 d, three different 0·8 l samples of rumen fluid were taken and their viscosities compared. The surfactant had caused a significant decrease in all cases. We also observed decreases in ruminal total volatile fatty acids ($P<0.01$), in Ac:Pr ($P<0.001$) and in the numbers of protozoa ($P<0.001$) especially *Entodinium* spp.

We conclude that, under these conditions, bulk viscosity plays little part in the rate of liquid turnover within the rumen.

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Stearns, R. S. (1970). *Kirk-Othmer Chemical Technology*, vol. 21. New York: Wiley Interscience.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Digestion of grass stem in nylon bags of different pore size in an artificial rumen (Rusitec). By R. E. BRICE and I. M. MORRISON (introduced by P. C. THOMAS), *The Hannah Research Institute, Ayr KA6 5HL*

The rumen simulation technique (Rusitec) (Czerkawski & Breckenridge, 1977) provides a useful model system to study the digestion of plant cell wall material by rumen microbes. The digestion of plant cell wall material has been investigated by suspending nylon bags, containing the material under study, in the rumen of suitably cannulated animals (e.g. Bacon *et al.* 1981 and references therein). Problems associated with this approach include the flow of particulate material, particularly fine particles of plant material, from the rumen into the bags.

To minimize this problem, digestion of plant materials in nylon bags has been carried out in Rusitec. Previously, we have employed bags of 1 mm pore size but in other studies on the digestion of plant cell walls (Morris & Bacon, 1977; Chesson, 1981) bags of 5 μm pore size were used. Bacon (1979) has discussed the possibility of using bags of different pore sizes to investigate the contributions of different organisms to cell wall digestion. Udén *et al.* (1979) have pointed out the effect of different pore sizes on the digestibility of grass.

In the experiment reported here, bags of 5, 24, 56 and 1000 μm pore size were used to investigate the effect of pore size on (1) dry matter (DM) digestibility, (2) the composition of the digesta, and (3) the activity of selected glycoside hydrolases and polysaccharidases in the liquid washed from the digesta.

The results show that although there was an increase in DM digestibility from the 24 through to the 1000 μm bags it was not statistically significant. However, there was a significant reduction in DM digestibility when grass stem was incubated in the 5 μm bag. There was a gradual increase in the number of D-xylose residues being removed with increasing pore size but approximately 30% more cellulose was removed from the 24 μm bags than from the 5 μm bags. The differences in enzyme activity associated with the different pore size bags were small.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The effect of diet composition and particle size on the outflow of chromium-mordanted protein supplements from the rumen of high yielding dairy cows. By M. E. ELIMAM and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Factors affecting the outflow of protein supplements from the rumen of high yielding dairy cows have been studied. A highly significant positive effect was observed for feeding level (Elimam & Ørskov, 1982). The aim of this study was to investigate the effect of diet composition and particle size on the outflow of chromium-mordanted protein supplements from the rumen.

Two experiments were conducted, each with four high yielding British Friesian dairy cows in mid-lactation. They were fed twice daily at twice their estimated energy requirements for maintenance per day, according to a 4×4 Latin-square design. In the first experiment they were given completely mixed diets ranging from 25% hay:75% concentrate to 100% hay. In the second experiment they were given long dried grass or dried grass, hammer milled through 40, 20 or 5 mm screens, the last of which was subsequently pelleted. The fractional rates of outflow of protein supplements from the rumen were determined using Cr-treated fish meal, as described by Elimam & Ørskov (1981) (see Table).

Table 1. *The effect of hay:concentrate and particle size on Cr-mordanted protein outflow from the rumen of high yielding dairy cows*

Expt 1			Expt 2	
Proportion of:				
Hay	Concentrate	Fractional rate of outflow/h	Form of diet	Fractional rate of outflow/h
0·25	0·75	0·064	Long	0·080
0·50	0·50	0·086	Ground through 40 mm screen	0·086
0·75	0·25	0·088	Ground through 20 mm screen	0·088
1·00	—	0·079	Ground through 5 mm screen and pelleted	0·047
SEM	—	0·0072	SEM	0·0054

When the proportion of hay was increased from 0·25 to 0·50, the outflow rate increased ($P<0·05$), but further increase in the proportion of hay had no significant effect on the outflow rate. Ganev *et al.* (1979) reported that roughages produced a faster outflow of protein supplements from the rumen of sheep than concentrates. Owens *et al.* (1979) made similar observations for liquid and solid phase digesta in steers. This could be due to increased salivation and rumination.

The decrease in particle size was associated with a very small increase in protein outflow, which was not significant. However, the ground and pelleted diet gave a significantly lower outflow ($P<0·05$) than the other three diets. This experiment suggested that the critical particle size which affects the rate of outflow of Cr-mordanted proteins from the rumen is below that which passes through a 20 mm screen.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The correlation between extent of pH depression and degradability of washed hay in sheep given hay and concentrate. By L. ISTASSE and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In recent studies (Stewart, 1977; Mould, 1982) the cellulolytic microflora of the rumen was inhibited when rumen pH fell below approximately 6. When a high level of concentrate diet was offered to sheep in different ways and over different time intervals during the day, rumen pH often fell below 6 but for different lengths of time. The work reported here was undertaken to determine the pH pattern which had the greatest effect on the degradability of washed hay.

Three sheep were used in a Latin-square design. A diet consisting of approximately 333 g hay and 666 g rolled barley/kg diet was offered *ad lib.* in three ways: as a complete mixed diet, with the hay always on offer and the barley on offer either two or four times a day. The degradability of washed hay in the rumen was determined using the nylon bag technique as reported by Mehrez & Ørskov (1977). Five incubation times were used: 8, 16, 24, 48 and 72 h. The rumen pH was recorded every 2 h during the first day. Since the eating pattern of the animals varied within treatments it was felt that analysis according to a Latin-square design was inappropriate. However, linear regressions of the degradability of washed hay in the rumen on the magnitude of the depression of pH below 6 (i.e. the lowest pH measured), the length of time when the pH was below 6 (duration) and a combination of both these criteria (the extent of the pH depression calculated by summing the products of time with each unit of pH below 6) were calculated.

The correlation coefficients are given in Table 1 for the five incubation times.

Table 1. *Correlation coefficients between washed hay degradability and different methods of describing the pH depression below* 6

	Incubation time (h)				
	8	16	24	48	72
Maximal depression below 6 (pH)	−0·59	−0·88	−0·79	−0·65	−0·61
Duration of depression below 6 (h)	−0·35	−0·76	−0·42	−0·50	−0·39
Summated depression below 6 (pH × h)	−0·66	−0·90	−0·87	−0·73	−0·66

From this table it can be seen that the highest correlations were obtained with the extent or summation of the pH depression below 6, rather than the maximal depression or duration of depression. The closest relationship (r −0·90 and r −0·87) were observed after 16 and 24 h incubations. The correlations tended to be maximal at 16 h and 24 h. The lower values at 8 h are probably due to small particle losses from the bags and by the degradation of some soluble carbohydrates. The lower correlations for the 48 and 72 h can be expected because the asymptote of the degradability of washed hay is being reached.

Mehrez, A. Z. & Ørskov, E. R. (1977). *J. agric. Sci., Camb.* **88**, 645.
Mould, F. (1982). PhD Thesis. University of Aberdeen.
Stewart, C. S. (1977). *App. Environ, Microbiol.* 497.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Rumen volatile fatty acids and blood constituents in sheep given either molasses or a mixture of sugars. By R. H. GODOY MONTÁNEZ,* D. S. PARKER and D. G. ARMSTRONG, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Molasses-based diets given to ruminants are characterized by a slow, even intake and high rates of fermentation of the soluble sugars in the rumen. The high mineral content (10–12% dry matter (DM)) may influence these characteristics by affecting the osmolarity of rumen contents. We have given sheep diets based either on cane molasses or a mixture of sugars (sucrose 36%, dextrose 12%, water 52%) simulating those present in molasses with 2% urea at the same level of intake (2·4% body-weight) together with forage (0·5% body-weight as DM). When given the sugar-based diet, feed was consumed in 8 h whereas when given molasses this time was extended to 20 h. Results of analyses of eight samples of rumen liquor and nine samples of jugular venous blood taken from each sheep between 2 and 8 h after feeding are shown in the Table.

(Mean values plus standard errors for the four sheep given sugar; individual mean values for the sheep given molasses)

	Rumen volatile fatty acids (mol %)						Jugular blood (mM)					
	Acetate		Propionate		Butyrate		Acetate		Butyrate		Glucose	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sugar (*n* 4)	44·9	2·8	18·4	3·0	24·8	1·9	1·94	0·31	0·22	0·08	3·19	0·03
Cane molasses (*n* 2)	59·4		36·7		7·5		1·5		0·03		4·2	
	55·1		33·4		6·8		1·3		0·03		4·3	
Cane molasses (*n* 1)	50·6		12·2		31·6		1·97		0·03		3·10	

On the sugar-based diets all four sheep showed a similar pattern of fermentation in the rumen, had a significant concentration of butyrate in jugular plasma and high levels of plasma insulin (>100 μU/ml). Two of the animals on molasses showed a propionate type of rumen fermentation with raised glucose and low butyrate concentrations in venous plasma. Although the third sheep on molasses had a butyrate pattern of fermentation in the rumen, plasma butyrate concentration was not raised. All the sheep, when given molasses, had lower insulin levels (20–40 μU/ml) than the values determined while on the sugar diet. These results suggest that rapid consumption of sugars by the ruminant animal can result in elevated butyrate levels in peripheral blood and the concomitant release of insulin (Bassett, 1978).

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Endocrine responses to cessation of lactation in sheep. By J. M. BASSETT, *Nuffield Institute for Medical Research, University of Oxford, Headley Way, Headington, Oxford OX3 9DS* and *CSIRO, Division of Animal Production, Blacktown, NSW, 2148, Australia*

Weaning results in a relatively sudden cessation in the need for the endocrine and metabolic adaptations supporting milk synthesis.

Changes in blood hormone and metabolite concentrations associated with this adaptation have been investigated in *ad lib.* fed crossbred ewes kept in pens and suckling single or twin lambs. Lactation was terminated by removal of lambs 90 d or less after birth. Blood samples were obtained on alternate days by jugular venipuncture or by means of a catheter in the jugular vein.

Following weaning, intake of the lucerne chaff/oats diet declined to 70% of the preweaning intake (3·43 kg/d) within 4 d before recovering to a stable value of approximately 80% of the earlier intake. Although food intake fell and there were only small changes in prefeeding glucose and free fatty acid concentrations there was a marked increase in plasma insulin and a decline in growth hormone during the first few days after weaning. Values at 4 and 14 d after weaning are compared with preweaning values in the Table.

Prefeeding plasma and metabolite concentrations in weaned ewes

(mean ± SEM; n 13)

	Days relative to weaning					
	−4		+4		+14	
	Mean	SEM	Mean	SEM	Mean	SEM
Glucose (mmol/l)	3·20	0·07	3·48	0·07	3·19	0·05
Free fatty acids (μmol/l)	502	40	473	47	448	33
Insulin (ng/ml)	1·21	0·19	2·70	0·28*	1·28	0·12
Growth hormone (ng/ml)	3·95	0·65	1·48	0·17*	1·91	0·26

Significantly different from preweaning value: *$P<0·05$.

More frequent observations after feeding during the first few days after weaning indicate that there are very large increases in plasma insulin, suggesting that this mechanism may be involved in the resetting of appetite and metabolic adaptation, following reduction in the mammary gland's requirements for nutrients.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Endocrine and metabolic responses to intravenous glucose or insulin injection in sheep during pregnancy and lactation. By J. M. BASSETT, *Nuffield Institute for Medical Research, University of Oxford, Headley Way, Headington, Oxford OX3 9DS* and *CSIRO, Division of Animal Production, Blacktown, NSW, 2148, Australia*

In most species glucose tolerance and the sensitivity to insulin is blunted during late pregnancy (Freinkel, 1980). However, adult ruminants are considered relatively insensitive to insulin so this effect may be of little significance in them.

On separate days, insulin (0·25 U/kg body-weight) and glucose (0·25 g/kg body-weight) were injected intravenously (i.v.) into eighteen crossbred ewes, twelve with a single lamb and six with twins, first during late pregnancy and again during early lactation.

There were no consistent differences in the rate at which injected glucose was removed. The half-times values for glucose were (mean ± SEM) 23·3±2·73 min *v.* 35·8±4·56 min in ewes with a single lamb and 29·9±4·10 *v.* 30·3±5·30 min in ewes with twins during pregnancy and lactation respectively. Similar rates were observed in non-lactating adult sheep. Similarly, there were no differences between pregnancy and lactation in the insulin response to glucose nor in the rate at which plasma glucose concentration decreased after i.v. insulin injection, although the recovery from hypoglycaemia was significantly prolonged during pregnancy. There was a difference in the rate at which plasma free fatty acid (FFA) concentrations declined after i.v. glucose injection in the two situations. The mean half-times (min) over the first 40 min were 41·3±4·5 in pregnancy and 25·4±20 during lactation. However, FFA concentrations were higher during lactation (868±74·4 *v.* 273±21·8 μmol/l) than during pregnancy.

There were, however, marked differences between pregnant and lactating sheep in plasma growth hormone (GH) concentrations both before injection of insulin or glucose and in the subsequent responses to them. Basal concentrations of GH were lower during pregnancy (3·73±0·73 ng/ml) than in lactation (7·94±1·48 ng/ml) but the maximum GH level 20 min after i.v. insulin injection was far less during pregnancy than in lactation (6·39±2·03 *v.* 47·8±5·79 ng/ml) although the pattern of GH responses was similar to that observed earlier (Wallace & Bassett, 1970). There were also far larger changes in GH following glucose injection into lactating sheep.

The observations do not indicate reduced carbohydrate tolerance or marked resistance to insulin action during late pregnancy in the sheep, but do indicate enhanced sensitivity of GH release to stimulation during lactation.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Relationship between dental caries increment and dietary habits assessed over 2 years in 405 English schoolchildren. By A. J. RUGG-GUNN, A. F. HACKETT, D. R. APPLETON*, G. N. JENKINS and J. E. EASTOE, *Department of Oral Biology and *Department of Medical Statistics, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4BW*

The aim of this study was to rank some dietary factors in the order in which they correlate with a 2-year dental caries increment in 11–13-year-old children.

Between September 1979 and July 1981, 193 boys and 212 girls all initially aged $11\frac{1}{2}$ years from a non-fluoridated area of south Northumberland, each kept five separate dietary diaries of three consecutive days each. They were asked to record all food and drinks taken, the time of consumption and give an estimate of how much was consumed in household measures. This information was then cross-checked at an interview on the 4th day. They were each dentally examined in October 1979, 1980 and 1981.

A variety of dietary factors thought to influence the progress of dental caries were calculated and averaged for the 15 d intake recorded. These were then correlated against the observed 2-year caries increment.

Both the caries experience at $11\frac{1}{2}$ years (mean 4·9 decayed missing filled surfaces (DMFS)) and the subsequent 2 year increment (mean 3·6 DMFS), were lower than expected. 58% of the caries increment were in tooth fissures, which were also the most sensitive sites to dietary intake. However, dietary intake was found to be very variable, e.g. mean (15 d) sweets intake was 22·0 g/d with a range of 0–107·7 g/d.

The following correlations were found with the fissure caries increment:

Dietary variable (15 d average)	Correlation coefficient
Total sugars (weight)	+0·143**
Total sugars minus lactose (weight)	+0·129**
Items >10% sugars (weight)	+0·118*
Confectionery + biscuits + cakes + puddings (weight)	+0·116*
Items >60% sugars (weight)	+0·115*
Sweets (weight)	+0·111*
Confectionery (weight)	+0·108*
Hot sugared drinks (excluding tea) (frequency)	+0·108*
Lactose (weight)	+0·103*
Milk (weight)	+0·102*
Items >1% sugars (weight)	+0·101*
Time between last food >10% sugars and bedtime	−0·101*

*$P<0.05$, **$P<0.01$.

We have concluded that sugars intake was positively correlated with caries increment, but that the weight of sugars consumed was more highly correlated than the frequency of sugars intakes. Fissure surface caries correlated more highly with dietary variables than other surfaces in these children. The proximity of the last sugary intake to bedtime was correlated with caries increment.

This work was supported by an MRC project grant.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The effect of hormone status on the rate of outflow of a chromium-mordanted protein concentrate from the rumen of ewes. By R. G. A. STEPHENSON,* N. T. NGONGONI, J. J. ROBINSON and T. ATKINSON, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and J. M. CHESWORTH, *Department of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD*

Late pregnancy in the ewe is accompanied by an increase in the amount of amino nitrogen reaching the abomasum (Thompson *et al.* 1978), and at least part of this increase arises from an enhanced rate of outflow of protein supplements from the rumen (Gonzalez, J. S., Robinson, J. J. and Fraser, C., unpublished results). To test if rumen outflow rates are influenced by endocrine changes, twenty non-pregnant, non-lactating Finn Dorset Horn ewes were individually penned under natural daylength hours (mean 16·3 h light, 7·7 h dark) and injected with one of four treatments: saline (9 g sodium chloride/l) (control), progesterone + oestrogen to mimic hormone status in pregnancy, bromocryptine (CB 154) or thyrotrophin releasing factor (TRF) to suppress and elevate respectively the concentration of prolactin in plasma. Details of the administration procedures are given in the Table.

The effects of altering the hormone status of ewes on the rate of outflow of a chromium-mordanted protein supplement from the rumen

(mean ± SE for five ewes per treatment)

	Control		Progesterone + oestrogen (20 mg + 80 μg/24 h)		CB 154 (1 mg/12 h)		TRF (50 μg/6 h)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Progesterone (ng/ml)	1·2	0·28	4·3	0·66	1·1	0·50	2·2	0·50
Oestradiol-17β (pg/ml)	5·2	0·82	32	11	3·8	0·34	4·3	0·45
Prolactin (ng/ml)	84	21	91	47	15	4	299	21
Fractional outflow rate/h	0·051	0·0033	0·045	0·0016	0·049	0·0045	0·064	0·0046

The ewes were given daily 1600 g of a diet of milled hay and concentrates which contained 8·4 MJ of metabolizable energy and 140 g crude protein/kg. Outflow rate from the rumen of a supplement of chromium-treated fish meal was estimated by linear regression of ln Cr_2O_3 concentration in the faeces against time.

Altering the circulating concentrations of progesterone and oestrogen had no significant effect on the rate of outflow of the protein supplement from the rumen but the use of TRF to elevate prolactin increased it by 30% ($P<0·025$).

Thompson, J. L., Robinson, J. J. & McHattie, I. (1978). *Proc. Nutr. Soc.* **37**, 71A.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Prolactin infusion causes increased nitrogen retention in lambs in continuous darkness. By B. R. BRINKLOW and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

It has often been noted that sheep grow faster during the summer than the winter months, and evidence suggests that this is an effect of photoperiod. Forbes, Driver *et al.* (1979) and Forbes, El Shahat *et al.* (1979) found that growth rate and circulating prolactin (PRL) levels were both higher in 16 h photoperiod days compared with 8 h photoperiod days. The present study was carried out to investigate whether the increased PRL secretion and increased growth found under conditions of long photoperiods were causally related.

Four castrated male lambs born in March, which had been reared in natural daylight, were transferred at the end of September with a mean (± SD) live weight of 37·7 ± 1·8 kg, to metabolism crates in two light-proof rooms. A concentrate diet (nitrogen content, 25 g/kg) was given at 70 g/kg $W^{0.75}$ per d. The animals were maintained in continuous darkness to produce low levels of PRL secretion. Half of the animals in turn were infused with 10 mg ovine PRL/d in 250 ml saline (9 g sodium chloride/l) into the jugular vein for 10 d in a pattern designed to mimic that produced by a 16 h photoperiod day, the other two animals in each case acting as non-infused controls. For the final 7 d of each 10 d period, faeces and urine were collected from all four animals for determination of N retention. On the penultimate day of each treatment hourly blood samples were taken for 24 h for PRL determination by radioimmunoassay. The results of the experiment are summarized in the Table.

	N input (g/7 d)		Faecal N output (g/7 d)		Urinary N output (g/7 d)		Percentage N retention		Plasma PRL concentrations (ng/ml)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
PRL infusion	196·5	2·2	56·2	2·7	83·4	4·4	28·9*	1·0	84·5**	7·7
Control	195·6	1·4	55·5	1·22	96·3	2·8	22·4	1·0	30·6	4·3

*$P<0.02$, **$P<0.005$.

There was an increase in N retention in the animals infused with PRL which was due predominantly to a decrease in urinary output.

These results suggest that PRL increases the rate of net protein synthesis in lambs and might therefore mediate the effects of photoperiod on growth.

This work was supported by a grant from the Agricultural Research Council.

The prolactin was a gift from the NIH (OPRL-14) with a growth hormone contamination of less than 0·5% as determined by radioimmunoassay.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Effect of insulin infusion on nitrogen excretion in sheep. By R. SUMNER and T. E. C. WEEKES, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

A combined infusion of insulin and glucose into pigs decreased urinary excretion of nitrogen and urea and plasma urea concentrations when compared to infusions of saline or glucose alone (Fuller *et al.* 1977). Experiments have now been carried out to determine the effect of constant intravenous infusion of insulin and glucose on N excretion in sheep.

Seven castrate male lambs weighing 25–39 kg were given dried grass pellets (17–19 g N/kg dry matter (DM)) at 80 g DM/kg $W^{0.75}$ per d in twenty-four equal hourly portions. Animals were fitted with two intrajugular catheters 24 h before infusions began. Infusion periods were: isotonic saline (0·33 ml/min) for 5–6 d followed by a combined infusion of insulin (1 mU/kg body-weight per min) and glucose (3·4–4·0 mg/kg body-weight per min) for 6–9 d, and finally an infusion of glucose (3·4 mg/kg body-weight per min) alone for 3–4 d. During the combined insulin and glucose infusion plasma glucose was monitored and the glucose infusion rate adjusted to maintain normoglycemia. Each day, three to four plasma samples were taken and 24 h urine and faecal collections made. Results are shown in the Table.

Infusion ...	Saline		Insulin + glucose		Glucose	
	Mean	SEM	Mean	SEM	Mean	SEM
Plasma insulin (μU/ml)	14	4	35	5***†	25	4
Plasma glucose (mg/l)	730	60	780	170	920	80
Plasma urea (mg/l)	41	3	34	6*	43	21
Plasma α-amino-N (mg/l)	57	6	46	7**	40	9
Urine N (g/d)	8·0	1·3	7·7	1·1	8·0	1·6
Urine urea (g/d)	13·6	1·7	13·3	1·4	13·0	2·3
Faecal N (g/d)	16·1	6·1	14·8	7·1	17·0	7·2
N intake (g/d)	24·6	2·2	24·6	2·2	21·5	4·1

Statistical significance between saline and insulin + glucose infusion: *$P<0.05$, **$P<0.01$, ***$P<0.001$; between insulin + glucose and glucose alone infusion: †$P<0.01$.

During the glucose alone infusion animals began to refuse feed, results are uncorrected for this.

It is concluded that a modest elevation in plasma insulin concentration in growing lambs does not significantly alter N excretion in urine and faeces. Plasma urea and α-amino-N concentrations were reduced significantly by the insulin and glucose infusion compared to the saline infusion.

This work was supported by SERC and Pfizer Central Research.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Maternal and foetal metabolism and its endocrine regulation in alloxan-diabetic pregnant ewes. By J. M. BASSETT, A. H. BURKS and R. A. PINCHES, *Nuffield Institute for Medical Research, University of Oxford, Headley Way, Headington, Oxford OX3 9DS*

The modifying effects of pregnancy on the metabolic abnormalities following pancreatic β cell destruction with alloxan in pregnant ewes was clearly shown by the studies of Reid *et al.* (1963). However, consequences for foetal metabolism and growth due to maternal insulin lack and the associated abnormalities in disposition of dietary nutrients have not been defined.

Destruction of pancreatic β cell function by rapid intravenous (i.v.) injection of alloxan (40 mg/kg body-weight) into Mule ewes with chronically cannulated foetuses (123–136 d gestation) caused marked disturbances in maternal glucose homoeostasis, even when replacement insulin was given. When insulin was not given, or was inadequate, marked hyperglycaemia accompanied by increased plasma glucagon concentrations was observed as in other situations of experimental diabetes (Unger *et al.* 1977).

In diabetic ewes maintained by continuous i.v. insulin infusion, pre-feeding glucose concentrations were in the normal range but after feeding (800 g sheep pellets and 1600 g grass nuts daily in two feeds) there was frequently an exaggerated increase in plasma glucose compared to the negligible change seen during control studies, although there were no significant changes in plasma glucagon. Likewise, the increment in plasma glucose concentration (mean ± SEM) (5·78±0·46 mmol/l) attained after 2 h i.v. glucose infusion (1·02 mmol/min) was significantly greater ($P<0·001$) than that observed during glucose infusions before β cell destruction (3·11±0·30 mmol/l). There was no correlation between plasma glucose and insulin concentrations, in contrast to the close relation seen in all ewes during control infusions.

Foetal hyperglycaemia and hyperinsulinaemia were associated with maternal hyperglycaemia in the diabetic ewes. The increment in foetal glucose during maternal glucose infusion was significantly greater in the diabetic ewes (1·62±0·13 *v.* 1·05±0·10 mmol/l; $P<0·01$). In most foetuses plasma insulin and glucose concentrations were highly correlated, but increases in insulin remained proportionate to the degree of hyperglycaemia and were similar to those in foetal lambs infused with glucose (Bassett & Madill, 1974). Foetal plasma α-amino acid nitrogen concentrations before feeding were decreased after induction of maternal diabetes (8·09±0·19 *v.* 9·10±0·31 mmol/l; $P<0·01$) but did not change significantly after feeding. Prolonged maintenance of marked maternal hyperglycaemia resulted in foetal lactic acidaemia and rapid demise.

The results re-emphasize the central role of regulated insulin release in maintaining normal metabolic homoeostasis in the pregnant ewe and her foetuses.

We thank the British Diabetic Association for financial support.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Effects of undernutrition and exercise on uterine uptake of D(-)3-hydroxybutyrate in the late-pregnant ewe. By K. D. CHANDLER, P. MCN. HEMPHILL, A. R. BIRD and A. W. BELL, *School of Agriculture, La Trobe University, Bundoora, Victoria* 3083, *Australia*

In foetal sheep, ketone utilization is negligible, even in hyperketonaemic ewes (Battaglia & Meschia, 1978), but the capacity of the uteroplacental tissues to metabolize significant amounts of non-carbohydrate substrates is unknown (Meschia *et al.* 1980). Therefore, we have measured rates of net uterine uptake of D(-)3-hydroxybutyrate (3HB) in well-fed and undernourished pregnant ewes at rest and during acute exercise.

Ten well-fed, single-pregnant Merino ewes at 101–121 d gestation had catheters implanted in the abdominal aorta, the main vein draining the pregnant uterine horn and the foetal posterior vena cava. After surgery they were given lucerne chaff (metabolizable energy content approximately 8·5 MJ/kg) at 1000–1200 g/d ('fed', *n* 6) or 350 g/d ('underfed', *n* 4). At least 7 d later, uterine blood flow was measured by the antipyrine placental clearance method, together with uterine arteriovenous (A-V) concentration differences of 3HB and oxygen, while the ewe stood at rest and then while it walked at 0·7 m/s on a 10° slope. Net uterine uptake of 3HB was calculated as blood flow × (A-V) 3HB concentration, and the maximum contribution of 3HB to uterine oxidative metabolism as (A-V) 3HB × 4·5/(A-V) O_2. The results are presented in the Table.

Arterial concentration, net uterine uptake and uterine oxygen quotient of D(-)3-hydroxybutyrate

	Fed (nine Expts, six ewes)				Underfed (five Expts, four ewes)			
	Rest		Exercise		Rest		Exercise	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Arterial concentration (mmol/l)	0·61	0·03	0·75	0·08	2·28	0·28	1·86	0·25
Uterine uptake (μmol/min)	40	5	43	8	100	21	84	24
(A-V) 3HB uptake × 4·5/(A-V) O_2 uptake	0·117	0·014	0·124	0·022	0·350	0·065	0·248	0·054

Effects of undernutrition were highly significant for each measurement ($P<0·001$, analysis of variance). Exercise caused a significant increase in arterial 3HB concentration in fed ewes ($P<0·05$) and a significant decrease in underfed ewes ($P<0·01$).

Our findings that 3HB utilization by the pregnant uterus is far from negligible in well-fed ewes, and markedly increased during severe undernutrition have been independently corroborated (Pethick & Lindsay, 1982). Rates of 3HB uptake have not been previously measured; present values could account for 20–25% of total 3HB entry rate in fed and underfed ewes, as predicted from the results of Pethick & Lindsay (1982).

The exercise-induced decline in arterial 3HB levels in underfed ewes may have been due to increased utilization by contracting muscle.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The effect of glucagon and 3′–5′ cyclic AMP on the metabolism of palmitate by isolated hepatocytes from adult sheep. By M. A. LOMAX, I. A. DONALDSON* and C. I. POGSON**, *Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ*

Glucagon plays an important role in the development of ketonaemia in non-ruminants by altering the partition of hepatic fatty acid metabolism towards oxidation and away from esterification. This action of glucagon has been reported to be mediated by a decrease in the rate of *de novo* fatty acid synthesis in the liver (McGarry & Foster, 1980). However, ruminant liver has been shown to synthesize fatty acids at low rates (Ballard *et al.* 1969) and a study was therefore undertaken to examine the role of glucagon in the control of palmitate metabolism in sheep liver.

The preparation and incubation of hepatocytes was as described by Donaldson *et al.* (1979) and Lomax *et al.* (1979). The results are presented in the Table.

(Mean values with their standard errors. Number of animals in parentheses)

		Production (nmol/h per mg dry wt)					
		$^{14}CO_2$		^{14}C esterified lipid		Ketone body release	
Substrate	Addition	Mean	SEM	Mean	SEM	Mean	SEM
Palmitate (1 mM)	None	3·5	1·1 (3)	41·7	4·6 (3)	10·4	2·3 (3)
	Glucagon (10^{-6}M)	4·3	1·5 (3)	38·0	4·4 (3)	11·5	2·7 (3)
	Dibutyryl cyclic AMP (10^{-4}M)	2·8,2·6 (2)		43·9,35·4 (2)		14·7	4·8 (3)
Palmitate (1 mM) + carnitine (1 mM)	None	3·9	0·4 (3)	ND		66·2	3·3 (3)
	Glucagon (10^{-6}M)	3·1	1·3 (3)	ND		63·4	4·3 (3)
	Dibutyryl cyclic AMP (10^{-4}M)	3·0,2·8 (2)		ND		73·5	7·8 (3)

ND, not determined.

Glucagon did not influence either the rate of ketone body synthesis or the rates of [1-^{14}C] palmitate oxidation to $^{14}CO_2$ and metabolism to esterified lipid by sheep hepatocytes. Glucagon was similarily without effect on palmitate metabolism in the presence of carnitine but addition of carnitine to incubations did stimulate the rate of ketogenesis. These results were not due to a lack of glucagon receptor function since dibutyryl 3′–5′ cyclic AMP also did not affect fatty acid metabolism in sheep liver cells.

It is concluded that glucagon does not have a direct action on the metabolism of long chain fatty acids by sheep liver.

This work was supported by the ARC and Roussel Uclaf S.A.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Effects of trenbolone on corticosterone production by isolated rat adrenal cells. By K. M. THOMAS and R. G. RODWAY, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Trenbolone acetate (TBA) administration has been shown to reduce plasma concentrations of glucocorticoids in female rats and sheep. Treated animals also exhibited a lowered glucocorticoid response to a challenge dose of ACTH (Thomas & Rodway, 1982). To investigate the possibility of a direct action of trenbolone (TB) on the adrenal gland we have studied the effect of addition of trenbolone to incubations of adrenal cells from female rats.

Initial incubations contained approximately 3×10^4 adrenal cells/ml, and a basal production of corticosterone was found to be (mean ± SEM) $0{\cdot}81\pm0{\cdot}05$ ng/10^4 cells per 2 h. Addition of ACTH (Synacthen; CIBA, Sussex) at 10^{-10}M or 10^{-12}M gave significant increases in corticosterone production. Inclusion of 10^{-6}M TB together with ACTH resulted in a reduction of corticosterone production compared with ACTH alone, i.e. 44% reduction in 10^{-10}M ACTH incubations ($P<0{\cdot}05$); 81% reduction in 10^{-12}M ACTH incubations ($P<0{\cdot}05$). Inclusion of a lower concentration of TB (10^{-7}M) together with ACTH (10^{-10}M) resulted in a smaller reduction of corticosterone production (see Table).

The effects of ACTH and trenbolone on corticosterone production

(All results are expressed as ng corticosterone/10^4 cells per 2 h)

ACTH concentration (M)	Trenbolone concentrations (M): Nil		10^{-7}		10^{-6}	
	Mean	SEM	Mean	SEM	Mean	SEM
Nil	0·81	0·05	—	—	—	—
Nil	0·91	0·05*	0·53	0·05*	0·52	0·04*
10^{-12}	4·96	0·40	—	—	0·93	0·10
10^{-10}	110	13	76	5	62	8

*In this experiment 5×10^5 cells/ml were incubated. In all other experiments 3×10^4 cells/ml were used.

In order to examine the basal production rate of corticosterone, incubations using larger numbers of cells were performed (5×10^5 cells/ml). Basal production rate of corticosterone was $0{\cdot}91\pm0{\cdot}05$ ng/10^4 cells per 2 h, and inclusion of TB (10^{-6}M or 10^{-7}M) reduced the rate of production to $0{\cdot}52\pm0{\cdot}04$ and $0{\cdot}53\pm0{\cdot}05$ ng/10^4 cells per 2 h respectively ($P<0{\cdot}01$). This represented a reduction of 43% and 42% respectively.

In a separate experiment oestradiol (10^{-6}M) or testosterone (10^{-6}M) also reduced the cells' response to ACTH (10^{-10}M) by 26% and 42% respectively. Again trenbolone (10^{-6}M) reduced the response by 40%.

These results support previous in vivo studies where TBA administration was shown to decrease plasma glucocorticoids and adrenal glucocorticoid production. However, the specificity of these in vitro effects reported here is uncertain since both testosterone and oestradiol also reduced the corticosterone response to ACTH.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Growth and hormonal status of female Zucker rats maintained at 22° and 30°. By J. M. FLETCHER, P. HAGGARTY, K. W. J. WAHLE and P. J. REEDS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Characteristic features of the genetically obese Zucker rats (fa/fa) include a post-weaning hyperphagia, hypothermia at ambient temperatures below thermoneutrality, several endocrine abnormalities and an enhanced efficiency of energy utilization (Bray & York, 1971; Godbole *et al.* 1978). The casual relationships between these manifestations of the fatty genotype are incompletely understood. Female lean and obese Zucker rats were maintained below (22°) and at thermoneutrality (30°) and the consequences for food intake, body composition and hormonal status were investigated.

Female rats were weaned at 21 d and placed in individual cages at either 22 or 30°. Diet (Oxoid, Breeder) and water were available to appetite and food intake was measured daily. Rats at both temperatures were maintained on a 12 h light/dark cycle (0900–2100 hours). When they were 34 d old the rats were killed at 1100–1200 hours and blood samples taken. Carcases were analysed for crude protein (N × 6·25) and total lipid (see Table).

	Lean rats					
	22°			30°		
	Mean	SD	*n*	Mean	SD	*n*
Initial body-weight (g)	45·2	6·3	(11)	40·8	4·0	(10)
Final body-weight (g)	99·0	6·9	(11)	91·2	8·7	(10)
Carcase lipid content (g)	7·89	2·0	(11)	7·52	1·5	(9)
Carcase protein content (g)	16·1	1·2	(11)	15·4	2·4	(9)
Food intake from 21–34 d (g)	161·6	7·9	(11)	124·7	12·7	(10)
Plasma insulin (ng/ml)	1·2	0·8	(11)	1·46	0·9	(10)
Plasma corticosterone (μg/100 ml)	3·3	2·3	(11)	1·6	1·3	(7)
	Obese rats					
	22°			30°		
	Mean	SD	*n*	Mean	SD	*n*
Initial body-weight (g)	44·0	7·6	(8)	45·8	4·9	(8)
Final body-weight (g)	116·5*	10·2	(8)	91·6†	10·7	(8)
Carcase lipid content (g)	26·1*	5·4	(7)	22·3*	4·5	(7)
Carcase protein content (g)	14·7	5·4	(7)	12·8*†	1·4	(7)
Food intake from 21–34 d (g)	240·5*	30·5	(8)	176·1*†	21·0	(8)
Plasma insulin (ng/ml)	6·51*	2·7	(5)	5·23*	5·0	(7)
Plasma corticosterone (μg/100 ml)	7·8*	5·7	(5)	26·3*†	14·0	(7)

*Significantly different (at least $P<0·05$) from lean phenotype at the same ambient temperature.
†Significantly different (at least $P<0·05$) from obese phenotype at 22°.

At thermoneutrality both phenotypes ate less than they did at 22°, but fatty rats were still hyperphagic compared with lean rats. The carcases of fatty rats had higher lipid contents at both temperatures, but only at thermoneutrality was a difference in protein content evident. The voluntary reduction in food intake of female fatty rats maintained at thermoneutrality was accompanied by increased plasma glucocorticoid concentrations, unaltered plasma insulin levels and reduced protein deposition compared with the same phenotype at 22°.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Milk, lactoferrin and heavy-metal absorption. By J. QUARTERMAN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Milk feeding is known to increase the absorption of a number of heavy metals by rats (Quarterman & Morrison, 1981) and human milk is believed to be more effective in this respect than cows' milk. Hambidge *et al.* (1979) found that breast-fed infants had higher plasma zinc concentrations than those given cows' milk. Cox *et al.* (1979) showed that the uptake of iron by human enterocytes from human lactoferrin was several-fold greater than from bovine lactoferrin, indicating species specificity. We wished to know if lactoferrin could stimulate in vivo the absorption of other metals.

Hooded Lister rats in groups of six and weighing 100–120 g were given a semi-purified diet (Williams & Mills, 1970) or cows' milk as their only food for 3 d. Some were given human lactoferrin, 0·4 mg in 0·2 ml saline (9 g sodium chloride/l) by mouth twice a day for 3 d in addition to the milk or the semi-purified diet. At the end of this time each rat was given by mouth approximately 100 kBq ^{59}Fe, ^{65}Zn or ^{203}Pb. 2 h later the rats were killed and activity measured in blood, liver, kidney and gut-free carcass.

Activity of isotope in blood (disintegrations/min $\times 10^{-3}$ *per ml) or in gut-free carcass (disintegrations/min* $\times 10^{-3}$*)*

	Semi-purified diet		Semi-purified diet + lactoferrin		Cows' milk		Cows' milk lactoferrin	
Treatment group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
^{59}Fe Blood	20	2	42	4*	—	—	—	—
Carcass	19	2	29	2*	—	—	—	—
^{203}Pb Blood	1·2	0·2	4·7	1·0*	5·0	0·6*	—	—
Carcass	2·9	0·6	5·1	0·4*	4·7	0·4*	—	—
^{65}Zn Blood	—	—	—	—	52	10	81	11
Carcass	—	—	—	—	47	6	83	7*

*Significantly different from treatment without lactoferrin ($P<0·05$).

Lactoferrin administration increased the absorption of Fe, Zn and Pb and may thus be responsible for the stimulating effect of milk on heavy metal absorption. Human milk contains much higher concentrations of lactoferrin than cows' milk and this may account for the superior absorption of Zn from human milk reported by Hambidge *et al.* (1979) and others.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Biliary copper secretion in cattle. By M. PHILLIPPO and D. S. GRACA (Introduced by I. BREMNER), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

A negative exponential relationship exists between the rate of loss of liver copper and liver Cu concentration (McDonald *et al.* 1979). Little information, however, exists on the mechanism of liver Cu depletion and the present work was undertaken to determine whether changes in biliary Cu excretion accounted for this relationship.

Four animals weighing between 80 and 120 kg live weight were surgically prepared according to the method of Symonds *et al.* (1982) so that bile could be collected quantitatively. At least 1 month elapsed after surgery before any measurements were made. The animals were maintained for 3–4 months on barley–straw diets containing either 4 (basal) or 12 mg Cu/kg dry matter (DM). The total biliary excretion of Cu was measured over an 8 h period, both 4 d before and 3 d after obtaining a liver sample by biopsy. Cu was estimated by atomic absorption spectrometry on samples of bile directly and on liver after digestion with HNO_3:$HClO_4$:H_2SO_4 (4:1·0:0·5).

The animals weighed between 140 and 350 kg during the course of the experiments. Initially the mean (± SE) liver Cu concentration was 367±56 (range 222–500) mg Cu/kg DM and decreased to 40–247 mg Cu/kg DM at the end of the study. The total loss of Cu in bile appeared to be determined by the liver Cu concentration and was not significantly affected by different dietary Cu concentrations. The total daily biliary excretion of Cu (y mg/d) was related to the liver Cu concentration (x mg/kg DM) by the equation:

$$y = 0{\cdot}164 + 0{\cdot}0035x \ (\pm 0{\cdot}00038) \quad r^2\ 0{\cdot}88 \quad (n\ 24)$$

The daily loss of Cu from the liver was calculated using the equation of McDonald *et al.* (1979) for estimating liver DM, and the liver Cu concentration. The amount of Cu excreted in bile accounted for 39·9±2·1% (n 6) of the total Cu lost from the liver over the range of liver concentrations studied.

These results indicate that biliary Cu excretion accounts for less than half of the total Cu lost from the liver. Endogenous loss of Cu would seem to depend on the Cu status of the animal and to be more variable than suggested by the Agricultural Research Council (1980). The other processes whereby Cu is lost from the liver and from other body tissues during depletion remain to be identified.

D. S. Graca acknowledges the support of C.N.Pq. Brasil.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The retention of ^{59}Fe and ^{65}Zn in lambs given milk substitute diets based on either casein or soya-bean protein. By R. HILL, *The Royal Veterinary College, Boltons Park, Potters Bar, Herts.* and D. M. WALKER, *Department of Animal Husbandry, University of Sydney, Sydney, 2006, New South Wales, Australia*

Concentrations of trace elements, suitable for milk replacers containing casein or separated milk as the sole source of proteins, have been published (Roy, 1977). However, the growing practice of including soya-bean proteins in milk replacers may affect the availability of individual trace elements. This proposition has been tested with the elements iron and zinc in the experiment described below.

Eight lambs, 2–3 d of age, were placed in separate metabolism cages and bottle fed twice daily on milk substitutes based on either casein or a soya-bean protein concentrate (Gibney & Walker, 1978). After a brief period of acclimatization, four lambs were given the casein diet and four the soya-bean protein diet for 10 d. During a second 10 d period the diets were reversed. The lambs grew well on both diets.

On the first morning of each 10 d period, 10 ml of an aqueous solution containing 10 $\mu Ci^{59}Fe$ as ferric chloride and 10 ml of a similar solution containing 10 $\mu Ci^{65}Zn$ as zinc chloride were added to the milk substitute given to each lamb. Faeces and urine were collected daily and determinations of ^{59}Fe and ^{65}Zn were made on these separately. In fresh urine, radioactivity was too low for measurement but from determinations on the ash of 100 ml urine samples, it was established that the proportion of each element in the urine was around 0·2–0·3% of the dose.

Six lambs completed both experimental periods in good health. Mean (± SE) retention of ^{59}Fe in lambs given the casein diet was 50±11·1% and in those given the soya-bean protein diet 6±5·0%. There were large variations among animals but the treatment effect was significant at $P<0·02$. Retention of ^{65}Zn was significantly greater than of ^{59}Fe for all lambs but the effect of dietary treatment was similar to that for iron. ^{65}Zn retention values for casein and soya-bean protein diets were 84±6·4% and 52±3·4% respectively, and the difference was significant at $P<0·01$.

The possible importance of phytate in reducing the availability of Fe and Zn has been discussed (Young & Janghorbani, 1981) and the phytate content of the soya-bean protein diet used in this experiment was more than sufficient to bind all the Fe and Zn present in that diet. However, phytate is not the only metal binding agent present in plant products (Walker & Welch, 1982).

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Glucagon-induced lipolysis and plasma Mg levels in normal and hyperthyroid sheep. By S. C. BOLTON, T. E. C. WEEKES, P. M. M. GODDEN and D. G. ARMSTRONG, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Martens & Rayssiguier (1976) proposed that increased lipolysis may render a ruminant animal susceptible to hypomagnesaemic tetany, either by causing a movement of magnesium from extracellular fluid to intracellular space, or by increased chelation of Mg by plasma free fatty acids (FFA). Aikawa & Reardon (1965) showed that thyroxine accelerates the uptake of ^{28}Mg by rabbit tissues; moreover, Keynes & Care (1967) observed hypermagnesaemia in thyroidectomized sheep. In the present study, the relationship between glucagon-induced lipolysis and plasma Mg levels has been examined in normal and hyperthyroid sheep.

Four mature wether sheep fed twice daily on dried grass pellets (1 kg fresh weight/d) were subjected to two consecutive, 4 week, test periods. During the control period, each animal received a daily subcutaneous injection of 1 ml saline (9 g sodium chloride/l). During the thyroxine treatment period, the daily subcutaneous injection contained 1 mg thyroxine dissolved in 0·1M-NaOH and diluted in 1 ml saline. This treatment caused a significant ($P<0·01$) rise in plasma total thyroxine levels (mean ± SEM) from 57±2·0 μg/ml (control) to 178±14·0 μg/ml (hyperthyroid). During the last 2 weeks of each period animals received a single intravenous injection of glucagon (5·0 μg/kg live weight) and blood samples were taken between 30 min before and 3 h after glucagon injection. Jugular catheters were used for injection and sampling. Food was withheld from the animals from 12 h prior to the glucagon injection until the end of the sampling period.

Glucagon injection into animals during the control period caused a significant rise in plasma FFA ($P<0·05$), but had no effect on plasma Mg. During the hyperthyroid period, pre-glucagon plasma FFA were significantly higher ($P<0·05$) and plasma Mg significantly lower ($P<0·01$) than during the control period. Injection of glucagon during the hyperthyroid period resulted in a further rise in plasma FFA, the magnitude of which was not significantly different from that induced by glucagon during the control period; however, there was a further significant fall in plasma Mg ($P<0·001$).

These results would tend to suggest that increased hipolysis itself does not directly cause a fall in plasma Mg.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The chemical composition of mixed bacteria separated from different phases of rumen digesta. By R. J. MERRY and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Methods of estimating microbial-N entering the duodenum of ruminants are largely based on marker ratio techniques. Mixed bacteria used for establishing marker:N values have generally been separated from the liquid phase of rumen digesta and it has been assumed that these values are representative of the total bacterial population. The validity of this assumption was investigated.

Four steers with rumen cannulas were given diets of equal parts of barley and straw, supplemented with urea to contain 30 g N/kg dry matter (DM). Samples of whole rumen digesta were taken before the morning feed, strained and mixed bacteria were separated from the strained liquor by differential centrifugation (liquid phase bacteria (LPB)). The fibrous residue left after straining was resuspended in saline, homogenized and subjected to mechanical vibration. The treated material was strained and washed twice with saline. The strained liquors were combined and mixed bacteria were separated as before (solid phase bacteria (SPB)). Two of the steers had been infused with ^{35}S-labelled sodium sulphate for 10 d prior to sampling. Values for some chemical components of the bacteria are shown in the Table.

	LPB		SPB	
	Mean	SE	Mean	SE
Lipid (g/kg DM)	124·4	8·2	244·5**	8·7
Ash (g/kg DM)	157·1	20·2	86·6†	2·4
Total-N (g/kg DM)	80·3	2·6	70·1*	2·8
RNA-N:total N	0·093	0·002	0·075*	0·005
DAP-N:total N	0·000698	0·00067	0·00469*	0·00032
^{35}S (dpm/mg N × 10^4)‡	2·11	0·03	2·04	0·03

†$P \leqslant 0{\cdot}05$, $^{*}P \leqslant 0{\cdot}02$, $^{**}P \leqslant 0{\cdot}01$.
DAP, α,ε-diamino pimelic acid.
‡Values for two steers only. dpm, Disintegrations/min.

Significant differences were found between total N, ash and lipid contents of SPB and LPB. RNA-N:total N and DAP (α,ε-diamino pimelic acid)-N:total N values were both significantly lower in SPB compared with LPB. Values for ^{35}S:total N were only obtained for two animals and no significant differences were observed. These results suggest that under the conditions studied, amounts of microbial-N calculated to enter the duodenum based on RNA-N:total N or DAP-N:total N values in LPB would tend to be underestimates to varying degrees, depending upon the relative contribution of bacteria from each phase of the total digesta leaving the rumen.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Effect of dietary nitrogen supplementation on fibre digestion in the rumen.

By A. B. McAllan and R. H. Smith, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The amount of 'rumen degradable nitrogen' that just leads to maximum microbial protein production between mouth and duodenum is commonly regarded as indicating a supply that will be adequate to support all aspects of rumen microbial activity. This is tacitly assumed to be so in the N rationing scheme put forward by the Agricultural Research Council (1980).

The idea was tested in an experiment in which six Friesian steers, equipped with rumen and abomasal cannulas, were given isoenergic diets of approximately equal parts of concentrates (flaked maize + tapioca) and barley straw with no N supplement (BS) or with supplements of urea (BSU) or fish meal (BSF) or similar diets in which the straw had been treated with sodium hydroxide (BSA, BSAU and BSAF respectively). Degradable N:metabolizable energy values (g/MJ) were estimated to be about 0·9 for the basal and 1·4 for the N supplemented diets and organic matter (OM) intakes were 2·67, 2·42, 2·54, 2·27, 2·39 and 2·42 kg/d for BS, BSU, BSF, BSA, BSAU and BSAF respectively. ^{103}Ru-phenanthroline and polyethylene glycol were included as flow markers and estimates of microbial protein flows at the abomasum were made with RNA as a microbial marker. Results for which intakes and flows of cellulose and hemicellulose + pectin (HC/P) were assumed to be given by β-linked glucose and xylose + arabinose + galactose + mannose respectively, were as follows:

Diet . . .	BS	BSU	BSF	BSA	BSAU	BSAF
Proportion of OM intake truly digested in the rumen						
In total	0·54	0·56	0·56	0·63	0·65	0·61
As cellulose	0·057	0·063	0·103	0·069	0·082	0·125
As HC/P	0·033	0·043	0·055	0·059	0·074	0·091
Efficiency of microbial-N synthesis (g/kg OM truly digested in the rumen)	14·3	21·6	22·0	11·5	17·9	18·8

For the basal diets, cellulose and HC/P digestibilities were improved by replacing untreated with alkali treated straw. Supplementing either basal diet with either N source significantly improved (generally $P<0·05$) the efficiency of microbial protein synthesis but significant improvements (generally $P<0·05$) in digestibilities of cellulose and HC/P were achieved only with fish meal supplementation.

It is suggested that a supply of N that optimizes the flow of microbial protein to the duodenum does not necessarily optimize fibre digestion. This may explain, in part, marked differences reported in the literature for optimal rumen ammonia concentrations (Satter & Slyter, 1974; Mehrez *et al.* 1977).

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The effect of portal vein infusion of lysine on the intake of high and low protein diets by the chicken. By AUDREY A. RUSBY and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Shurlock & Forbes (1981) have shown that the liver is sensitive to the concentration of glucose in the hepatic portal vein. The following experiments were carried out to investigate the role of the liver in protein intake responses to L-lysine.

Four cockerels with hepatic portal vein (HPV) catheters (Rusby, 1982) were given a choice of high protein (295 g crude protein/kg dry matter (DM); HP) and low protein (60 g crude protein/kg DM; LP) diets. On two separate occasions each of the following were infused at a rate of 1 ml/h for 3 h: (i) isotonic saline and phosphate buffer (control), (ii) 50 g lysine, (iii) 100 g lysine, (iv) 150 g lysine/l saline and phosphate buffer.

The results showed that there was little effect on the intake when the birds had continuous access to food. When they were fasted overnight (Expt 2) and offered food at the start of the infusion, however, intake of both foods was depressed in a dose-related manner.

To see whether this effect was local to the liver, three starved birds with jugular vein (JV) and HPV catheters received the following treatments: (i) control into both catheters (control), (ii) control into JV, 100 g lysine into HPV, (iii) 100 g lysine into JV, control into HPV (Expt 3).

	Feed intakes (g)					
Time from start of infusion . . .	0–3 h		0–6 h		0–24 h	
Diet . . .	HP	LP	HP	LP	HP	LP
Expt 2 (*n* 8)						
Control	30·8	42·5	35·5	54·0	58·6	77·6
50 g Lysine HPV	24·8	34·1	29·5	38·3	52·6	61·5
100 g Lysine HPV	11·0	24·6	13·0	29·6	36·4	50·0
150 g Lysine HPV	13·3	22·0	13·8	22·8	35·3	61·3
SEM	11·0	11·0	10·4	10·4	14·0	19·0
Expt 3 (*n* 6)						
Control	12·1	32·0	18·2	46·8	32·7	66·0
100 g Lysine HPV	11·8	19·1	13·5	22·0	26·2	43·4
100 g Lysine JV	14·0	29·6	17·5	38·8	33·0	59·8
SEM	4·5	8·5	5·7	8·8	9·7	10·8

Lysine infused into the HPV significantly depressed the total intake of both feeds whereas when infused into the JV it had no effect. The uptake of lysine by the liver appears to be involved in the control of feed intake in the chicken.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Fasting heat production of broilers selected for increased growth rate, food consumption or conversion of food to gain. By D. J. FARRELL, *Department of Biochemistry and Nutrition, University of New England, Armidale, NSW, Australia* and R. A. E. PYM, *Poultry Research and Advisory Station, Seven Hills, NSW, Australia*

Changes in the heat production (HP) of animals may help to explain observed changes in biological performance as a consequence of genetic selection. In this study calorimetric measurements were made on eleven to thirteen groups each comprising three 6-week-old male chickens taken from the tenth generation of three lines selected during days 35–63 for increases in live-weight gain (W), food consumption (F) or improved food conversion efficiency (E). An unselected control line (C) was also measured (see Pym & Farrell, 1977). Three birds from a line were placed in a closed-circuit indirect respiration chamber at 22° (Farrell, 1972; Farrell & Swain, 1977) without food for 24 h. Measurements were made for the next 22–24 h. Two lines were tested concurrently in two chambers and each line was tested a total of eleven to thirteen times.

Broilers selected for food consumption had a higher ($P<0.05$) HP (kJ/kg W per d) than other lines, while HP was lower in the weight gain and food efficiency lines ($P<0.05$) than in the controls (see Table). These results differ slightly from earlier findings (Pym & Farrell, 1977) for the same lines in their third generation when F line birds also had the highest fasting HP, but values for lines E, C and W did not differ.

				Fasting heat production	
Line	*n*	Weight (kg)	Respiratory quotient	(kJ/d)	(kJ/kg W per d)
W	13	2.35^{a}_{ab}	0.741^{a}_{b}	1134^{a}_{b}	483^{a}_{b}
F	11	2.22_{b}	0.731_{ab}	1255_{c}	568^{a}
E	13	2.13_{c}	0.735_{a}	1030_{c}	484_{c}
C	12	1.96^{c}	0.741^{a}	993^{c}	509^{a}
Least significant difference$_{0.05}$		0.16	0.006	71	23

Values within columns with the same superscripts are not significantly different ($P>0.05$).

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

A comparison of the heat production of heat-induced dwarf and normal chicks. By D. J. FARRELL* and N. X. TINH†, **Department of Biochemistry and Nutrition* and *†Department of Animal Science, University of New England, Armidale, NSW, Australia*

Dwarfism can be induced in chicks by increasing temperature from 37·5 to 41·7° during the middle stage of incubation (Tinh *et al.* 1977). Fasting heat production (FHP) of groups of dwarf chicks and groups of normal-sized chicks (controls) was measured in four respiration chambers (Farrell, 1972) in a thermoneutral environment for 24 h in two series of calorimetric experiments during the first few weeks of post-hatching life.

Up to 25 d of age the weight of the heat-treated chicks was always less than the controls. In Expt 1, using unsexed cross-bred chicks, there was no difference ($P<0.05$) in FHP between the two groups at any age to 23 d. Results of Expt 2 on unsexed broiler chicks are given in the Table. Values are the mean of two replicates at each age.

Age (d) . . .	2		11		25	
Chamber temperature (°) . . .	33		30		27	
	Controls	Treated	Controls	Treated	Controls	Treated
Body-weight (g)	412	403	978	818	1658	1389
No. of chicks	12	12	12	12	6	6
FHP (kJ/d)	266	327	767	742	1156	999
FHP (kJ/kg W per d)	646	811	784	907	697	719
Respiratory quotient	0·73	0·72	0·72	0·73	0·71	0·73

At 2 and 11 d the heat-treated chicks had higher ($P<0.05$) FHP when expressed as kJ/kg W per d or kJ/kg $W^{0.75}$ per d than the corresponding controls. In Expt 1, the similar FHP between the two groups may have been due to the slow-growing cross-bred chicks in which differences in body-weight were much less than in broiler chicks in Expt 2. These latter chicks were incubated from day 12 at 41·7° for 4 d rather than for 3 d as in Expt 1. Differences in FHP between the two groups at day 2 and day 11 are unlikely to be explained by the need for higher ambient temperature by the dwarf chicks to maintain thermoneutrality but stem from the increased temperature during incubation.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Effects of milling and diet form on the metabolizable energy of maize, barley and wheat for poultry at two ages. By D. J. FARRELL, *Department of Biochemistry and Nutrition, University of New England, Armidale, NSW, Australia* and J. R. ASHES and J. P. HOGAN, *CSIRO, Division of Animal Production, Blacktown, NSW, Australia*

Grinding of grain is necessary for adequate diet mixing, yet there is little information on the effects of particle size and diet presentation on the metabolizable energy (ME) of grains in poultry diets. Three grain-based diets containing 700 g/kg of either maize, barley or wheat that had been milled to three different moduli of fineness (MF) by hammer mill (fine) or Ripple-Flo Mill (medium and coarse) were fed as a mash or as pellets/crumbles to growing chickens (8–15 d) and to adult cockerels (1 year). The 108 determinations of ME using total collection of excreta over 4–5 d included three grains × three grists × two diet forms × two ages × three replicates. Results are given as MJ/kg dry matter in the Table.

		Chicks		Cockerels	
	MF	Mash	Crumbles	Mash	Pellets
Maize	2·56	$15{\cdot}32^{a}_{ab}$*	$16{\cdot}10^{b}_{b}$	$15{\cdot}27^{a}_{a}$	$14{\cdot}94^{a}_{a}$
Maize	2·73	$15{\cdot}50^{a}$	$15{\cdot}89_{b}$	$15{\cdot}13_{b}$	$15{\cdot}11^{a}$
Maize	3·10	$15{\cdot}06^{a}$	$15{\cdot}89$	$15{\cdot}55$	$14{\cdot}89^{a}$
Barley	2·39	$13{\cdot}11^{a}$	$13{\cdot}26^{a}$	$13{\cdot}00^{a}$	$13{\cdot}01^{a}$
Barley	2·65	$13{\cdot}04^{a}_{a}$	$13{\cdot}09_{bc}$	$13{\cdot}05_{ac}$	$13{\cdot}29^{a}_{a}$
Barley	3·11	$12{\cdot}98^{a}$	$13{\cdot}63$	$13{\cdot}26^{a}$	$13{\cdot}09$
Wheat	2·17	$13{\cdot}65^{a}_{a}$	$13{\cdot}83^{a}_{bc}$	$13{\cdot}60^{a}_{b}$	$13{\cdot}84^{a}_{c}$
Wheat	2·48	$13{\cdot}31^{a}_{a}$	$14{\cdot}16_{bc}$	$13{\cdot}77_{ab}$	$14{\cdot}37_{c}$
Wheat	2·65	$13{\cdot}35^{a}$	$14{\cdot}23$	$13{\cdot}78^{a}$	$14{\cdot}64$

*Values within a row with the same superscripts are not significantly ($P<0{\cdot}05$) different. Least significant difference$_{0.05}$ = 0·457.

These results indicate that ME values within grains may differ when given as mash or as pellets/crumbles to growing or adult birds; in addition, coarser grinds than currently used can be adopted without a significant fall in ME.

We thank Californian Pellet Mill (Australia) for financial support for this project.

https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

The effects of trenbolone acetate on plasma lipid concentrations in mature female sheep. By MARGO E. BARKER, J. R. SCAIFE and H. GALBRAITH, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD, Scotland*

Recent studies have shown that subcutaneous implantation of sheep with a combination of trenbolone acetate (TBA) and oestradiol-17β can alter the concentrations of plasma cholesterol and triglycerides (Scaife, *et al.* 1982) and is associated with changes in the in vitro activity of certain lipolytic and lipogenic enzymes (Burch *et al.* 1982).

In a preliminary investigation, blood samples were collected immediately before implantation and at weekly intervals thereafter until slaughter from thirty-two Scottish Blackface ewes treated with TBA as described by Sulieman *et al.* (1982). Those in group C were sham implanted, those in groups T_1, T_2 and T_3 received 20, 40 and 60 mg TBA respectively.

Plasma cholesterol (PC) concentrations fluctuated considerably in the control group throughout the experiment. In groups T_2 and T_3 there was a marked decrease in PC concentrations to minimum values of 14·2 and 18·8 mg/100 ml respectively at week 4. These values were significantly ($P<0·05$) different from those of groups C and T_1. From week 4 onwards the PC levels in groups T_2 and T_3 rose and were not significantly different from those of the other groups at weeks 6 and 8.

Plasma triglyceride (PT) concentrations in groups C and T_2 were significantly different from those of groups T_1 and T_3 at week 0. At weeks 2, 4, 6 and 8 all implanted animals had significantly lower PT concentrations than the controls and at week 8 PT levels in groups T_2 and T_3 were 0·6 and 1·1 mg/100 ml while those in groups C and T_1 were 21·0 and 15·3 mg/100 ml respectively.

Plasma phospholipid (PP) concentrations in all four treatment groups showed an overall decrease between weeks 0 and 8, but the decrease was more marked in the implanted than in the control animals. At weeks 4, 6 and 8 groups T_1, T_2 and T_3 had significantly ($P<0·05$) lower PP concentrations than the control group. There were no significant differences between groups of implanted animals.

Plasma total lipid (PTL) concentrations decreased in all treatment groups during the experimental period. Groups T_1 and T_2 differed significantly ($P<0·05$) from group C at week 0 (246 and 252 *v.* 305 mg/100 ml). Between weeks 2 and 6 PTL levels in group T_2 fell to the lowest value of 76·2 mg/100 ml and then rose slightly at week 8. At week 8 groups T_1 and T_2 had significantly ($P<0·05$) lower PTL concentrations than the control group (142 and 116 *v.* 166 mg/100 ml).

Our results suggest that TBA can significantly influence the concentration of plasma lipids in sheep. These changes may reflect alterations in partitioning of energy between protein and lipid deposition.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Response of mature female sheep to trenbolone acetate. By A. H. SULIEMAN, H. GALBRAITH and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD, Scotland*

Trenbolone acetate (TBA) has been shown to improve growth in female rats and cattle (Galbraith & Topps, 1981). The work reported here studied the response of mature female sheep to this steroid.

Thirty-two ewes, weighing approximately 45 kg, were allocated to be sham-implanted controls (group C) or to be subcutaneously implanted with 20, 40 or 60 mg TBA (groups T_1, T_2 and T_3 respectively) 60 d before slaughter. They were fed to appetite a diet containing 11 MJ metabolizable energy and 0·125 kg crude protein/kg dry matter (DM). The left-hand side of the chilled carcass (CW) was dissected into components including lean tissue (LT) and intermuscular fat (IF).

The results, which are mean values for each group, were compared as follows: contrast (A) = group C *v.* mean of groups $T_1 + T_2 + T_3$, contrast (B) = T_1 *v.* mean of groups $T_2 + T_3$, contrast (C) = T_2 *v.* T_3 (see Table).

	Treatment group					Standard error of difference	Contrast		
Weight (kg)	C	T†	T_1	T_2	T_3		A	B	C
Live-weight gain	8·63	9·60	8·55	11·17	8·88	1·56	—	—	—
Dry matter intake	94·10	98·10	94·80	101·40	98·10	4·21	—	—	—
Gut fill	10·11	9·00	8·65	9·78	8·41	0·61	•	—	•
Carcass weight	22·06	23·40	22·70	24·07	23·37	0·54	••	•	—
Lean tissue	5·60	6·05	5·82	6·21	6·13	0·16	••	•	—
Intermuscular fat	1·34	1·54	1·46	1·60	1·56	0·09	•	—	—
Gut + caul fat	0·76	1·10	0·97	1·13	1·21	0·12	••	—	—
Kidney + channel fat	0·73	1·00	0·97	0·99	1·04	0·11	••	—	—

•$P<0·05$, ••$P<0·01$.
†T, mean of groups $T_1 + T_2 + T_3$.

The hormonally treated ewes had, on average, a greater live-weight gain and DM intake than the controls. Implantation of TBA significantly reduced the gut fill but significantly increased the mean weights of CW, LT, IF, gut plus caul fat and kidney plus channel fat. Differences between the dose levels of TBA were significant ($P<0·05$) for CW and LT ($T_1 < T_2 + T_3$) and for gut fill ($T_2 > T_3$). The ewes in group T_2, had the greatest weight gain and carcass weight.

The results suggest that TBA increased the deposition of carcass lean tissue and internal depot fat in female sheep.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Steroid concentrations in sheep implanted with trenbolone acetate. By LESLEY J. MACVINISH, H. GALBRAITH and J. M. CHESWORTH, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD, Scotland*

An important consideration in the use of anabolic steroids in meat-producing animals is the residual concentration of these compounds in blood and tissues after slaughter. Concentrations of exogenous trenbolone acetate (TBA) plus its major metabolite trenbolone hydroxide (TBOH) and of endogenous oestradiol-17β (OE_2) in twenty-eight mature female sheep have been investigated. These animals were either sham-implanted controls (group C) or implanted with 20, 40 or 60 mg TBA (groups T_1, T_2 and T_3 respectively) as described by Sulieman *et al.* (1982). Steroid concentrations were measured by radioimmunoassay following solvent-extraction. Concentrations in blood of TBOH increased with increasing dose of TBA and peaked at weeks 1 to 2 and declined thereafter (Fig. 1). A similar pattern was observed for endogenous OE_2. Maximum mean concentrations of OE_2 for groups T_1, T_2 and T_3, which were observed at week 1, were 21·1, 26·5, 33·0 ng/l respectively compared with 8·3 ng/l for group C. These concentrations declined to 7·1, 10·6, 17·9 ng/l respectively and to 5·2 ng/l for group C at slaughter.

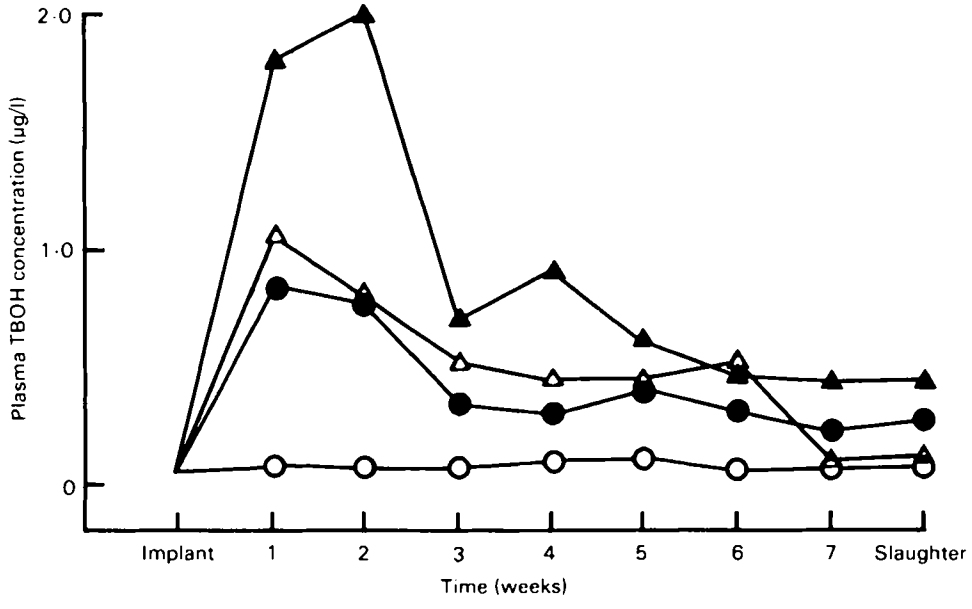

Fig. 1. Mean plasma trenbolone concentrations in sheep. Group C, ○; T_1, ●; T_2, △; T_3, ▲.

Significant ($P<0·01$) increases in the concentration of residual TBA + TBOH in samples of shoulder muscle taken after slaughter, were obtained in treated animals compared with those in controls: mean values (± SEM), in ng/kg, for groups C, T_1, T_2 and T_3 respectively were 16·6 (7·8), 78·4 (13·6), 63·2 (13·0), 181 (46).

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Protein turnover in sheep treated with trenbolone acetate and zeranol.

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Treatment with trenbolone acetate (TBA) plus oestradiol (OE), or zeranol (Z) has been reported to increase weight gain, carcass weight and protein deposition in wether lambs (Maund, 1976; Coelho *et al.* 1981). In rats TBA treatment decreases protein synthesis and protein degradation in muscle (Vernon & Buttery, 1978).

Muscle protein fractional synthetic rate (measured by constant infusion of L-[4,5-^{3}H]-leucine) and cathepsin D activity (Barrett, 1972) were determined in entire female lambs implanted with TBA (80 mg Finapliz; Roussel-Uclaf, Paris) or Z (12 mg Ralgro; Crown Chemical Co. Ltd., Lamberhurst, Kent) 4 weeks before slaughter and unimplanted controls.

TBA and Z treatment significantly increased weight gain in these animals and improved food conversion efficiency ($P<0.025$). Skeletal muscle protein synthesis was significantly reduced at $P<0.1$ in TBA treated animals. Muscle 'free' cathepsin D activity was significantly reduced by treatment with TBA and Z but total cathepsin D activity was not altered (see Table).

(Results are expressed as means + SE; number of observations in parentheses)

	Control		Trenbolone acetate		Zeranol	
	Mean	SE	Mean	SE	Mean	SE
Initial weight (kg)	30·6	3·46 (5)	31·7	1·31 (6)	31·2	1·15 (6)
Final weight (kg)	36·4	4·09 (5)	42·4	1·25 (6)	40·6	1·50 (6)
Weight gain (kg)	5·8[a]	1·00 (5)	10·7	1·14 (6)	9·4	0·78 (6)
Food conversion efficiency (kg/kg body-weight gain)	6·1[b]	0·26 (5)	4·9	0·34 (6)	5·0	0·42 (5)
Muscle protein fractional synthetic rate/d	0·060[a]	0·011 (5)	0·039	0·003 (6)	0·041	0·006 (5)
Free cathepsin D activity (dpm per mg protein)	3·79[b]	0·22 (5)	2·06	0·56 (4)	2·00	0·54 (3)
Total cathepsin D activity (dpm per mg protein)	15·74	1·25 (6)	14·02	1·42 (5)	16·00	0·93 (6)

Statistical significance between treatments [a]$P<0.1$, [b]$P<0.025$.
dpm, Disintegrations/min.

Despite differences in TBA metabolism in rats and ruminants (Pottier *et al.* 1981), it appears that TBA has similar effects on muscle protein turnover.

We thank the ARC for financial support and Roussel-Uclaf and The Crown Chemical Co. Ltd., for their donations of Finaplix and Ralgro respectively.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press

Reduction of urea synthesis in growing heifers implanted with trenbolone acetate. By I. A. DONALDSON, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT* and R. J. HEITZMAN, *ARC Institute for Research on Animal Diseases, Compton, Newbury RG16 0NN*

As part of a study of the action of anabolic agents on nitrogen metabolism, three Friesian × Ayrshire heifers were implanted at 350 kg live weight with 300 mg trenbolone acetate (TBA, Finaplix; Hoechst, Hounslow, Middlesex) and a further three weight-matched controls were sham-implanted. The animals were given a concentrate/hay ration, adjusted weekly to live weight, which provided 9·4 g crude protein/kg $W^{0.75}$ per d and a calculated metabolizable energy intake of 0·915 MJ/kg $W^{0.75}$ per d. Average daily live weight gain (ADLG) over the 12-week experimental period was increased by TBA (0·99 *v.* 0·67 kg/d; $P<0.05$), feed conversion was improved (9·19 *v.* 13·5; $P<0.02$) and the molar ratio of urea:creatinine excreted in urine was reduced (11·5 *v.* 15·3; $P<0.05$). Urea metabolism was measured immediately prior to implantation and on days 3, 10, 38 thereafter by injecting a single dose of 250 μCi of [^{14}C]urea (Nolan & Leng, 1970) into animals fed frequently during that day and the preceding 4 d (Donaldson, 1977). The results shown in the Table are mean values for the three post-treatment times for each group.

	TBA	Control	SED*	Significance of difference
Nitrogen intake (g N/d)	120	121	4	NS
Plasma urea (mmol/l)	3·82	4·82	0·31	$P<0.05$
Urea pool (mol)	1·04	1·13	0·07	NS
Urea synthesis (mol/d)	2·74	3·24	0·09	$P<0.02$
Urea synthesis (mmol/kg $W^{0.75}$ per d)	32·7	38·4	1·6	$P<0.05$
Urea space (l)	261	239	20	NS

*Standard error of difference between means, 3 degrees of freedom.
NS, not significant.

Plasma urea was significantly lowered by TBA but neither urea pool size nor the volume of its distribution reached significance. Urea synthesis over the whole period to 38 d was significantly lowered by TBA and within time was first significant on day 10 (2·55 *v.* 3·39 mol/d; $P<0.01$) and the difference was sustained beyond day 38. [^{14}C]urea traces the sum of *de novo* and recycled urea production but while recycling may have been reduced in consequence of the lower plasma urea concentration this response would not be anabolic and it is suggested that mainly *de novo* urea synthesis was reduced by TBA. On this assumption, the overall treatment difference of 0·5 mol/d urea synthesis can be calculated (Nolan & Leng, 1970) to equate with the sparing of 86 g/d of protein from oxidation, and specifically on day 10 the sparing of 143 g/d protein. This represents 430–715 g/d as lean tissue (protein content 20%). This rate of accretion was more than adequate to explain the increment in ADLG.

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https://doi.org/10.1079/PNS19830013 Published online by Cambridge University Press