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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Thirtieth Meeting of the Nutrition Society was held in the Curtis Auditorium, University of Newcastle upon Tyne on Tuesday and Wednesday, 16/17 *September* 1986, *when the following papers were read:*

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Dose-related effects on hay intake of acetate infused into the rumen of lactating cows. By M. H. ANIL, J. N. MBANYA, H. W. SYMONDS and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

The end-products of fermentation in the ruminant stomach are potential feedback signals in the control of food intake as well as being major energy sources (Baile & Forbes, 1974). Intraruminal infusion of acetate depresses intake in cattle (Bhattacharya & Warner, 1968; Montgomery *et al.* 1963) but these experiments were carried out on dry cows and no dose-response relation was demonstrated. It would be anticipated that greater amounts of acetate would be required to depress intake in lactating cows, where acetate is removed more rapidly from the rumen.

Seven rumen-fistulated lactating cows were used in an experiment of Latin-square design. They were given free access to ryegrass hay; in addition, compound food was given three times a day in amounts which depended on the stage of lactation. Treatments involved 3-h intraruminal infusions of 4·5 litres water containing 0–1500 g sodium acetate. Voluntary intake of hay was monitored and rumen fluid samples were taken every 30 min for pH measurement and volatile fatty acid analysis.

The Table shows the weight of hay eaten during the infusion and for the subsequent 2 h, when acetate concentrations were still raised.

	Wt of sodium acetate infused (g/3 h)						
	0	250	500	750	1000	1250	1500
Hay intake (g/5 h)	3516	3640	3475	3298	3001	2476*	2605*
SE	254	240	408	316	170	311	227

*Significantly different from 0 g treatment ($P<0·05$).

Rumen pH was not affected by the infusions. Rumen acetate concentrations rose from 53·7 mmol/l to a maximum of 178 mmol/l, which was significantly correlated with the weight of acetate infused (r 0·60); the weight eaten during infusion and in the subsequent 2 h was also correlated with the weight of acetate infused (r −0·50).

Although large amounts of acetate were required to depress intake, under physiological conditions acetate production is coupled with other satiating signals such as propionate, distension and heat, so that the lower amounts normally produced probably contribute to satiety.

This work was supported by the AFRC.

Baile, C. A. & Forbes, J. M. (1974). *Physiological Reviews* **54**, 160–214.
Bhattacharya, A. N. & Warner, R. G. (1968). *Journal of Dairy Science* **51**, 1091–1094.
Montgomery, M. J., Schultz, L. H. & Baumgardt, B. R. (1963). *Journal of Dairy Science* **46**, 1380–1383.

Influence of different levels of dietary energy on the performance of cows suckling two calves. By M. A. S. KHAN, J. H. TOPPS and P. J. BROADBENT, *School of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD*

Twin calves can be produced by embryo transfer and other methods of twin-calf production may become feasible. However, there is little objective information on the degree of undernutrition which cows suckling double and twin calves can sustain in early lactation. Khan *et al.* (1986) observed that cows can suckle double calves when given 90 to 100 MJ metabolizable energy (ME)/d but there may be a risk of ketosis when low-quality forages are given. A subsequent experiment has examined three levels of energy using nine Hereford × Friesian cows suckling double calves and nine cows suckling natural twin calves. The cows, in their second lactation, were on experiment for 21 weeks starting from the 22nd day of lactation. The diets consisted of grass silage, barley straw, barley and white fishmeal. The three diets supplied 80, 90 or 100% (105, 115 and 125 MJ ME/d) of energy requirements according to the Ministry of Agriculture, Fisheries and Food (1984). The calves received hay and a concentrate mixture. Animals were weighed, milk consumption estimated and blood samples taken fortnightly; intake of hay and concentrates were measured daily.

Table 1. *Performance of cows suckling double and twin calves and growth rate of calves*

				SED	Significance
Energy supplied (% of requirements)	80	90	100		
Energy intake (MJ ME/d)	105	115	125		
Cows:					
Live weight loss (kg/d)	0·10	0·06	0·11	0·09	NS
Milk yield (kg/d)	13·2	13·5	14·3	1·10	NS
Plasma free fatty acids (μmol/l)	356	325	256	55·7	NS
Calves:					
Growth rate (kg/d)	0·90	0·92	0·98	0·04	NS

NS, not significant.

Concentrate intakes (kg/d per calf) were 1·31 for double-suckled calves and 0·94 ($P<0·05$) for twin calves. This difference was probably due to premature birth, hence, the smaller size of the twin-born calves. Otherwise there was no difference in the performance of cows suckling double calves and their calves when compared with those suckling twins and their calves. All comparisons between treatments for both cows and calves were not significant. Since the underfed cows did not lose more weight it is probable that body fat was depleted, and protein and water deposited in the body differentially between the treatments.

Khan, M. A. S., Topps, J. H. & Broadbent, P. J. (1986). *Proceedings of the British Society of Animal Production*, paper no. 106.

Ministry of Agriculture, Fisheries and Food (1984). *Technical Bulletin no. 433*. London: H.M. Stationery Office.

Acute changes in mammary gland blood flow, lipogenesis and circulating insulin levels during refeeding of starved lactating rats. By S. W. MERCER and D. H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

Refeeding rapidly reactivates lipogenesis in the mammary gland of starved lactating rats and this is prevented by acute insulin deficiency (Robinson *et al.* 1978). However, the time-course of changes in circulating insulin levels during refeeding is not known. The aim of the present study was to investigate the temporal relation between plasma insulin concentration and lipogenesis in the mammary gland of lactating rats during the starved–refed transition.

Rats at 11–14 d of lactation were fasted overnight (18 h) and refed 5 g of chow diet, which they consumed within 20 min. Immediately before and during refeeding, blood samples were taken from conscious animals from the carotid artery which had been cannulated 2 d earlier. Plasma insulin concentrations rose rapidly on refeeding and by 20 min were some twenty-fold higher than the basal, starved level. However, between 20 and 30 min there was a sharp decline in insulin concentration, which remained at near basal values for the remainder of the 90 min experimental period (mean plasma insulin concentration (mU/l): 20 min, 98 (SE 16); 30 min, 26 (SE 6); $P<0.005$). A significant increase in plasma glucose concentration was also observed on refeeding.

Lipogenic rate in the mammary gland (assessed in vivo with ^3H_2O), measured from 30–90 min after the onset of refeeding, was elevated twenty-fold compared with the starved value. Thus following the transient hyperinsulinaemia induced by refeeding, there was a surge in lipogenic activity in the gland. Measurement of relative blood flow over the same time period, using [^{14}C]dichloro-diphenyl-trichloroethane (Herd *et al.* 1968; Rofe & Williamson, 1983), revealed only a minor increase to the gland during refeeding. A time-course of lipogenesis indicated little reactivation in the gland during the first 30 min of refeeding, followed by a fifty-fold increase above the starved level over the 30–60 min refeeding period. Thus the lipogenic response of the mammary gland cannot be temporarily correlated with circulating insulin concentration nor with the rate of hormone delivery to the gland; this suggests that the sensitivity of the tissue to insulin acutely increases during this period of refeeding.

These results imply that the control of lipogenesis in the mammary gland is acutely tuned to the availability of carbohydrate substrate, as suggested previously (Mercer & Williamson, 1986).

Herd, J. A., Goodman, H. M. & Grose, S. A. (1968). *American Journal of Physiology* **214**, 263–268.

Mercer, S. W. & Williamson, D. H. (1986). *Proceedings of the Nutrition Society* **45**, 116A.

Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978). *Biochemical Journal* **176**, 343–346.

Rofe, A. M. & Williamson, D. H. (1983). *Biochemical Journal* **212**, 899–902.

Food intake and carcass composition of chickens given glucose in the drinking water. By E. A. ENGKU AZAHAN and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

A solution of glucose (100 g/l) is perceived by chickens to be a drink rather than a food (Shaobi & Forbes, 1985) and does not depress food intake (Injidi, 1981). The present experiment examined the fate of the extra energy intake.

Twelve cockerels of an egg-laying strain (Thornber 404), aged 41 d, were allocated to three groups. One group was killed for the estimation of initial carcass composition, one group was offered food and tap water *ad lib.*, while the third was offered food and a solution of glucose (90 g/l) *ad lib.* for 42 d. The birds were caged individually so that food and fluid intakes and faecal output could be monitored. During the last 3 weeks the oxygen consumption of each bird was measured for 23 h so that heat production could be estimated. At the end of the experiment the birds were killed for carcass analysis. The results are summarized in the Table.

	Control		Glucose	
	Mean	SEM	Mean	SEM
Food energy intake (MJ/42 d)	60·8	1·3	47·3*	2·7
Fluid energy intake (MJ/42 d)	0·0		10·2	0·4
Faecal + urine energy output (MJ/42 d)	18·7	0·9	15·1 NS	1·2
Estimate of heat production (MJ/42 d)	29·2	1·4	28·5 NS	1·7
Gain in carcass energy (MJ/42 d)	8·6	0·3	8·7 NS	0·9
Protein content of carcass (g/kg DM)	677	21	613 NS	24
Fat content of carcass (g/kg DM)	231	20	295*	14

DM, dry matter; NS, not significant; *$P<0.05$.

Food intake was depressed in those birds offered glucose solution so that total energy intake and energy stored in the carcass were unaffected. There was, however, a slight decrease in weight gain but a significant increase in body fat with the glucose treatment, suggesting that the reduced intake of protein had limited growth.

The results do not suggest that any increase in heat production occurred in response to the intake of glucose solution but we do not know why food was depressed in this experiment when it was not in the earlier work (Injidi, 1981).

Injidi, M. H. (1981). Involvement of melatonin thyroid hormones and glucose in the control of food intake and growth of chickens. PhD Thesis, University of Leeds.

Shaobi, T. S. & Forbes, J. M. (1985). *Proceedings of the Nutrition Society* **44**, 57A.

Food intake of intact and vagotomized chickens infused with lysine and glucose into the hepatic portal vein. By A. A. RUSBY and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Infusion of either glucose (Shurlock & Forbes, 1981) or lysine (Rusby & Forbes, 1983) into the hepatic portal vein of 21-h fasted cockerels has a marked, dose-dependent effect on food intake. The present experiment was designed to see whether the effects of the two are additive and whether they are attenuated by vagotomy.

Sixteen cockerels of an egg-laying strain, aged 15–16 weeks, were prepared under general anaesthetic with catheters into the hepatic portal vein. In eight birds, both vagus nerves were cut as they traverse the proventiculus. On separate occasions, after a 21-h fast, 3-h infusions were made at 10 ml/h into the portal vein of solutions of (i) saline (9 g sodium chloride/l), (ii) 300 mg lysine, (iii) 1260 mg glucose, (iv) 300 mg lysine plus 1260 mg glucose/3 h.

In the intact birds, intakes during the 1st hour of the infusion were significantly depressed by lysine (45·7 g) and glucose (44·9 g), compared with the saline control (57·3 g) (standard error of difference between two means 3·9). The combined infusion of lysine and glucose was significantly more effective (33·0 g) than either lysine or glucose alone. These effects persisted throughout the infusion period. In the vagotomized birds there was no significant effect of treatment, intakes being (i) 43·6, (ii) 38·6, (iii) 39·1 and (iv) 38·4 g (SED 7·2) for the four treatments.

These results show that there is an additive effect of hepatic infusions of lysine and glucose on food intake and that effects of both may be blocked by abdominal vagotomy, implicating the liver in the control of food intake in the bird.

Rusby, A. A. & Forbes, J. M. (1983). *Proceedings of the Nutrition Society* **42**, 51A.
Shurlock, T. G. H. & Forbes, J. M. (1981). *British Poultry Science* **22**, 323–331.

Energy and nitrogen metabolism in genetically fat and lean chickens. By P. A. GERAERT, *Station de Recherches Avicoles, INRA Nouzilly* 37380 *Monnaie, France* and M. G. MACLEOD, T. R. JEWITT and JULIE ANDERSON, *AFRC Institute of Grassland and Animal Production, Poultry Division, Roslin, Midlothian EH25 9PS*

In order to understand the mechanisms which control fattening in broilers, two lines of chickens were divergently selected on the basis of low (LL) or high (FL) abdominal fat content of males at 9 weeks of age (Leclercq *et al.* 1980).

Energy and protein utilizations were investigated in twelve male chickens of each line at 5 and 7 weeks of age and at two different ambient temperatures, 10° and 25°, using indirect calorimetry. Two birds from the same line were placed in each calorimeter chamber for a 6 d period. The results from 5-week-old birds kept at 25° are presented in the Table.

	LL		FL		Statistical significance
	Mean	SEM	Mean	SEM	
Body-wt (W; kg)	0·706	0·020	0·695	0·014	NS
ME intake (kJ/kg $W^{0·75}$ per d)	1484	36	1460	96	NS
Heat production (kJ/kg $W^{0·75}$ per d)	780	19	778	24	NS
Net availability of ME (%)	82·0	0·9	84·2	0·9	NS
ME maintenance (kJ/kg $W^{0·75}$ per d)	624	16	645	11	NS
Gross efficiency of energy retention (%)	47·4	0·3	46·1	20	NS
Energy retained as protein (kJ/kg $W^{0·75}$ per d)	375	9	332	22	$P<0·05$
Energy retained as fat (kJ/kg $W^{0·75}$ per d)	329	14	350	61	NS
Gross efficiency of protein retention (%)	48·8	1·4	42·0	1·6	$P<0·001$
Uric acid excreted (g/d)	1·27	0·03	1·88	0·11	$P<0·05$

ME, metabolizable energy; NS, not significant.

ME intake, heat production, net availability of ME, ME maintenance and gross efficiency of energy retention were similar in both lines. However, the partition of retained energy between fat and protein did differ with increased protein deposition and conversely decreased fat retention in LL chickens. Similar results were observed at 7 weeks old.

The differences in fat content between FL and LL birds seemed, therefore, to be mainly a consequence of changes in protein metabolism, with more amino acids being catabolized for energy in FL birds. This was confirmed by the increase in uric acid excretion.

Leclercq, B., Boyer, J. P. & Blum, J. C. (1980). *British Poultry Science* **21**, 107–113.

The anabolic effects of growth hormone and thyroxine on tissue growth in Snell dwarf mice. By P. C. BATES, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE* and A. T. HOLDER, *Institute of Child Health, 30 Guilford Street, London WC1 1EH*

The homozygote (*dw/dw*) Snell dwarf mouse lacks growth hormone (GH) and thyroid hormones and grows to only about one-third of the size of its heterozygote normal littermates. We have investigated the consequences of these hormone deficiencies on tissue protein metabolism in full-grown dwarf-mice and in young mice treated with GH or thyroxine (T_4).

Protein synthesis rates were measured 15 min after an intraperitoneal injection of a flooding dose of [^{3}H]phenylalanine (20 ml/kg body-weight, 150 mM-phenylalanine, 50 μCi [4-^{3}H]phenylalanine/ml). Values given are means for six animals and in all cases significance of difference (*t* test) was $P<0.005$. In adult (11–12-week-old) dwarf mice the rate of protein synthesis was depressed, compared with that in normal littermates, in skeletal muscle (2·9 *v.* 5·1 %/d), due to a reduced RNA activity (5·7 *v.* 11·3 g protein synthesized/d per g RNA), and in liver (47·1 *v.* 87·5 %/d), due to a reduced RNA concentration (RNA:protein, 40·2 *v.* 50·1) as well as a reduced RNA activity (12·0 *v.* 17·2 g protein synthesized/d per g RNA).

In younger animals (5–6 weeks), treatment of dwarf mice with GH (daily subcutaneous injection of 40 mU/mouse for 10 d) induced body-weight growth (19% increase above that of controls injected with saline) with concomitant growth in muscle, liver and heart. In muscle there was an increase in the protein synthesis rate (9·3 *v.* 7·4 %/d; GH *v.* saline) due to an increased RNA concentration (RNA:protein, 8·6 *v.* 6·9) with no change in RNA activity or in the degradation rate. T_4 administration (daily subcutaneous injection of 8 μg/mouse for 10 d) resulted in a similar increase in whole body growth as that found with GH; heart growth increased but skeletal muscle and liver weights were unchanged. T_4 caused a large increase in skeletal muscle protein synthesis (13·1 *v.* 7·4 %/d), due to an increase in both RNA concentration (RNA:protein 9·6 *v.* 6·9) and RNA activity (13·5 *v.* 10·7 g protein synthesized/d per g RNA) but there was a similarly large increase in the degradation rate. Thus the underlying mechanisms accounting for the apparent anabolic responses to the two hormones are different when examined for individual tissues.

The differing responses of zinc and protein metabolism to *Escherichia coli* endotoxin in rats fed on diets containing maize, coconut and fish oils.

By G. BROWN, V. HUNT, J. WAN and R. F. GRIMBLE, *Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

E. coli endotoxin produces a fall in serum zinc, loss of muscle protein and an increase in hepatic proteins which are prevented, in rats, by diets low in linoleate (Wan & Grimble, 1986). Liver, kidney and muscle are rich in protein-bound Zn. Serum Zn changes may thus depend on the extent of sequestration of Zn by liver and kidney. Serum, liver and kidney Zn contents were examined after administration of endotoxin to rats fed on diets containing various fats.

Male Wistar rats (100 (SE 1) g) were fed *ad lib.* for 5 weeks on standard chow (Labsure CRM (X)) enriched with 90 g maize, coconut or fish (Maxepa; Seven Seas Health Care, Hull) oils/kg and 180 mg butylated hydroxytoluene/kg. Vitamin E was added to the maize- and coconut-oil diets (90 mg/kg) as Maxepa contains vitamin E (1 mg/g). Diets were stored at −20° and food hoppers refilled daily. Body-weights after 5 weeks were 303 (SE 3), 305 (SE 3), 315 (SE 4) g for the maize-, coconut- and fish-oil groups respectively. Each group was divided in two, one received sterile saline intraperitoneally and the other 400 μg *E. coli* endotoxin (strain 055:B5 Difco Labs) in saline/kg body-weight. Food was removed from all groups which were then decapitated 24 h after injection. Blood was collected and liver, kidneys and a sample of thigh muscle rapidly excised. Homogenates (1:4 w/v) of liver and kidney were prepared in ice-cold 0·25 M-sucrose in 10 mM-Tris acetate buffer, pH 7·4. A sample was retained for total protein assay and the remainder centrifuged at 100 000 *g* for 1 h at 4°. Cytosolic fractions, tissue samples and serum were analysed for Zn, protein and corticosterone (Wan & Grimble, 1986).

(Mean values for four animals per group)

	Maize oil		Coconut oil		Fish oil	
	Saline	*E. coli*	Saline	*E. coli*	Saline	*E. coli*
Muscle protein (g/kg)	187	177*	185	183	183	182
Total liver protein (g)	1·68	2·49**	1·73	2·10*	1·98	2·42
Serum corticosterone (ng/ml)	268	339*	254	282	267	297*
Cytosolic Zn (μg/g protein)						
Liver	389	441	384	511**	443	545*
Kidney	369	349	326	377	345	389
Serum Zn (μg/ml)	2·12	1·73***	2·04	2·29	2·08	1·89

Significantly different from saline group (*t* test): *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Coconut and fish oils impaired the fall in serum Zn and muscle protein without reducing the gain in Zn by liver. The response of serum Zn to endotoxin, and its inhibition by fat, are not related to sequestration in liver or kidney in fasted rats.

Wan, J. & Grimble, R. F. (1986). *Proceedings of the Nutrition Society* **45**, 38A.

The use of [^{3}H]leucine as a marker for microbial protein synthesized in the rumen. By J. M. DAWSON, C. I. BRUCE, M. J. LAMMIMAN and P. J. BUTTERY, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics* and E. M. GILL and D. E. BEEVER, *The Animal and Grassland Research Institute, Hurley, Maidenhead, Berks*

Four Friesian steers (mean 180 kg), fitted with rumen and duodenal cannulas, were continuously fed on grass silage (Beever *et al.* 1987) at 24 g dry matter (DM)/kg live weight with and without 150 g fishmeal/kg silage DM. L-[4,5–^{3}H]leucine and $(^{15}NH_4)_2SO_4$ were infused intraruminally over 140 h. Samples of strained rumen fluid and duodenal digesta were taken at plateau and microbial fractions were isolated by differential centrifugation. Microbial protein synthesis was estimated from the specific activity of [^{3}H]leucine (Bruce *et al.* 1985), the enrichment of ^{15}N or diaminopimelic acid (DAPA) concentration in the microbes relative to the whole digesta.

Table 1. *Estimates of microbial N flow (g/d) by different markers*

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	Marker	Silage	Silage + fishmeal	SE of difference	Silage *v.* silage + fishmeal*
Rumen	^{15}N	47·69	63·89	3·99	$P<0·05$
	[^{3}H]leucine	57·06	69·37	6·73	NS
	DAPA	47·67	66·60	4·98	$P<0·05$
Duodenum	^{15}N	60·90	77·47	4·25	$P<0·05$
	[^{3}H]leucine	58·71	75·84	8·34	NS
	DAPA	39·73	59·19	5·75	$P<0·05$

*Statistical significance tested by analysis of variance.
NS, not significant.

All three markers in both rumen- and duodenally-derived microbes indicated a trend for increased microbial N flow at the duodenum when silage diets were supplemented with fishmeal, although these were only significant ($P<0·05$) with ^{15}N and DAPA. Statistical comparison by paired *t* test of estimates determined by the individual markers indicated that [^{3}H]leucine values were higher ($P<0·05$) than ^{15}N or DAPA values in ruminally-derived microbes. Estimates obtained with [^{3}H]leucine did not differ significantly with the source of microbes ($P>0·05$), whereas ^{15}N estimates were significantly ($P<0·001$) higher in duodenally-derived microbes and DAPA estimates were significantly ($P<0·05$) lower. [^{15}N] and [^{3}H]leucine estimates were not significantly different in duodenally-derived microbes ($P>0·05$) but both were significantly ($P<0·01$) higher that DAPA estimates.

DAPA is a constituent of some bacterial cell walls and $^{15}NH_4^+$ would be expected to label both protein and nucleic acids. With [^{3}H]leucine, only bacterial and protozoal protein becomes labelled giving a more realistic estimate of the microbial contribution to the duodenal protein flow.

The support of the AFRC is gratefully acknowledged.

Beever, D. E., Gill, E. M., Evans, R. T., Gale, D. L. & Wilton, J. C. (1987). *Proceedings of the Nutrition Society* **46**, 38A

Bruce, C. I., Marsden, M. & Buttery, P. J. (1985). *Proceedings of the Nutrition Society* **44**, 143A.

The effect of fishmeal supplementation of grass silage on nitrogen metabolism in growing cattle. By D. E. BEEVER, E. M. GILL, R. T. EVANS, D. L. GALE and J. C. WILTON, *Animal and Grassland Research Institute, Hurley, Maidenhead, Berks SL6 5LR*

Marked responses in animal performance to fishmeal supplementation of grass silage have been observed (Gill *et al.* 1987) whilst Gill & Beever (1982) noted a non-linear response in duodenal amino acid flow to the supplement. The present study was undertaken, using the silage diets described by Gill *et al.* (1987), to provide information on nutrient digestion and supply in order to interpret the production responses obtained, and to investigate the non-linearity of response to fishmeal supplementation.

Six Friesian steers (initially 5 months old, 120 kg), each fitted with simple cannulas in the rumen, proximal duodenum and terminal ileum, were allocated to two 3×3 Latin squares with three diets consisting of grass silage offered alone (diet C) or with 50 (FML) or 150 (FMH) g fishmeal dry matter (DM)/kg total DM. The level of feeding was 24 g total DM/kg live weight, offered in two equal meals at 09.00 and 21.00 hours. Each period, including diet changeover, lasted 6 weeks.

	C	FML	FMH	SEM
Rumen ammonia (mg NH_3-N/l)	113	109	149	5·2
Rumen fractional outflow rate, water (/h)	0·1904	0·1356	0·1171	0·0107
Nitrogen (g/d)				
Consumed	90	108	143	1·9
Entering small intestine*	106[a]	111[a]	140[b]	5·2
Leaving small intestine	36	48	43	2·4
Absorbed	70[a]	63[a]	97[b]	3·53
Total amino acids (g/d)				
Entering small intestine	443[a]	496[a]	653[b]	32·9
Leaving small intestine	131	168	165	18·0
Absorbed	312[a]	328[a]	488[b]	25·0

*Excludes all ammonia, i.e. non-ammonia-N.
[a,b]Values in rows with unlike superscript letters were significantly different ($P<0·05$).

Rumen ammonia concentration was increased only on diet FMH whilst rumen fractional outflow rate declined significantly on both FM diets (see Table). FM supplementation (compared with diet C) significantly ($P<0·05$) increased nitrogen disappearance from the intestines by 40% on diet FMH compared with a 10% decline on diet FML where availability in the intestines was reduced. These results were confirmed by estimates of total amino acid disappearance from the small intestine, whilst variable responses in the small intestinal disappearances of lysine, methionine, tyrosine and histidine were observed. Microbial N synthesis and total volatile fatty acid production were unaffected by dietary treatment.

Reasons for this variability in response to fishmeal inclusion are not yet clear, but indicate that caution is required in the use of standard 'protein values' for dietary supplements such as fishmeal.

Gill, E. M. & Beever, D. E. (1982). *British Journal of Nutrition*, **48**, 37–47.
Gill, E. M., Beever, D. E., Buttery, P. J., England, P., Gibb, M. J. & Baker, R. D. (1987). *Journal of Agricultural Science, Cambridge* (In the Press).

The metabolism of L-[4,5-^{3}H]leucine labelled fishmeal protein by rumen micro-organisms in vitro. By A. YHOKHA AND P. J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD*

To measure the uptake of free amino acids and peptides by rumen micro-organisms, labelled fishmeal (FM) protein was prepared. Brown trout (*Salmo trutta*) were injected intraperitoneally with L-[4,5-^{3}H]leucine and processed into fishmeal (see Windsor, 1981). In excess of 92% of the label was in the form of protein-bound leucine.

Triplicate Rusitec vessels were used (Rumen Simulation Technique; Czerkawski & Breckenridge, 1977). The basal diets, given once daily in the two different experiments were (1) chopped hay (5 g), ground straw (5 g), fishmeal (1·5 g) (10·58 g dry matter (DM)), and (2) straw (10 g), starch (5 g), fishmeal (2 g) (15·28 g DM). The ^{3}H-labelled FM replaced the non-radioactive FM during a period of 96 h of sampling. Samples were taken at various time intervals from the free fluid fraction (compartment 1) and particle-associated fraction (compartment 2) by washing with McDougall's buffer. Microbial leucine specific activity (SA) plateau was attained at 60–70 h after feeding of ^{3}H-labelled FM. The proportion of microbial protein leucine derived from free plus peptide-bound leucine was obtained from the ratio, microbial leucine SA at plateau:[^{3}H]FM protein-bound leucine SA. Results are given as means and standard errors of the triplicate vessels.

	Diet 1		Diet 2	
	Mean	SE	Mean	SE
Input SA of protein-bound leucine fishmeal ($\times 10^5$ dpm/μmol)	2·85		1·76	
Microbial protein leucine SA at plateau ($\times 10^5$ dpm/μmol)				
Compartment 1	1·29	0·035	1·50	0·012
Compartment 2	2·31	0·088	1·38	0·019
Proportion of microbial protein leucine derived from [^{3}H]FM protein hydrolysis				
Compartment 1	0·45	0·013	0·85	0·005
Compartment 2	0·81	0·075	0·78	0·011

dpm, Disintegrations/min.

A very high proportion of performed units were incorporated into microbial protein. In diet 1, the uptake between the microbial compartments was markedly different, but in this case approximately 18% of the total precursor leucine could have been contributed from hay.

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Feed antibiotics and buffers to control rumen lactic acid: 1. dose rates. By C. L. MCDONALD, E. M. AITCHISON and J. B. ROWE, *Western Australian Department of Agriculture, Baron-Hay Court, South Perth* 6151, *Western Australia*

Under conditions where rapid fermentation of starch reduces rumen pH, some groups of bacteria which ferment lactic acid (e.g. *Veillonella alcalescens*) are inhibited while the lactate-producing bacteria are not affected. This leads to a build-up of lactic acid in the rumen which in severe cases can cause death. The present experiment investigated methods to control lactic acid accumulation, by using either buffers (bicarbonate, bentonite clays) to maintain stable rumen pH, or by using a specific gram-positive antibiotic (avoparcin) to inhibit the bacteria which produce lactic acid (e.g. *Streptococcus bovis*, *Lactobacillus*).

Sheep that had previously been adapted to a roughage diet were given gristed wheat (14 g/kg live weight) in a slurry directly into the rumen using a stomach tube. Treatments were administered with the wheat as follows. Controls: no additive (n 12); additives (n 6): avoparcin 30, 60, 90 mg/kg wheat; sodium bicarbonate, Steecofeed bentonite and Cudgen bentonite each at 30, 50, 70 g/kg wheat. Samples of rumen fluid were taken from all sheep at 4, 8, 12, 24 and 48 h after administering the wheat.

L-Lactic acid concentrations did not increase until 12 h after dosing with wheat, and were reduced in many animals by 48 h. Lactic acid concentration was bimodally distributed in all treatments, falling into two distinct groups which statistically were analysed separately. Animals with 'high' lactate (n 57) were defined as those with lactate concentrations $>$ 5 mmol/l. These had a mean lactate concentration of 48·8 mmol/l (SE 2·9) and pH 5·2 (SE 0·1) after 12 h. The 'low' group (n 27) had a mean lactate concentration of 1·5 mmol/l (SE 0·3) and pH of 6·2 (SE 0·1). The pattern of response after 24 h was similar. No treatment significantly affected the proportion of sheep with 'high' lactate. Avoparcin was the only additive that significantly reduced lactate concentrations at 12 h ($P<0·05$) and 24 h ($P<0·01$) in the 'high' lactate group. The reduction in lactate concentration (Y, mmol/l) was closely related to increasing levels of avoparcin (X mg/kg wheat):

$$Y = 58·1 + 0·36X \ (r^2\ 0·95).$$

It was concluded that avoparcin reduced the concentration of lactic acid in animals developing a lactate pattern of fermentation but that more animals per treatment group were required to measure the effect of this additive on the incidence of this pattern of fermentation.

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Feed antibiotics and buffers to control rumen lactic acid: 2. pre-dosing. By E. M. AITCHISON, C. L. MCDONALD, P. CASSON and J. B. ROWE, *Western Australian Department of Agriculture, Baron-Hay Court, South Perth* 6151, *Western Australia*

McDonald *et al.* (1987) reported that the concentration of lactic acid in the rumen of sheep given high levels of starch was reduced by the antibiotic avoparcin. The present experiment determined the effect of antibiotics (avoparcin and flavomycin) and period of treatment, before introduction of wheat into the rumen, on the proportion of animals subsequently developing high concentrations of lactic acid.

Treatments were imposed on 240 Merino wethers (mean weight 51 kg) adapted to a roughage diet as follows. Two groups (*n* 24) were drenched individually with a slurry (10 ml/d) containing either 90 mg avoparcin or 36 mg flavomycin for 4 d before being given 800 g gristed wheat containing either 90 mg avoparcin (A4) or 36 mg flavomycin (F4); two groups were drenched for 2 d before being given the medicated wheat (A2, F2); two groups received antibiotic with the wheat (Ao, Fo); one group received wheat containing 90 g sodium bicarbonate (B); control animals (*n* 72) received wheat with no additives. Samples of rumen fluid were taken from all sheep 24 h after dosing with wheat.

Rumen L-lactate concentrations were bimodally distributed, falling into two distinct groups, defined as 'high' and 'low' respectively (McDonald *et al.* 1987).

Treatment . . .	Control	Ao	A2	A4	Fo	F2	F4	B
'High' rumen lactate								
No. of animals	48	1	0	1	13	12	15	13
Lactic acid (mmol/l)	53·3	11·1	—	12·6	54·1	52·9	55·0	62·1
Total VFA (mmol/l)	7·7[a]	53·0	—	51·3	7·0[a]	25·9[b]	20·3[b]	6·7a
pH	4·9	5·4	—	4·7	4·9	4·8	4·8	4·8
'Low' rumen lactate								
No. of animals	24	22	24	23	11	10	8	11
Lactic acid (mmol/l)	1·1	0·2	0·1	0·1	0·4	0·8	0·5	0·5
Total VFA (mmol/l)	68·5[a]	65·8[a]	71·1[a]	82·0[b]	70·8[a]	60·8[a]	65·1[a]	66·5a
pH	6·2	6·1	6·0	6·1	6·2	6·3	6·3	6·4

VFA, volatile fatty acids.
[a,b]Means in the same row with unlike superscript letters are significantly different ($P<0·05$). Values where *n* 1 were not included in analysis of variance.

The proportion of animals with 'high' lactate in groups treated with avoparcin (2/72) was significantly ($P<0·001$) lower than that for controls (48/72).

This work was partially funded by the Australian Meat and Livestock Research and Development Corporation, Hoechst Australia Ltd and Cyanamid Australia Pty Ltd.

McDonald, C. L., Aitchison, E. M. & Rowe, J. B. (1987). *Proceedings of the Nutrition Society* **46**, 40A.

Evaluation of avoparcin, flavomycin and lasalocid for wool production. By E. M. AITCHISON, I. G. RALPH and J. B. ROWE, *Western Australian Department of Agriculture, Baron-Hay Court, South Perth* 6151, *Western Australia*

Protein availability to sheep may be increased by ionophore feed additives (e.g. lasalocid) through decreased degradation of dietary protein in the rumen, or by antibiotics such as avoparcin which increase absorption of amino acids from the intestines. The present experiment investigated whether these compounds, plus a third antibiotic, flavomycin, increased wool growth in sheep fed at maintenance.

A total of 176 Merino wethers (2–6 years of age, 37·5 (SD 5·3) kg), were given either oaten chaff (C) or a pelleted mixture (P) containing (g/kg) 600 lucerne, 250 lupins and 150 barley, at a level calculated to maintain live weight, for 9 weeks. Animals were individually given either the control diets (n 16) or diets containing additives (n 8) as follows; avoparcin (A: 25, 50, 75 mg/kg feed); flavomycin (F: 5, 15, 30 mg/kg feed); lasalocid (L: 30, 50, 70 mg/kg feed). Wool growth was measured by clipping midside patches. Samples of rumen fluid were taken during week 6 for analysis of volatile fatty acids (VFA) and ammonia.

Live weight changes for the animals eating diets C or P with no additive were 27 and −3 g/d respectively. No dietary treatment produced live weight changes which were significantly different from those of their controls. Animals receiving F gained weight ($P<0·05$) relative to those given A or L (main effect of additives 26, 7, 9 g/d respectively, SED 6·8). Animals eating diet P grew significantly ($P<0·001$) more clean wool (64 mg/m^2 per d) than those on diet C (51 mg/m^2 per d). Treatment F increased wool growth on both diets compared with either A or L (62 *v.* 53, 56 mg/m^2 per d respectively; SED 0·025, $P<0·01$). No additive effect was significantly different from the controls, and changes in wool growth were not dose-related.

Total rumen VFA concentration was higher on diet P (72·0 mmol/l) than on diet C (58·2 mmol/l; $P<0·001$) but none of the additives gave significant effects that were dose-related.

Although compounds A and L changed rumen fermentation by significantly increasing the molar proportion of propionate relative to acetate and NH_3 concentration, they had no effect on wool growth or live weight change in sheep fed at maintenance. Flavomycin, which had no measurable effect on rumen fermentation, increased both wool production and live weight relative to A or L.

This work was supported by a grant from the Australian Wool Corporation; and by Cyanamid Australia Ltd., Hoechst Australia Ltd and Roche Products Pty Ltd.

The effect of Actaplanin on the composition of cow's milk. By J. L. CLAPPERTON, W. STEELE and W. BANKS, *Hannah Research Institute, Ayr KA6 5HL*

Actaplanin (Eli Lilly plc, Basingstoke) is an additive which, when introduced into the rumen of cattle, reduces the proportion of acetic acid and increases that of propionic acid in the rumen fluid. These changes should result in a reduction in the proportion of fat in the milk (Banks *et al.* 1983).

Four Friesian Cross cows in about the 8th week of lactation were offered a diet of hay (5 kg/d), sugar-beet pulp (2 kg/d) and a mixture of rolled barley and extracted soya-bean meal (8 kg/d). The experimental plan was a 4×4 Latin square with 3-week periods and up to 1 g Actaplanin/d was mixed with barley and soya-bean meal. The diet was given in two equal meals immediately after milking. Two of the cows were each fitted with a rumen cannula so that samples of rumen fluid could be obtained for the measurement of volatile fatty acids (VFA).

	VFA (mmol/l)				Milk		
Actaplanin (mg/d)	Acetic	Propionic	Butyric	Total	Fat (g/l)	Protein (g/l)	Yield (l/d)
0	71	22	16	113	41·5	34·7	16·1
250	65	19	12	99	39·0	33·3	18·0
500	63	26	12	103	38·2	33·3	16·8
1000	55	22	10	88	34·4	32·6	17·0
LSD	5	2	1	7	4·8	1·5	2·2

LSD, least significant difference.

The results are shown in the Table. The addition of Actaplanin depressed the total concentration of VFA in the rumen fluid, especially that of acetic and butyric acids, whilst the concentration of propionic acid was unaffected. Increasing the amount of Actaplanin added to the diet progressively reduced the fat content of the milk and each of the levels of Actaplanin used reduced the milk-protein content. There was a small but non-significant increase in milk yield when Actaplanin was added.

Banks, W., Clapperton, J. L. & Steele, W. (1983). *Proceedings of the Nutrition Society* **42**, 399–406.

Effect of undernourishment and subsequent refeeding on hind-limb muscle protein metabolism in growing lambs. By L. A. CROMPTON and M. A. LOMAX, *Department of Physiology and Biochemistry, University of Reading, Whiteknights, PO Box 228, Reading RG6 2AJ*

Muscle protein synthesis is sensitive to food intake, increasing after meals and decreasing again in the post-absorptive state (Millward *et al.* 1983). However, the influence of nutrient intake on protein degradation remains unclear.

We have used a hind-limb model similar to that of Oddy & Lindsay (1986) to measure muscle protein synthesis, gain and degradation. This technique measures the net exchange and gross uptake of [^{3}H]tyrosine across the hind-limb of growing lambs using arterio-venous difference and blood flow rate procedures. Muscle protein synthesis and degradation can then be estimated assuming that the gross uptake of tyrosine is equivalent to intracellular tyrosine synthesized into muscle protein and that all tyrosine coming from protein degradation is released from the muscle.

Four wether lambs, live-weight range 28·6–32·8 kg, were given 700 g commercial barley-based concentrate and 75 g hay, twice daily, to achieve a growth rate of 350 g/d (16·6 MJ metabolizable energy/d). Muscle protein metabolism was studied in the fed state, after 2 d of undernourishment and on the day of refeeding, when feed was offered at hourly intervals. Results are shown in the Table.

		Nitrogen intake (g/d)		Muscle protein turnover (%/d)					
				Net gain		Synthesis		Degradation	
Treatment	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fed	4	$32{\cdot}4^{c}$	1·4	$1{\cdot}8^{a}$	0·8	$4{\cdot}3^{a}$	0·3	2·6	0·8
Undernourished	4	$1{\cdot}6^{d}$	0·0	$-1{\cdot}7^{bc}$	0·3	$1{\cdot}6^{b}$	0·5	3·3	0·7
Refed	4	$33{\cdot}4^{c}$	1·6	$1{\cdot}5^{d}$	0·4	3·0	0·5	1·5	0·7

Values in the same column with unlike superscript letters are significantly different: $^{a,b}P<0{\cdot}01$, $^{c,d}P<0{\cdot}001$.

Undernourished lambs showed a net loss of hind-limb muscle protein due to a 62% decrease in protein synthesis and a 29% increase in protein degradation. However, 5 h after initial refeeding, a net gain of muscle protein had been restored due to an increase in protein synthesis and a decrease in protein degradation.

The changes in muscle protein synthetic rate in response to nutrition show good agreement to those obtained in rats (e.g. Millward *et al.* 1983). The results also suggest a pronounced role for protein degradation in controlling changes in muscle mass that occur in response to altered nutrient intake.

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The relation between hind-limb muscle protein metabolism and growth hormone, insulin, thyroxine and cortisol in growing lambs. By L. A. CROMPTON and M. A. LOMAX, *Department of Physiology and Biochemistry, University of Reading, PO Box 228, Reading RG6 2AJ*

The increase in muscle protein synthesis that occurs on refeeding fasted rats is significantly correlated with the increase in insulin and the decrease in glucocorticoid concentrations (Millward *et al.* 1983). The extent to which growth hormone (GH) is involved in the restoration of muscle protein synthesis, following refeeding, is unknown.

We have assessed the relation between changes in the concentration of GH, insulin (I), total thyroxine (T_4) and cortisol (C) and changes in muscle protein metabolism during altered nutritional states.

A hind-limb model for muscle tyrosine metabolism was used to determine muscle protein synthesis, gain and degradation in four wether lambs in the fed state, after 2 d of undernourishment and on the day of refeeding, as described previously (Crompton & Lomax, 1987). During the measurement of muscle protein metabolism, arterial blood samples were taken every 30 min to measure hormone concentrations.

The significant relations between muscle protein synthesis and degradation (X) (%/d) and plasma hormone concentrations (Y) are shown below:

X	Y	Equation	r	P
Synthesis	GH (ng/ml)	$Y = 20{\cdot}6 - 4{\cdot}0X$	0·832	<0·001
Synthesis	I (ng/ml)	$Y = 0{\cdot}09 + 0{\cdot}10X$	0·793	<0·01
Synthesis	T_4 (nmol/l)	$Y = 32{\cdot}8 + 12{\cdot}5X$	0·645	<0·05
Degradation	C (ng/ml)	$Y = 3{\cdot}6 + 2{\cdot}2X$	0·519	<0·05

The increase in muscle protein synthesis and gain observed by refeeding undernourished lambs was associated with a rise in I concentrations and a fall in C and GH concentrations. This is consistent with the work in rats (Millward *et al.* 1983).

The significant correlations between muscle protein synthesis and degradation and hormone concentrations in lambs support a role for I and T_4 as regulators of muscle protein synthesis and suggest a role for C in the control of muscle protein degradation in response to refeeding after a period of undernourishment. The results also show that plasma GH and muscle protein synthesis are inversely related and therefore the increase in muscle protein synthesis seen on refeeding does not require an increase in GH concentrations.

This work was supported by the AFRC.

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Effects of recombinant-DNA-derived growth factors on cultured sheep muscle cells. By JANE M. M. HARPER and P. J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD*

Epidermal growth factor (EGF) is known to be a potent mitogen for epithelial and endothelial cells, but its wider functions in the body are unknown. It has recently been produced on a large scale for trial as a chemical defleecing agent in sheep (Allen *et al.* 1985). Insulin-like growth factor-1 (IGF-1), otherwise known as somatomedin C, is thought to be important in the regulation of the metabolism of many cell types, but its role in sheep muscle has not been studied. The effects of both these factors on basal serum-free protein turnover in cultured sheep muscle cells were examined as part of a study on the control of growth in ruminants.

Cells were released from the hind-limb of 8-week-old fetal sheep by digestion of the tissue with a neutral protease and were cultured in vitro (Harper *et al.* 1986). Differential plating (Richler & Yaffe, 1970) was used to select a sub-population in which at least two-thirds were muscle precursors (myoblasts). These were grown in a medium (Dulbecco, modification of Eagles medium) containing fetal calf serum (80 ml/l) and allowed to fuse, forming contractile myotubes, in a medium containing 20 ml serum/l. [^{3}H]tyrosine was used as a radioactive tracer to measure protein synthesis in replicate cultures over a 6-h period, or protein breakdown over 24 h, using modifications of methods described by Ballard (1982).

These differentiated sheep muscle cells were poorly responsive to IGF-1; the maximal stimulation of protein synthesis was only 13% ($P<0.01$) and under the culture conditions described protein degradation was not significantly affected. Half-maximal stimulation of protein synthesis occurred at concentrations between 10^{-10} and 10^{-9}M, similar to those found by other workers for IGF-responsive processes. EGF produced greater effects: synthesis was stimulated by 27% ($P<0.001$) and degradation diminished by 6% ($P<0.01$). The cells were also more sensitive to this factor. Half-maximal effects were between 10^{-11}M and 10^{-10}M. When used in combination, IGF-1 and EGF produced additive effects on protein synthesis, suggesting the involvement of either separate intracellular pathways or separate cell populations in the two responses.

It is clear that either or both growth factors could be involved in the control of deposition of muscle protein in the whole ruminant.

The support of the AFRC is gratefully acknowledged. EGF was the kind gift of Coopers Animal Health and IGF-1, produced by Amgen Biologicals Inc., was purchased from Amersham International plc, Amersham, Bucks.

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Insulin binding to skeletal muscle microsomes during lactation in sheep.

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Although insulin is required to maintain lactation the hormone does not stimulate uptake of glucose or amino acids by the ruminant mammary gland, whereas uptake by skeletal muscle is responsive to insulin. The decrease in whole body sensitivity to insulin which occurs in lactation will therefore increase the availability of nutrients to the mammary gland. The present experiment determined whether these changes are mediated through alterations in binding of insulin to skeletal muscle membrane receptors.

We determined insulin binding to microsomal preparations of skeletal muscle from control (non-lactating) and peak lactating (21 d post-partum) ewes. Microsomes were prepared from three skeletal muscles of differing function and fibre type, and their capacity to bind [^{125}I]insulin was determined at a range of insulin concentrations (0–10 nM). Non-specific binding was assessed using a very high (45 nM) insulin concentration and deducted. Results are expressed relative to the 5′-nucleotidase (*EC* 3.1.3.5) activity (a plasma membrane marker) of the preparations. Preliminary results showed that this enzyme activity was unaltered by lactation.

Table 1. *Binding of [^{125}I]insulin to microsomal membranes from sheep muscles*

	Non-lactating			Lactating		
Muscle	Mean	SEM	*n*	Mean	SEM	*n*
Gastrocnemius	19·4	3·16	5	13·8	4·84	4
Vastus lateralis	8·4	1·10	3	9·6	1·56	4
Sternocephalicus	6·0	2·22	3	7·3	1·30	4

Results were obtained using 0·8 nM-insulin and are expressed as pmol insulin bound/unit 5′-nucleotidase activity. 1 Unit 5′-nucleotidase is 1 μg phosphate released/mg protein per h.

There were no differences in insulin binding at any concentration, between control and lactating ewes. The number of insulin receptors per adipocyte is also unchanged in lactation (Vernon *et al.* 1981), suggesting that the decrease in whole body sensitivity in lactation is mediated by post-receptor changes.

Insulin binding tended to be greater for the gastrocnemius muscle, which has predominantly white, fast-twitch fibres, than for the other two muscles, which have a lesser proportion of white fibres.

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Vernon, R. G., Clegg, R. A. & Flint, D. J. (1981). *Biochemical Journal* **200**, 307–314.

Insulin-like growth factor-1 and growth hormone in sheep. By J. M. PELL, L. A. BLAKE, H. L. BUTTLE, I. D. JOHNSSON and A. D. SIMMONDS, *Animal and Grassland Research Institute, Shinfield, Reading RG2 9AT* and D. J. MORRELL, *Institute of Child Health, 30 Guilford Street, London WC1N 1EH*

Daily administration of growth hormone (GH) to ruminants causes a decrease in whole-body fat content, sometimes accompanied by an increased rate of lean-tissue deposition. A consistent anabolic response to GH would be desirable for animal production. The anabolic actions of GH are mediated, at least in part, via the insulin-like growth factors (IGFs) (Isaksson *et al.* 1985) and therefore interactions between GH and IGF-1 were investigated.

Blood was collected from lambs in two separate growth trials consisting of control (C) and GH-treated (T, 0·1 mg/kg per d) animals and assayed for plasma GH and total IGF-1. In Expt 1, daily gain increased by 22% (284 to 347 g/d, $P<0.05$, n 8 per group) and lean tissue content increased by 24% (9·2 to 11·4 kg/carcass, $P<0.05$) in T. No anabolic response to GH was found in Expt 2 despite almost identical nutritional and environmental conditions (C daily gain, 228 g/d; lean tissue content, 7·9 kg/carcass, n 8 per group). GH concentrations increased four- to eightfold in all T lambs. IGF-1 concentrations also increased in all T lambs (mean and SEM: Expt 1, 235 (27) to 460 (74); Expt 2, 418 (72) to 1302 (174) ng/ml). Thus, elevated total IGF-1 concentrations can occur in the absence of increased growth rates.

The contribution of muscle to circulating IGF-1 was investigated using sheep prepared with chronic catheters in both external iliac arteries and veins. GH was infused for 24 h into the left artery and blood was sampled from the remaining catheters. From the Table it can be concluded that muscle and connective tissue may not be major sources of total blood IGF-1.

			GH (ng/ml)			IGF-1 (ng/ml)		
Treatment	n		A	LV–A	RV–A	A	LV–A	RV–A
Saline infused	4	Mean	1·34	0·03	0·06	461	8	34
i.a., 24 h		SEM	0·25	0·04	0·03	47	22	31
GH, infused i.a.,	3	Mean	3·45	0·82	−0·09	601	−16	−60
5 mg/24 h		SEM	1·40	0·38	0·10	67	59	37
GH, infused i.a.,	3	Mean	5·46	0·45	−0·01	747	19	57
10 mg/24 h		SEM	2·47	0·09	0·19	189	27	98
GH injected s.c., 20 mg	4	Mean	8·90	−0·36	−0·17	945	−25	16
		SEM	0·60	0·24	0·28	200	65	64

A, artery; LV, left vein; RV, right vein; saline, 9 g sodium chloride/l; i.a., intra-arterially; s.c., subcutaneously.

We wish to thank A. R. Jones and J. R. Abbey for their expert care of the animals.

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The effect of sodium bicarbonate on nitrogen metabolism in the rumen of sheep fed on silage-based rations. By C. J. NEWBOLD, P. C. THOMAS and D. G. CHAMBERLAIN, *Hannah Research Institute, Ayr KA6 5HL*

In sheep given grass silage diets supplemented with sugars, the inclusion of sodium bicarbonate in the diet reduced ammonia levels (Chamberlain *et al.* 1985) but the question remains whether this was due to increased absorption from the rumen or increased incorporation of ammonia into microbial protein.

To examine this further, four sheep, fitted with permanent cannulas in the rumen and proximal duodenum, were given a basal diet of grass silage (651 g dry matter (DM)/d) and a rolled barley–sucrose concentrate (1:1 w/w, 405 g DM/d). This was supplemented with sodium bicarbonate at rates of 0, 25, 50 or 75 g/d. The diets were all given in twenty-four hourly meals each day with the sodium bicarbonate mixed with the concentrate part of the diet. The experiment was a 4×4 Latin square with 2-week periods.

The effect of sodium bicarbonate on nitrogen metabolism in sheep given a diet of silage and sugar–barley

	Sodium bicarbonate (g/d)				
	0	25	50	75	SED
Total N intake (g/d)	20·1	20·1	20·1	20·1	
Rumen ammonia (mg/l)	170	152	143	125	14·7*
Non-ammonia N flow to the small intestine (g/d)	19·8	19·7	22·4	21·4	1·0*

*$P<0.05$.

Addition of sodium bicarbonate to the diet led to a significant reduction in rumen ammonia concentrations (see Table). This was associated with an increase in the duodenal flow of non-ammonia nitrogen and a 14% increase in the amount of diaminopimelic acid reaching the small intestine when 75 g sodium bicarbonate/d was given compared with the control. Sodium bicarbonate had only a small effect on rumen pH (6·14 and 6·43 for the 0 and 75 g sodium bicarbonate/d respectively) but there was also an approximately 50% increase in outflow rate and a 30% increase in dilution rate when 75 g sodium bicarbonate/d was given compared with that of the control.

The results indicate that, with silage diets of the type used here, modifications in rumen environment induced by sodium bicarbonate inclusion in the diet can improve the fixation of dietary N leading to an increased flow of microbial protein to the small intestine.

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