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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Sixteenth Meeting of the Nutrition Society was held in The Main Assembly Hall, School of Agriculture, University of Nottingham, Sutton Bonington, near Loughborough on Tuesday and Wednesday, 9/10 *July* 1985, *when the following papers were read:*

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Protein synthesis during implantation in ewes: interaction between the endometrium and trophoblast. By D. PULLAR, MARJORIE K. JEACOCK and D. A. L. SHEPHERD, *Department of Physiology & Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ*

Histological evidence shows that pre-implantation embryos are capable of pinocytotic capture of maternally secreted proteins (Winterberger-Torrès & Fléchon, 1974) whereas post-implantation embryos rely completely on free amino acids for their supply of nitrogen. The period of change from one form of N supply to the other may be critical for the nutrition of the developing embryo and hence for the establishment of pregnancy. The experiment reported here was designed to test the hypothesis that the presence of an embryo in the uterine lumen influences protein synthesis in the maternal endometrium at the time of implantation.

Rates of protein synthesis were measured in caruncular endometrium and in trophoblast by determining the rate of incorporation of L-[4,5-^{3}H]leucine into the trichloroacetic-acid-insoluble fraction. The tissue was obtained from six Blue-Face Leicester × Swaledale ewes, which were killed 17 d after tupping. Explants were then maintained in culture in a modified Minimum Essential Medium with Earle's Salts (Gibco Ltd) supplemented with insulin and non-essential amino acids for 24 h in an atmosphere of air:carbon dioxide (95:5, v/v) at 37°.

	Rate of incorporation of leucine into protein (nmol/mg tissue protein per d)	
	Mean	SE
Trophoblast	14·6	0·75
Trophoblast co-cultured with caruncular tissue	13·5	0·79
Caruncular tissue co-cultured with trophoblast	14·9	1·08
Caruncular tissue	24·3	1·00

The results indicate that the rate of protein synthesis in the trophoblast was unaffected by the presence of caruncular tissue, but the presence of trophoblast suppressed the rate of protein synthesis in caruncular tissue significantly ($P<0·01$). This effect was also reflected in the release of protein from the tissue since the sum of the protein released into the medium when trophoblast and caruncular tissue were cultured individually was twice that released when both tissues were cultured together.

It is concluded that factors originating from the trophoblast of the embryos regulate protein synthesis in the endometrium at the time of implantation.

D.P. is the recipient of a MAFF Postgraduate Agricultural Studentship.

Winterberger-Torrès, G. & Fléchon, J. E. (1974). *Journal of Anatomy* **118**, 143–153.

The effect of shearing on plasma glucose, free fatty acid, growth hormone and insulin concentrations in the pregnant ewe. By M. E. SYMONDS[1], M. J. BRYANT[2] and M. A. LOMAX[1], *Departments of [1]Physiology & Biochemistry* and *[2]Agriculture, University of Reading, Whiteknights, Reading RG6 2AJ*

The pregnant ewe responds to shearing by mobilizing body tissue (Symonds *et al.* 1985). Growth hormone (GH) and insulin may play an important role in the utilization of this increased endogenous supply of nutrients. However, little is known about the long-term effects of shearing on these hormones or on plasma glucose and free fatty acid (FFA) concentrations.

Two groups of three unshorn (US) and four shorn (S) (at 8 weeks before lambing) Blue faced Leicester cross Swaledale ewes were housed at ambient temperature (−3 to 12°) and 24 hourly blood samples were taken from each group for 24 h at 14-d intervals. Animals were fed as described by Symonds *et al.* (1985).

There were no significant differences between the S and US groups in plasma glucose, FFA, GH or insulin concentrations over the period of 49–20 d before lambing. However, shearing resulted in a significant increase in plasma glucose concentrations over the final 14 d of pregnancy (S, 2·92 (SE 0·15) (*n* 7); US, 2·42 (SE 0·14) (*n* 6) mM; $P<0·05$). There were no significant differences in plasma FFA (S, 0·55 (SE 0·05); US, 0·67 (SE 0·08) mM), GH (S, 6·74 (SE 1·56); US, 5·85 (SE 0·31) ng/ml) or insulin (S, 0·32 (SE 0·04); US, 0·26 (SE 0·03) ng/ml).

Period before lambing (d)		MXT (°)		Plasma GH (ng/ml) S (*n* 4)		Plasma GH (ng/ml) US (*n* 3)		Plasma glucose (nM) S (*n* 4)		Plasma glucose (nM) US (*n* 3)	
Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
41	1	10·1[a]	0·6	5·60[a]	0·97	4·98	0·42	2·91[a]	0·10	2·96[a]	0·05
27	1	6·4[c]	0·7	8·68[b]	0·50	5·25	0·99	3·43[b]	0·15	3·14[a]	0·06
13	1	10·7[a]	0·4	5·28[a]	0·74	5·54	0·41	2·84[a]	0·19	2·21[b]	0·20

Values in the same columns with different superscipt letters are significantly different: [ab]$P<0·05$, [ac]$P<0·01$.

A significant increase in plasma glucose and GH was observed between days 41 and 27 in the S ewes which did not occur in the US group. This was related to a significant decrease in the mean maximum temperature (MXT) recorded on the 7 d up to that of blood sampling. Regression analysis between plasma glucose (x) and GH (y) concentrations showed that these two indicies were significantly correlated over the final 50 d of pregnancy in the S animals.

$$x = 2·02 + 0·145 .y,\ r\ 0·685,\ P<0·05.$$

It is concluded that shearing may inhibit insulin secretion as plasma insulin concentrations were similar in both S and US groups despite an increased plasma glucose concentration over the final 2 weeks of pregnancy. Also, the direct correlation between plasma glucose and GH concentrations in the S group suggests a diabetogenic effect of GH and that this may be a response to long-term changes in ambient temperature.

M.E.S. acknowledges the support of a MAFF studentship.

Symonds, M. E., Bryant, M. J. & Lomax, M. A. (1985). *Proceedings of the Nutrition Society* **44**, 53A.

Effect of tonicity in the contents of the stomach and duodenum and in blood on parotid salivary secretion in sheep. By R. R. CARTER, W. L. GROVUM and W. W. BIGNELL, *Department of Biomedical Sciences, University of Guelph, Ontario, Canada N1G 2W1*

Total salivary flow in sheep is known to be inversely related to the tonicity of ruminal digesta and plasma (Warner & Stacy, 1977). However, the effect of tonicity in digesta *per se* on salivation was not examined. Consequently, its role in the reticulo-rumen, abomasum and duodenum is reported here along with information on sites where tonicity in blood might act to depress salivation.

Anaesthetized sheep with cannulated parotid ducts were used to study secretion in response to solutions of sodium chloride and polyethylene glycol-400 in the reticulo-rumen (3·5 litres) and abomasum (0·5 litres) which were free of digesta, washed with saline (9 g NaCl/l) and isolated to prevent outward fluid flow. The solutions flowed by gravity (137 ml/min) through a 450 mm duodenal segment.

The tonicity of the solutions had no significant effect on total parotid salivary flows over a 10 min collection period. The combined results are presented in the Table.

Total flow rates (ml/min) of parotid saliva in response to tonicity (mOsmol/l)

		Tonicity					
	n	210	290	360	440	SEM	Significance
Reticulo-rumen	5	1·46	1·56	1·61	1·45	0·09	NS
Abomasum	4	1·70	1·66	1·52	1·52	0·07	NS
Duodenum	4	1·56	1·55	1·54	1·56	0·06	NS

Carr & Titchen (1978) demonstrated a greater reduction in parotid secretion with infusions of hypertonic saline into the portal vein compared with the jugular vein. Our infusions into the jugular vein in four sheep (6960 mOsmol/l saline delivered at 0·76 or 0·38 ml/min for 2–4 min) produced no effect or a relatively small effect but infusions via the carotid artery below the arterial supply to the parotid gland markedly inhibited secretion. The osmolality of blood collected above this site of infusion showed that increases of 7–12 mOsmol/l could produce detectable inhibitions in salivation. Infusions into the mesenteric vein close to portal resulted in effects similar to those for the jugular vein in two sheep and similar to those for the carotid artery in the other two sheep. Single rapid injections of 3–5 ml (1740 mOsmol/l) produced a greater inhibitory effect ipsilaterally when made into the carotid artery than into either of the two venous routes (*n* 5).

These results indicate that elevating tonicities of contents in the reticulo-rumen, abomasum and duodenum will not inhibit salivation but inhibition is possible once the electrolyte is absorbed. The head and the liver were identified as possible sites mediating this effect.

Carr, D. H. & Titchen, D. A. (1978). *Quarterly Journal of Experimental Physiology* **63**, 1–21.

Warner, A. C. I. & Stacy, B. D. (1977). *Quarterly Journal of Experimental Physiology* **62**, 133–142.

Effect of protein infusion on excretion of purine derivatives in steers nourished by intragastric nutrition. By T. FUJIHARA, E. R. ØRSKOV and N. A. MACLEOD, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Lack of information about the excretion of endogenous purine derivatives in ruminants and problems of changes in the proportion of xanthine, hypoxanthine, uric acid and allantoin in ruminant urine has limited the possible use of the excretion rates as a measure of the production of nucleic acids by rumen microbes. These problems have largely been overcome. Firstly, the technique of intragastric nutrition (Ørskov *et al.* 1979) enables animals to be nourished without production of microbial protein. Secondly, a technique has been developed (T. Fujihara, P. J. Reeds and E. R. Ørskov, unpublished) which by addition of appropriate enzymes ensures that purine derivatives in the urine are quantitatively converted to allantoin. Using these methods, two steers (260 kg live body-weight (W)) were infused with volatile fatty acids into the rumen and casein into the abomasum to supply 450 kJ/kg $W^{0.75}$. The supply of casein varied from 0 to 1120 mg nitrogen/kg $W^{0.75}$; it was estimated that the upper level was equal to twice the maintenance need for N. The allantoin excretion after conversion of hypoxanthine, xanthine and uric acid was determined by the method of Young & Conway (1942). Protein infusion and allantoin excretion are compared in Fig. 1.

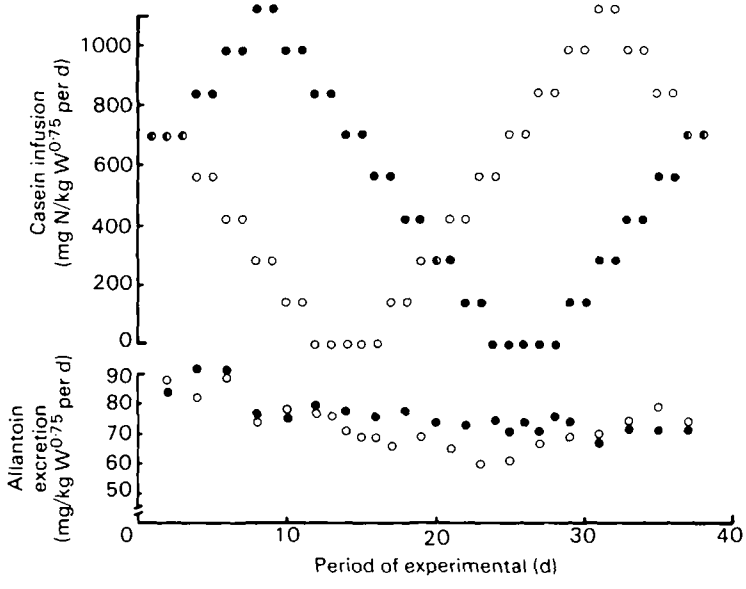

Fig. 1. The excretion of purine derivative in the urine of steers 1 (○) and 2 (●) associated with different levels of casein infusion.

During the first 6 d allantoin excretion was slightly higher than subsequently, presumably a carry-over effect after withdrawal of food when there would have been microbial protein produced. When this period was excluded the average allantoin excretion was 70 and 74 mg/kg $W^{0.75}$ per d for steers 1 and 2 respectively. The excretion was apparently not affected by the level of infusion of protein and so represented the basal level of excretion arising from purine metabolism in the tissues of the steers.

Ørskov, E. R., Grubb, D. A., Wenham, G. & Corrigall, W. (1979). *British Journal of Nutrition* **41**, 553–558.

Young, E. G. & Conway, C. F. (1942). *Journal of Biological Chemistry* **142**, 839–853.

The response in insulin, glucose and amino acids in the plasma of sheep to changes in rumen fermentation. By HEATHER J. FINLAYSON, D. S. PARKER and B. SLOAN, *Department of Agricultural Biochemistry and Nutrition* and *Department of Agriculture, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Two isonitrogenous, isoenergetic diets were given to three mature Suffolk × wethers resulting in daily dry matter intakes of 627 g silage and either 290 g of a low-starch (265 g/kg), high-fibre (242 g NDF/kg) concentrate (diet A) or 329 g of a high-starch (501 g/kg), low-fibre (86 g NDF/kg) concentrate (diet B). Diets were given in two equal portions at 08·00 and 15·30 hours and samples of rumen fluid and jugular blood were taken before feeding and in the 6 h period after the first feed of the day. Volatile fatty acid (VFA) concentrations and molar proportions were determined in the rumen fluid, and plasma insulin, glucose and amino acid concentrations were determined in the jugular blood.

One hour after feeding, total VFA concentrations increased from a value of 53 (SE 6) mM to 110 (SE 7) and 94 (SE 7) mM for diets A and B respectively. At 1, 2, 3 and 4·5 h post-feeding the molar proportion of propionate in animals fed on diet A (mean value over these times 0·31 (SE 0·01)) was significantly higher ($P<0·05$) than the corresponding value of 0·25 (SE 0·01) for diet B.

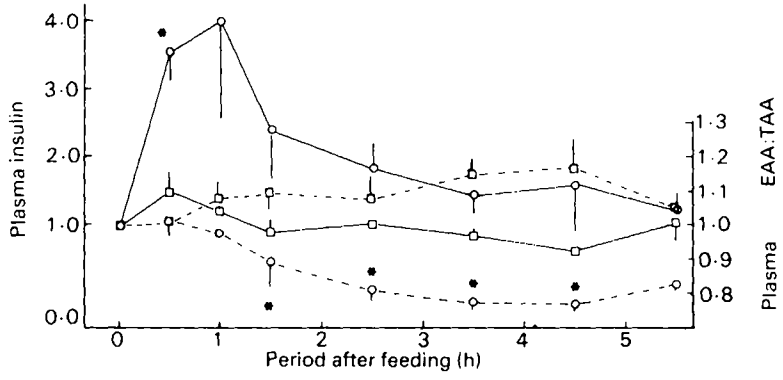

Fig. 1. Plasma insulin (——) and EAA:TAA (– – –) both expressed relative to values before feeding (time 0 = 1·0) in sheep fed on diet A (○) or diet B (□). *Values were significantly different from those for diet B ($P<0·05$).

Despite variation between individual sheep there were consistent trends within each diet in the concentration of plasma insulin and ratio, essential amino acids: total amino acids (EAA:TAA) in the plasma when the values were expressed relative to the pre-feeding value (Fig. 1).

The actual pre-feeding (time = 0) concentrations of insulin were 23 (SE 7·8) and 40 (SE 22·2) μU/ml for diets A and B respectively and the values EAA:TAA were 0·42 (SE 0·03) for diet A and 0·33 (SE 0·06) for diet B. There was no significant difference ($P>0·05$) in plasma glucose concentrations between the diets at any time during the sampling period. In this experiment the diet which resulted in a significant increase in the molar proportion of propionate in the rumen was associated with a higher response in plasma insulin after feeding and a fall in the value of plasma EAA:TAA.

B.S. is in receipt of a postgraduate scholarship from Dalgety Agriculture Ltd.

A calorimetric investigation of heat production during glucose fermentation in rumen fluid. By A. ARIELI, *Department of Animal Sciences, Faculty of Agriculture, Hebrew University, Rehovot, Israel*

Estimates of heat produced by rumen fermentation (Hf) vary between 6 and 13% of fermented substrate energy (Thomas & Rook, 1981), the higher values being obtained on high-roughage diets. These values are based on direct and indirect determinations of Hf, performed over relatively long time periods, during which the population of micro-organisms could change. This variation of experimental Hf values contrasts with the stochiometric balance (Wolin, 1975) according to which a constant Hf of hexoses of 6·4% is expected, regardless of fermentation characteristics.

The present study was undertaken to assess Hf during a short-term fermentation of glucose. Samples of strained rumen fluid (15 ml), taken from sheep fed on a hay and concentrate diet, mixed with 45 ml McDougall's artificial saliva, were kept for stabilization in 100-ml Erlenmeyer flasks for at least 2 h at 39° under anaerobic conditions. These samples were thereafter introduced into an adiabatic calorimeter (Tronac, model 450, Orem, Utah; with a precision of 40 mJ), at 39°, where their heat production before, during and after addition of glucose (0·4–6·4 mg per sample) was measured.

Mean (n 120) basal heat production of the rumen samples was 0·35 (SE 0·05 mW/ml rumen fluid. The addition of glucose was followed by an immediate, dose related increase in the rate of heat production. Maximal heat production was 1·13 mW/ml (SE 0·23) (n 24).

The effect of glucose was short (lasting 2–5 min) and heat production subsequently returned to its basal level. The expression of energy dissipated as heat (Hf) as a percentage of fermented glucose energy yielded an asymptotic dose response curve:

$$y = 2{\cdot}46 + 3{\cdot}81\, e^{-7{\cdot}21\, x} \qquad \text{SD} = 0{\cdot}04,\ P<0{\cdot}001$$

where y is Hf (%) and x is glucose concentration (mmol/l).

These results indicate that maximal Hf of glucose fermented agrees with Hf predicted from stochiometric calculations. Such an Hf was found at low (e.g. 10^{-6} mol/l) glucose concentrations. An increase in glucose concentration was followed by a decrease in Hf. At a concentration of 10^{-3} mol/l, Hf is expected to be 40% of the stochiometric value. The results suggest that under high-glucose concentrations the substrate is only partially fermented, the remainder being possibly stored for anabolic processes.

In the present study, substrate availability was varied by altering glucose concentration. Further study is needed to examine whether the results presented here will be reproduced when hexoses from different sources will be used.

Thomas, P. & Rook, J. A. F. (1981). In *Recent Developments in Ruminant Nutrition*, pp. 157–183 [W. Haresign and D. J. A. Cole, editors]. London, Butterworths.

Wolin, M. J. (1975). In *Digestion and Metabolism in the Ruminant*, pp. 134–148 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, Australia: University of New England Publishing Unit.

The effect of phosphorus deficiency on rumen microbial activity. By S. KOMISARCZUK, R. J. MERRY and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

A continuous culture technique (Merry *et al.* 1983) has been used to study the effect of progressive reductions in phosphorus concentration on rumen microbial activity. Two culture vessels (control and experimental) were inoculated with 1·05 litres strained rumen contents from sheep adapted to the experimental diets. Pelleted feed (36 g dry matter (DM)/d) of a mixture of (g/kg): 490 barley straw, 280 sugar-beet pulp, 200 tapioca, 30 urea, was added continuously. The diet supplied 24 mg P/d. The experiments were divided into four periods. The control vessel was infused with artificial saliva (1·7 litres/d) containing 120 mg inorganic P (P_i)/l over the four periods (this resulted in levels of approximately 50 mg P_i/l in the vessel). The experimental vessel was infused with artificial saliva containing (mg P_i/l): 120 (period 1), 80 (period 2), 40 (period 3), 0 (period 4). Periods consisted of 2-d for equilibration (except for period 1 where 5 d were required), followed by 3 d of sampling. The experiments were replicated four times. ATP, ammonia and P_i concentrations and pH were measured daily in samples of filtered vessel contents. Total volatile fatty acid (TVFA) concentrations were estimated in samples of vessel effluent. Mean values for three collection days in the four experiments are presented in the Table.

			Vessel				
	Period	Infused P_i (mg/l)	P_i concentration (mg/l)	pH	NH_3 (mmol/l)	ATP (μmol/l)	Effluent TVFA (mmol/l)
Control vessel	1	120	50	6·55	8·7	13·48	110·1
	2	120	50	6·51	9·8	13·78	111·3
	3	120	52	6·61	8·2	14·11	107·4
	4	120	52	6·52	8·4	14·01	113·3
Experimental vessel	1	120	48	6·54NS	9·2NS	12·46NS	104·8NS
	2	80	28	6·52NS	9·2NS	10·69•	106·5NS
	3	40	4	6·82††	9·7•	8·92††	98·4NS
	4	0	<1	7·26•••	16·3••	2·21••	58·9••

NS, non-significant.
Significance of difference from control value for the same period: •$P \leqslant 0·05$, ††$P \leqslant 0·02$, ••$P \leqslant 0·01$, •••$P \leqslant 0·001$.

There were significant increases in pH and ammonia concentration when P_i content in the vessel fell to 4 mg/l, whereas TVFA concentration did not show any significant change until P_i concentration was <1 mg/l. ATP concentrations, on the other hand, were significantly and increasingly reduced for all vessel P_i concentrations less than 48 mg/l.

It has been shown that although the microbial population can survive conditions of quite severe P_i depletion, marked effects on microbial activity are encountered with P_i concentrations of less than 50 mg/l.

Merry, R. J., Smith, R. H. & McAllan, A. B. (1983). In *Proceedings of the 4th International Symposium on Protein Metabolism and Nutrition, Clermant-Ferrand,* Vol. II, pp. 227–230. Paris: INRA.

Release of diaminopimelic acid from bacterial cell walls by rumen protozoa. By ANNA M. DENHOLM and J. R. LING, *Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD*

The degradation of bacterial cell wall peptidoglycan is a logical prerequisite for the digestion of rumen bacteria which form a major nutrient source for rumen protozoa.

To further examine this process, a mutant bacterium, *Bacillus megaterium* GW1, with its peptidoglycan specifically radiolabelled with 2,6-diamino [G-^{3}H] pimelic acid ([^{3}H]A_2pm) was used as a 'model' bacterium. Suspensions of these radiolabelled bacteria ($1{\cdot}5\times10^9$/ml) were subjected to in vitro incubations of 2 h with mixed ciliate protozoa ($5{\cdot}1\times10^5$/ml) obtained from the rumen of sheep. Additional protozoal incubations containing the model ($1{\cdot}5\times10^9$/ml) plus mixed rumen bacteria ($1{\cdot}7\times10^{10}$/ml) were used to investigate the degradation of the latter. The concentration of 'free' and 'peptide-bound' A_2pm released were determined by ion-exchange chromatography of supernatant fractions (200 g for 30 s) which had been deproteinized (10 g/l picric acid) or deproteinized and acid hydrolysed (6 M-hydrochloric acid for 22 h). Additional details of the preparation of microbial suspensions, conditions for their anaerobic incubation and the analyses of samples have been described by Denholm & Ling (1984).

The percentage of the total radiolabel appearing in the incubation supernatant is assumed to be a measure of the degradation of the model radiolabelled bacteria. The results showed that when incubated with rumen protozoa, 66% of the radiolabel contained in the model bacteria had been released. These degradation products represented a total of 116 mg A_2pm/l; 56% as free A_2pm and the remainder as peptide-bound A_2pm. However, when mixed rumen bacteria were present in the incubation, the total A_2pm released was 131 mg/l with 26% of this as free A_2pm. In this instance, however, only 53% of the model radiolabelled bacteria were degraded. This would be equivalent to 93 mg A_2pm/l; the remainder (38 mg A_2pm/l) had presumably originated from the mixed rumen bacteria. Nevertheless, the addition of large numbers of rumen bacteria appeared to have had a disproportionately small (66 *v* 53%) effect on the degradation of the radiolabelled bacteria. The susceptibility of *B. megaterium* cell wall to protozoal enzymes (Coleman, 1980), as well as some discrimination against rumen bacteria, could account for this.

According to Ling & Buttery (1978), mixed rumen bacteria contain approximately 7 g A_2pm-N/kg total N and the ovine rumen contains from 0·9 to 2·0 g bacterial N/l. This calculated release of 38 mg A_2pm/l would then represent 40–88% of the bacterial cell A_2pm. Furthermore, this amount of free plus peptide-bound A_2pm entering the duodenum would constitute about 40% of the daily A_2pm passage. These observations should be considered when A_2pm is used as a marker of bacterial N.

The financial assistance of the SERC is acknowledged.

Coleman, G. S. (1980). *Advances in Parasitology* **18**, 121–173.
Denholm, A. M. & Ling, J. R. (1984). *Canadian Journal of Animal Science* **64**, 18–19.
Ling, J. R. & Buttery, P. J. (1978). *British Journal of Nutrition* **39**, 165–179.

Leucine metabolism by rumen bacteria in vivo. By C. I. BRUCE, M. MARSDEN and P. J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD*

Preformed amino acids may have a more important role in the growth of rumen bacteria than has been formerly supposed (Maeng & Baldwin, 1976; Cottrill *et al.* 1982). A technique has been developed to measure in vivo leucine flux through the rumen free leucine pool, and its direct incorporation into rumen microbial protein.

The approach was to continuously infuse over 72 h 0·5 mCi L-[4,5-^{3}H]leucine into the rumen of continuously fed sheep fitted with rumen and duodenal cannulas. Samples of rumen and duodenal contents were taken from time 0 for 96 h. Rumen bacteria were obtained from strained samples of rumen fluid. Rumen free leucine was isolated by *in situ* dialysis. Leucine specific activity was determined by preparative ion-exchange chromatography. The proportion of microbial leucine arising from direct incorporation from the rumen free pool was obtained from the ratio microbial:rumen free leucine specific activities at plateau.

In three sheep given 690 g silage dry matter (DM)/d (total nitrogen 26 g/kg. D value 66, metabolizable energy 10·5 MJ/kg, pH 3·7), the proportion of microbial leucine incorporated directly was 0·39, 0·44 and 0·42, daily leucine flux through the rumen free pool being 3·5, 4·0 and 4·5 g respectively.

L-[4,5-^{3}H]leucine may also be used as a marker to estimate bacterial protein synthesis. Three sheep were given 640 g DM/d of a grassmeal/barley based ration. The microbial flow was estimated by comparing the specific activity of the bacterial fraction with that of the whole digesta and compared with estimates obtained simultaneously using diaminopimelic acid (DAPA) as a marker.

Daily bacterial non-ammonia nitrogen flows (g/d) were respectively 7·19, 3·52 and 6·41 (DAPA); 8·33, 3·69 and 5·69 (L-leucine from rumen bacteria); and 7·09, 3·94 and 6·34 (L-leucine from duodenal bacteria).

This technique offers considerable scope for investigation of the influence of free amino acids on rumen bacterial growth in vivo since it follows the carbon skeleton of the amino acid and not the readily exchanged amino group.

C.I.B. is an SERC CASE scholar with Unilever Research and M.M. is the recipient of a MAFF Postgraduate Agricultural Studentship.

Cottrill, B. R., Beever, D. E., Austin, A. R. & Osbourn, D. F. (1982). *British Journal of Nutrition* **48**, 527–541.
Maeng, W. J. & Baldwin, R. L. (1976). *Journal of Dairy Science* **59**, 648–655.

The uptake of peptides and amino acids by rumen bacteria. By P. BRONWEN COPPER and J. R. LING, *Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD*

There are many reports of the protein, amino acid and ammonia metabolism of rumen micro-organisms, but surprisingly few concerning peptide utilization.

A mixture of ^{14}C-labelled peptides was produced from a chymotryptic digest of radiolabelled, water-soluble proteins isolated from barley (*Hordeum vulgare* cv Kym) seedlings. These peptides were fractionated into five groups (A to E; mean number of amino acid residues 45, 35, 20, 12 and 7 respectively) using Sephadex G-25 fine. A sample of each peptide group was acid hydrolysed (boiling 6M-hydrochloric acid for 22 h) and the amino acid content determined by ion-exchange chromatography.

Each peptide group and products of its acid hydrolysis were separately incubated under anaerobic conditions at 39° with mixed bacteria prepared from the rumen of sheep fed on a hay and concentrate diet. Substrate concentrations were equivalent to 1 μmol constituent amino acids per ml incubation medium. Samples were removed from these incubations at intervals from 20 s to 20 min and bacterial cells were separated from the medium by centrifugation (11 600 g for 60 s) through silicon oil into perchloric acid. The bacterial pellets were removed and their ^{14}C and protein contents measured by liquid scintillation counting and the biuret method.

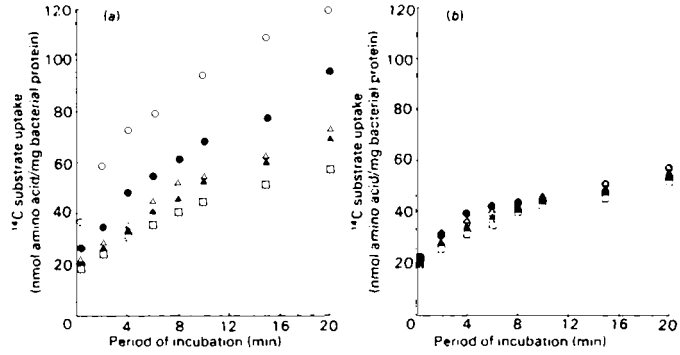

Fig. 1. The uptake of ^{14}C substrates by mixed rumen bacteria when incubated with A(○), B(●), C(△), D(▲) and E(□) as (*a*) peptide groups and (*b*) their products of acid hydrolysis.

The apparently rapid uptake before 20 s of incubation, shown in Fig. 1(*a*), is probably due to physical binding of the substrates to the bacteria, since this was not decreased in the presence of 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole, an inhibitor of active transport. Once binding had occurred, the total peptide carbon uptake by the bacteria was proportional to peptide size; the initial rates were 22·9, 10·1, 5·5, 4·1 and 3·9 nmol constituent amino acids per mg bacterial protein per min for peptide groups A to E respectively. When these peptides were acid hydrolysed, both total and initial rates of substrate uptake were considerably reduced (Fig. 1(*b*)).

These results suggest that the in vitro uptake of carbon from peptides by rumen bacteria is greater than that from amino acids. This process may confer energetic and nutritional advantages that can be exploited in the rumen.

P.B.C. acknowledges receipt of a MAFF Postgraduate Studentship.

Turnover of microbial nitrogen in the rumen of phosphorus-depleted sheep. By G. BREVES, H. HOELLER and H. W. LESSMANN, *Department of Physiology, School of Veterinary Medicine, Bischofsholer Damm* 15, *D*-3000 *Hannover* 1, *Federal Republic of Germany*

Three male castrated sheep (8–9 months of age and 48–50 kg body-weight) were adapted to a phosphorus-deficient, semi-synthetic diet consisting of pellets (660 g/d) and chopped straw (400 g/d) and providing 1·05 g P/d. Dietary N intake was 14·1 g/d, equivalent to 90 g crude protein (N × 6·25)/kg diet. The animals were fed four times daily and kept in metabolism cages. Water supply was by continuous infusion of 1·8–2·0 litres water/d. During the adaptation period and for P repletion they were supplemented by adding 3·6 g P as NaH_2PO_4. H_2O to the infusion fluid per day. Under both conditions, two experimental periods of 105–110 h each were conducted with each sheep. During these periods, CrEDTA (2·16 g/l) and $^{15}NH_4Cl$ (0·6 g/l; 96 atom %) were added to the infusion fluid. During the first 34 h after the start of marker infusion, rumen samples were taken every hour for Cr analysis, and every 4 h for isolation of rumen bacteria. From the 38th hour to the end of the experimental period, four samples were taken per day. Rumen bacteria fractions were prepared by differential centrifugation. Emission spectrometry was applied for measuring ^{15}N enrichment in the ammonia fraction of rumen fluids and in rumen bacteria.

Curves obtained for the increase of ^{15}N enrichment in microbial N were subjected to computer-aided exponential curve fitting after correcting the ^{15}N infusion rate for marker disappearance via net ammonia absorption and outflow from the rumen. The procedure described by Nolan & Leng (1983) was applied for calculating net ammonia absorption. Ammonia outflow was obtained from ammonia concentrations and fluid turnover.

Microbial N turnover results for the P depletion and repletion periods are shown in the Table.

	P depletion		P repletion		
Microbial N	Mean	SD	Mean	SD	Statistical significance
Turnover rate (/h)	0·055	0·009	0·056	0·010	NS
Pool (mg)	8100	2000	11 000	2600	$P<0·01$
Turnover (mg N/h)	430	65	590	39	$P<0·01$
Retention time (h)	18·8	3·4	18·5	3·3	NS

NS, not significant

These results are in accordance with previous findings when the flow of microbial N to the proximal duodenum measured in P depletion and repletion (Breves & Hoeller, 1985). They confirm the depressive effect of P depletion on microbial protein synthesis in the sheep rumen.

The investigations were supported by a grant of the German Research Foundation (DFG).

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Effect of monensin on net synthesis of thiamin and microbial protein in the rumen of cows. By H. HOELLER, G. BREVES, P. LEBZIEN and K. ROHR, *Department of Physiology, School of Veterinary Medicine, Bischofsholer Damm* 15, *D*-3000 *Hannover* 1, and *Department of Animal Nutrition, Agricultural Research Center, D*-3300 *Braunschweig-Voelkenrode, Federal Republic of Germany*

Ruminal thiamin net synthesis has been shown to be closely correlated with microbial protein synthesis as well as with organic matter digestion in vivo and fermentation activity in vitro (Breves *et al.* 1981; Hoeller *et al.* 1981). Since the feed additive monensin reduces microbial growth although not production of total volatile fatty acids, VFA (Wallace *et al.* 1981; Schelling, 1984), net synthesis of thiamin and of microbial protein were studied in vivo in cows with and without monensin supplementation.

Four dairy cows with rumen fistulas and duodenal cannulas were fed on a maize silage (620 g/kg)–concentrate (370 g/kg) diet, with and without sodium monensin (33 mg/kg food dry matter). Chromium oxide fixed to wheat flour was used as an indigestible marker, and labelling of microbial N was achieved by continuous infusion of [^{15}N]-urea into the rumen for 4 d. Thiamin net synthesis rate was obtained from the difference between thiamin intake and flow of the vitamin at the duodenum. Mean values for four cows are given in the Table.

	Control		Monensin	
	Mean	SD	Mean	SD
Thiamin intake (mg/d)	24·4	1·6	24·4	1·6
Thiamin at the duodenum (mg/d)	64·2	3·4	52·9*	5·9
Thiamin net synthesis (mg/d)	39·8	8·2	28·5*	1·6
Microbial N at the duodenum (g/d)	145·2	11·1	111·6*	7·2
Efficiency of microbial protein synthesis (g N/MJ ME)	1·47	0·02	1·12*	0·07

ME, metabolizable energy, *$P<0.05$.

Monensin reduced ruminal thiamin net synthesis by 28%, microbial protein synthesis by 23% and efficiency of microbial protein synthesis by 24%. Under both treatments 0·27 mg thiamin were synthesized per g microbial N leaving the rumen. The results indicate that thiamin synthesis is correlated with microbial growth rather than with fermentation activity.

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The use of growing rats in the assessment of the protein quality of bovine duodenal digesta. By M. D. EYRE and J. A. ROOKE, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

In this laboratory, Johnson *et al.* (1978) successfully included freeze-dried ovine duodenal digesta into a semi-purified diet as the only nitrogen source and measure the true digestibility (TD) and biological value (BV) of digesta N when the diets were given to rats. However, freeze-dried duodenal digesta (pH 2, 216 g ash and 42 g N/kg dry matter (DM)) obtained from cattle fed on grass silage-based diets was recently included (350 g digesta/kg diet) in a semi-purified diet as the only N source and given to rats weighing approximately 100 g. In this experiment, intakes of DM were low. Only 4·1 (SE 0·21) g DM daily (n 5) of 10 g DM offered were consumed, considerable weight losses ensued and the nutritive value of digesta N could not be measured. This is in agreement with Hewitt & Siddons (1985) who recently observed that when weanling rats were fed on a semi-purified diet containing freeze-dried ovine digesta as the only N source, rapid weight losses occurred and the rats died.

It was thought that the low pH of the digesta might have limited feed intake. Thus, sodium bicarbonate was incorporated into the semi-purified diet, where the only N source was again freeze-dried duodenal digesta (350 g digesta/kg diet), at two levels (10 or 20 g sodium bicarbonate/kg diet); diet pH was raised to 4 and 6 respectively. The duodenal digesta (pH 2, 216 g ash and 46 g N/kg digesta DM) was obtained from cattle fed on silage-based diets. Male rats, initial live weight 110 g, were offered 10 g daily of each of the digesta-containing diets or of diets containing 20 or 100 g egg albumen/kg diet (five rats/diet) for a 7 d preliminary period and then for a 7 d balance period. During the balance period, feed intakes observed for the digesta-containing diets were 59·3 (SE 2·37) g/7 d for the pH 4 diet and 62·5 (SE 0·00) g/7 d for the pH 6 diet. Mean weight gains were 3·0 (SE 0·89) g/7 d and 4·0 (SE 1·64) g/7 d for the pH 4 and 6 diets respectively. The TD and BV of the diets were 0·71 (SE 0·006) and 0·43 (SE 0·022) for the pH 4 digesta-containing diet, 0·72 (SE 0·004) and 0·50 (SE 0·020) for the pH 6 digesta-containing diet and 0·99 (SE 0·004) and 0·97 (SE 0·007) for the diet containing 100 g egg albumen/kg.

Thus, when diet pH was raised, the rats ingested most (pH 4 diet), or all (pH 6 diet), the diet offered and protein quality could be assessed. The observed values for TD and BV may be related not only to the protein source given but also to the low DM digestibility of the diets containing digesta (0·79 (SE 0·002)). Low DM digestibility of diets given to rats has been shown to affect the excretion of metabolic N in the faeces and endogenous N in the urine (Eyre, 1985) causing underestimation of TD and BV. Solutions to these problems are being sought.

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The use of the rabbit to study the effect of guar gum on the absorption of glucose from the small intestine. By A. BOBBOI and A. G. STEPHENS, *Department of Physiology & Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ*

Addition of guar gum to test meals of carbohydrate have been shown to lower post-prandial blood glucose and insulin levels in humans (Jenkins *et al.* 1976), and also to reduce the rate of glucose absorption in pigs (Rainbird *et al.* 1982). The aim of the present study was to establish an in vivo technique for investigating the effect of guar gum on glucose absorption from the small intestine in the rabbit.

Five male New Zealand White rabbits, live weight 2·0–2·5 kg, were fitted with simple T-piece cannulas in the duodenum. Following an overnight fast, 10 ml saline (9 g NaCl/l) or glucose (500 g/l) or glucose (500 g/l) with guar gum (Meyprogat 150; Meyhall UK Ltd) present at 5, 10 or 20 g/l, were infused within 1 min directly into the duodenum. Blood samples were taken from an ear vein before the infusion and at intervals up to 3·5 h after the infusion. Plasma was analysed for glucose and insulin concentrations. The experiment was conducted using a 5×5 Latin-square design.

	Plasma glucose		Plasma insulin	
Infusate (10 ml)	Peak height (mmol/l)	Area under the curve	Peak height (ng/ml)	Area under the curve
Glucose (500 g/l)	8·55	54	4·7	73
Glucose + guar gum (5 g/l)	6·72	38	4·1	52
Glucose + guar gum (10 g/l)	7·50	46	5·6	57
Glucose + guar gum (20 g/l)	6·55	30	3·4	37
SED	0·69	6·2	0·9	10

Plots of glucose and insulin concentrations against time were made and the peak heights (the difference between pre-infusion levels and the maximum concentrations observed) and the areas below the curves were measured (for mean values see Table). Following the saline treatment, plasma glucose and insulin levels were maintained at 7·50 mmol/l and 0·45 ng/ml respectively. Peak heights for glucose and insulin were generally reduced when guar gum was added although not to a significant level. The areas under the curves did show significant reductions ($P<0·05$) with the addition of guar gum.

Cannulated rabbits may prove a useful model for studying the effects of dietary fibre on nutrient absorption.

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Serine hydroxymethyltransferase (*EC* 2.1.2.1) activity in trenbolone acetate- and testosterone-treated rats. By H. P. S. MAKKAR and P. J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD*

Serine hydroxymethyltransferase (SHMT; *EC* 2.1.2.1) generates 'one-carbon' units and glycine from serine and is generally associated with growth (Schirch, 1982). The effect of trenbolone acetate (TBA) and testosterone on SHMT activity in liver, diaphragm and hind-limb muscle (gastrocnemius) is presented.

Female rats were injected with TBA or testosterone propionate (1 mg/kg initial body-weight per d) or maize oil placebo (0·1 ml) for 14 d (see Martinez *et al.* 1984 for details of diet). The SHMT activity was measured by the method of Taylor & Weissbach (1965). One unit of enzyme was equivalent to 1 μmol formaldehyde formed/min.

Both TBA and testosterone significantly increased the enzyme activity in liver, diaphragm and gastrocnemius muscle (Table). There was no change in the water content of any of the tissues studied in rats treated with TBA or testosterone.

Body-weight (g) and enzyme activity (units/g wet weight)

	Expt 1						Expt 2					
	Control			TBA			Control			Testosterone		
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*
Initial wt	112·6	0·67		113·1	0·62	18	118·4	1·10		118·6	1·49	8
Final wt	139·8	1·08		160·2	1·65	18	145·2	1·08		161·8	2·82	8
Liver	2·23	0·04	7	2·385*	0·05	7	2·71	0·02	8	2·98***	0·04	8
Diaphragm	0·0796	0·005	8	0·0996*	0·007	8	0·0825	0·004	7	0·0984**	0·004	8
Gastrocnemius muscle	0·057	0·003	12	0·0763***	0·003	12	0·05	0·001	7	0·063***	0·004	8

$P<0·05$, **$P<0·01$, ***$P<0·001$.

The increase in the SHMT activity explains the higher muscle intracellular glycine concentration observed in TBA-treated rats and increase in the efflux of glycine from perfused rat hind-limb of TBA-treated rats (see Buttery, 1978). TBA and testosterone both had similar effects on muscle SHMT activity in contrast to their different effects on muscle protein turnover (see Martinez *et al.* 1984).

H.P.S.M. is a Commonwealth Scholar.

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Some preliminary observations on the immediate effects of clenbuterol on heart rate, body temperature and nitrogen retention in lambs wholly nourished by intragastric infusion. By F. HERBERT, F. D. DeB HOVELL and P. J. REEDS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Clenbuterol, a β_2-agonist, has been shown to promote growth in sheep (Baker *et al.* 1984) but the rapidity with which it acts is unknown. The intragastric infusion of all nutrients (Ørskov *et al.* 1979) allows any immediate effects on nitrogen retention to be measured. Four lambs of between 30 and 35 kg body-weight (W) were nourished by a ruminal infusion of volatile fatty acids (650 kJ/kg $W^{0.75}$ per d) and an abomasal infusion of casein (1200 mg N/kg $W^{0.75}$ per d). Clenbuterol at 1·5 mg/d or 0·15 mg/d was administered via the abomasal infusate for 7 d. Two lambs were allocated to each level.

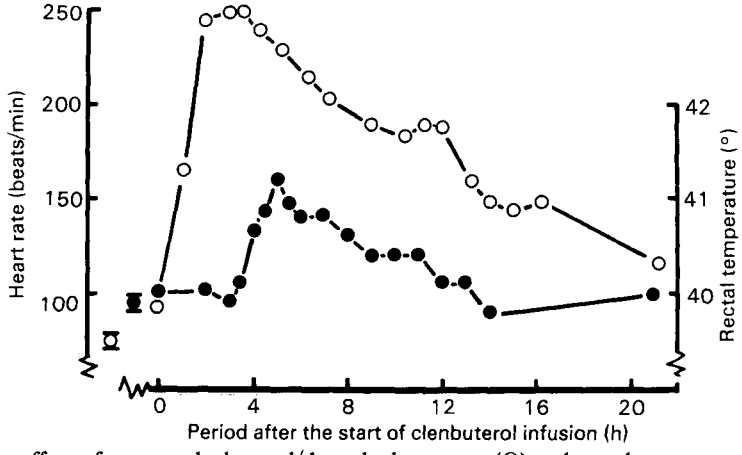

Fig. 1. The effect of 1·5 mg clenbuterol/d on the heart rate (○) and rectal temperature (●) of a 35 kg lamb. For the control period, values are means with their standard errors represented by horizontal bars.

Clenbuterol at 1·5 mg/d increased heart rate and rectal temperature in both lambs markedly. One lamb did not adapt and clenbuterol was withdrawn on the 2nd day. Both lambs increased N retention on the 1st day to 565 and 569 mg N/kg $W^{0.75}$ (control 325 and 365 (SE 24) mg N/kg $W^{0.75}$ per d). The lamb that did adapt (Fig. 1) maintained enhanced N retention at 458 (SE 37) mg N/kg $W^{0.75}$ per d. On a second challenge, 9 d later, the effect on heart rate and temperature was reduced, but N retention increased in both lambs to 457 (SE 25) and 630 (SE 45) mg N/kg $W^{0.75}$ per d (control 303 (SE 33) and 452 (SE 27) mg N/kg $W^{0.75}$ per d).

Clenbuterol at 0·15 mg/d had no effect on heart rate and temperature but N retention was enhanced in one lamb to 488 (SE 24) mg N/kg $W^{0.75}$ per d (control 352 (SE 18) mg N/kg $W^{0.75}$ per d). On a second challenge both lambs showed an improved N retention to 504 (SE 27) and 518 (SE 45) mg N/kg $W^{0.75}$ per d (control 292 (SE 14) and 263 (SE 13) mg N/kg $W^{0.75}$ per d).

Clenbuterol was a gift from Boerhinger-Ingelheim (Bracknell, Berks). F. H. acknowledges the support of a SERC studentship.

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Response of cows raising two calves to different levels of energy and undegradable protein. By M. A. SAMAD KHAN and J. H. TOPPS, *Biochemistry Division, School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Attempts to minimize the costs of calf production include the use of crossbred cows which have the ability to suckle two calves and to mobilize body reserves during periods of underfeeding in early lactation. In earlier work (Ross *et al.* 1981) it was found that the feeding of undegradable protein to such cows helped to sustain milk yields and high growth rates of calves. The present experiment has examined the response of crossbred cows and their calves to two different levels of energy and a source of undegradable protein.

Twelve Hereford × Friesian cows, each of which suckled two calves (natural and foster) were paired according to date of calving and allocated to one of two dietary treatments from the 22nd day of lactation, for 20 weeks. One diet was designed to supply the cow's requirements for energy and protein but did not contain a special source of undegradable protein (20 kg silage, 4 kg straw, 7·5 kg barley); the other was designed to supply 80% only of the cow's requirements for energy but it contained white fishmeal (20 kg silage, 4 kg straw, 3·75 kg barley, 1 kg fishmeal). The calves received good quality hay and a concentrate mixture of barley, oats, soya-bean meal, dried sugar-beet pulp and molassine meal in the ratio 8:6:3:2:1. Animals were weighed and milk consumption estimated weekly, intake of hay and concentrates by calves were measured daily and milk samples were obtained for analysis.

Cows supplied with adequate energy did not eat all the straw provided and as a result the energy intake was about 10 MJ ME less than planned. Results are summarized in the Table.

Diet supplying . . .	80% energy	Adequate energy	SED	Significance
Cows:				
W loss (kg/d)	0·28	0·21	0·104	NS
Milk yield (kg/d)	14·3	14·9	0·50	NS
Butterfat (g/kg)	37·5	45·9	2·61	$P<0·01$
Milk protein (g/kg)	32·6	30·7	0·60	$P<0·01$
Calves:				
Growth rate (kg/d)	1·05	0·98	0·048	NS
Milk intake (kg/d)	7·2	7·4	0·25	NS
Concentrate intake (kg/d)	1·27	1·10	0·294	NS
Hay intake (kg/d)	0·53	0·47	0·106	NS

NS, not significant.

The weight losses of the cows provided with an adequate level of energy were due to some animals giving more milk than anticipated and to the incomplete consumption of straw. All but one of the twenty-four calves grew well, the exception suffered from ill health which checked its growth. It may be concluded that cows raising two calves may be given either type of diet, the choice being dependent on the need to utilize the cow's body reserves and the costs of the dietary ingredients.

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A deterministic model to predict truly metabolizable energy of graminaceous forages. By R. J. DEWHURST and A. J. F. WEBSTER, *University of Bristol, Department of Animal Husbandry, Langford House, Langford, Bristol BS18 7DU* and F. W. WAINMAN and P. J. S. DEWEY, *Feedingstuffs Evaluation Unit, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Concern about the empiricism and imprecision of current equations for predicting 'truly' metabolizable energy, TME = gross energy − (faecal energy + methane energy), has encouraged the development of deterministic models to predict nutrient yield from first principles of ruminant fermentation and digestion. Our model predicts TME for graminaceous forages using feed evaluation techniques that may readily be applied in practice. The following components of the dry matter (DM) are recognized: ash, crude protein (CP; nitrogen × 6·25), ether extract, volatile fatty acids (VFA), lactate, acid-detergent lignin (ADL), β-glycans (neutral detergent fibre − ADL) and the remainder (α-glycans + simple sugars). TME is predicted from absorbed volatile and other fatty acids and amino acids. Microbial DM synthesis (0·486 CP) is the product of rumen ATP yield and its efficiency of utilization. VFA yields are calculated from the stoichiometric equations of Murphy *et al.* (1982) modified to match our forage description. Sugars and α-glycans are assumed to be wholly and rapidly fermented. β-glycans are assumed to be fermented incompletely at rates derived from Smith *et al.* (1972), also taking into account rumen outflow rate.

The model was tested on 116 forages examined by the Feedingstuffs Evaluation Unit (FEU) at the Rowett Research Institute (Wainman *et al.* 1975, 1978, 1984).

With one exception, the model predicted TME at least as well as the best simple predictor, which was, of course, only discovered retrospectively and was not consistent even within the same class of forage. Combining the eight groups yields eqn (1) which compares favourably with predictors based on in vivo measurements of digestible energy.

$$\text{Observed TME} = 1{\cdot}06 \times \text{model TME} - 0{\cdot}88 \quad r = 0{\cdot}95. \tag{1}$$

The model has the further advantage that it relates rumen outflow rate to DM intake for a given feed and species of ruminant and thus predicts the effect of plane of nutrition on TME. The decline in TME with increasing rumen outflow rate from 0·03 to 0·08/h is given by eqn (2).

$$\text{TME at } 0{\cdot}08/\text{h} = 1{\cdot}13 \times \text{TME at } 0{\cdot}03/\text{h} - 2{\cdot}43 \quad r = 0{\cdot}995. \tag{2}$$

This work was supported by SERC and Dalgety Agriculture Ltd.

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Estimation of truly absorbed amino nitrogen for grass forages. By C. J. WATERS, M. A. KITCHERSIDE and A. J. F. WEBSTER, *Department of Animal Husbandry, University of Bristol, Langford House, Langford, Bristol BS18 7DU*

The Agricultural Research Council (ARC) (1984) proposed that protein in feeds for ruminants be described in terms of truly absorbed amino nitrogen (TAAN). Webster *et al.* (1984) developed this concept to describe feeds in terms of quickly and slowly degraded N (QDN and SDN) which are equivalent to a and $(p - a)$ in the in vivo equation $p = a+bc/(c+k)$ (Ørskov & McDonald, 1979). The true digestibility of undegraded dietary N (UDN) was given by 0·9 (UDN–ADIN) where ADIN is acid-detergent insoluble N. The present paper describes a scheme for estimating TAAN in silages from their chemical composition.

Eight grass silages were first incubated in porous synthetic fibre (psf) bags in the rumen of three cows for periods of 0, 4, 8, 18, 24 and 48 h. Measurements were made of total N and ADIN in the bags after rumen incubation and subsequently after 48 h digestion in acid pepsin (*EC* 3.4.23.1). Observed values for disappearance rates of total N after incubation in the rumen agreed very closely with fitted values from the equation of Ørskov & McDonald (1979) describing such rates; residual standard deviation values for individual silages ranged from 1·03 to 4·34 mg/g. ADIN also disappeared from the psf bags, mean retention after 18 and 48 h being 507 (SE 48) and 340 (SE 22) mg/g total N respectively. If ADIN is undegradable and indigestible (Van Soest, 1982), this implies an overestimation of degradable N. However, the error incurred in ignoring ADIN disappearance for these grass silages is small since, on average, ADIN only constituted 122 mg/kg total N. Mean solubility of ADIN in acid pepsin was 212 mg/g (SE 22). This was not significantly affected by the earlier incubation time in the rumen.

Based on these and previous results (Webster *et al.* 1984), we propose the following equations to predict the yield of TAAN from grass forages when fermentable energy is not limiting.

Microbial N yield = 0·8 QDN + 1·0 SDN where QDN = a and SDN = $(p–a)$. SDN = 0·851 (N – (QDN–ADIN)) – 3·11 r^2 0·85. Truly digestible UDN, dUDN = 0·85 (UDN–ADIN) + 0·2 ADIN r^2 0·60.

This approach reduces estimated TAAN yield to 0·74–0·86 of that predicted by ARC (1984).

This work was supported by Dalgety Agriculture Ltd.

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Digestibility studies with sheep fed on diets based on concentrated grape must. By D. L. ROMNEY, D. S. PARKER and D. G. ARMSTRONG, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

The feeding of concentrated grape must (CGM) to farm livestock has been suggested as a means of reducing the surplus caused by over-production of wine in the EEC. In order to determine the potential of this product as a component of feeds for ruminants, a trial with wether sheep given increasing levels of CGM in the diet was carried out.

Eight mature sheep (mean body-weight 52·4 kg) were established on a basal diet of hay (300 g/d), soya-bean meal (80 g/d) and fishmeal (20 g/d) together with a mineral and vitamin supplement. The basal diet was then supplemented with increasing levels of CGM containing 20 g urea/kg CGM and 4 g $NaSO_4$/kg CGM. At each level of CGM consumption, such that it contributed 50, 60, 70 or 75% of total daily dry matter (DM) intake, digestibility and nitrogen balance studies were carried out. The digestibility of the basal diet when given alone was also determined. The results for the apparent digestibility coefficients are shown in the Table.

	Digestibility coefficient									
% total daily DM intake supplied as CGM . . .	0 (basal)		50		60		70		75	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DM	0·75	0·008	0·83	0·005	0·85	0·005	0·86	0·003	0·87	0·002
Organic matter	0·78	0·007	0·85	0·003	0·86	0·005	0·88	0·002	0·88	0·002
Crude protein	0·79	0·008	0·79	0·010	0·76	0·009	0·74	0·006	0·73	0·007
Acid-detergent fibre	0·70	0·011	0·63	0·009	0·62	0·011	0·56	0·013	0·54	0·010
Gross energy	0·77	0·008	0·80	0·003	0·82	0·006	0·83	0·004	0·84	0·006

CGM proved to be a palatable and highly digestible component of the feed which could be included in the diet in considerable quantities. From the results, at each level of CGM inclusion it could be calculated that the apparent digestibility of the organic matter of the CGM itself was 0·91 (SE 0·003). N balance results indicated that there was an increasing positive N balance up to the 60% level of total daily DM intake supplied as CGM but that this value declined at the higher levels. It also appears that CGM has an adverse effect on the digestibilities of the fibre and protein components of the diet when incorporated into the feed at very high levels.

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