ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Forty-first Scientific Meeting (Ninety-fifth Scottish Meeting) of the Nutrition Society was held at The Hannah Research Institute, Ayr, on Thursday, 9 March 1972, at 11.15 hours, when the following papers were read:

Energy metabolism in sheep receiving diets of barley, hay and flaked maize. By P. LAWRENCE and P. C. THOMAS,* *School of Biological Sciences, The University, Leeds*

Energy losses associated with particular patterns of ruminal fermentation were determined in five experiments each with one of two wethers fitted with a rumen cannula. In initial periods, animals received a diet of either chopped hay or a mixture of barley, flaked maize and hay. They were then transferred to a standard diet (22·5 g/kg body-weight/d) of a mixture of ground barley, ground hay and flaked maize (56:24:20). Food was provided each day in six equal meals at 4 h intervals and water (2 l/d) was infused continuously into the rumen. There was no access to drinking-water. These feeding conditions were similar to those described by Ishaque, Thomas & Rook (1971) and were designed to produce in individual sheep on different occasions fermentation patterns characterized by either a high molar percentage of propionic acid, or a high molar percentage of acetic plus butyric acids. Once a sheep was established on the standard diet, energy losses were determined over a 5 d period by respiratory quotient, and carbon and nitrogen balance techniques.

The results shown in Table 1 confirm the report of Ishaque *et al.* (1971) that 'propionic acid' fermentation patterns are accompanied by high faecal energy losses but indicate that the corresponding losses as methane and in urine are low and that there is an over-all gain in metabolizable energy. Heat losses varied between animals and between experiments but were closely correlated ($r=0·97$) with the molar percentage of acetic acid in the rumen fluid.

Table 1. *The composition of the mixture of short-chain fatty acids in the rumen fluid and intake and losses of energy in sheep receiving a diet of barley, hay and flaked maize*

Animal	C		A		
Expt no.	1	2	3	4	5
Short-chain fatty acids (molar %):					
Acetic	49·7	44·5	59·0	54·0	52·5
Propionic	28·0	29·0	18·1	27·0	29·3
Butyric	9·0	12·7	17·0	9·0	7·6
Intake (kcal/d)	5269	5300	5002	5029	4885
Losses (kcal/100 kcal dietary energy):					
In faeces	25·79	25·70	23·67	25·25	26·44
In urine	2·45	1·91	3·36	2·78	2·23
As methane	2·71	2·89	7·56	6·38	3·07
As heat	45·05	43·54	49·82†	48·59†	48·03

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†Small amounts of energy were lost as hydrogen.

REFERENCE

Ishaque, M., Thomas, P. C. & Rook, J. A. F. (1971). *Nature (New Biology)* **231**, 253.

The nutritive value of field beans (Vicia faba L.) for laying hens. By J. DAVIDSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Recent improvements in varieties of the field bean, *Vicia faba* L., in regard to yield per ha (acre) have resulted in a renewed interest in its use for feeding livestock in this country where it is one of the few crops that is rich in protein which can be grown successfully. In an experiment with Honegger laying hens, spring-sown (Maris Bead) and winter-sown (Throws MS) beans were compared with white fish meal as a source of supplementary protein in a cereal-based diet. Amino acid analysis indicated that sulphur amino acids would be deficient in the bean diets and so these diets were also tested with methionine supplementation.

Groups of forty-two Honegger hybrids, in their first laying year, were fed on diets based largely on oats supplemented with 4% protein from white fish meal of good quality or from the bean samples. The diets contained just under 12% protein, a level chosen to be sufficiently critical to demonstrate differences in quality of the supplementary protein. The predicted metabolizable energy (ME) concentrations were about 2·4 Mcal/kg. A commercial diet having about 15·5% protein and unknown ME concentration was also given.

The results given in Table 1 showed that when the bean diets were supplemented with 0·1% methionine to give a concentration of methionine similar to that in the fish meal ration, egg production did not differ significantly from that on the fish meal ration. The unsupplemented bean diets supported a significantly lower production. While production on the higher-protein commercial diet was greater than that on the fortified winter bean diet, it did not differ from that on the fortified spring bean or fish meal diets.

Table 1. *Egg production of laying hens over three 8-week periods*

(Mean values per bird)

Diet type		Egg nos.	Egg total wt (kg)	Food eaten (kg)
Fish meal		42·1	2·43	7·14
Winter bean:	−methionine	36·5	1·94	6·81
	+methionine	42·0	2·36	7·07
Spring bean:	−methionine	37·5	1·99	6·48
	+methionine	43·1	2·45	7·01
Commercial		44·1	2·55	6·58
Approximate standard error of difference between means		±1·2	±0·068	±0·20

The distribution of free amino acids between plasma and blood cells of chicks fed on different proteins. By A. G. STEPHENS* and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caernarvonshire*

It has been shown that the blood cells carry most of the free amino acids that

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are circulating in the blood of chicks, and that the distribution of free amino acids between cells and plasma is modified when the chick is deprived of dietary protein (Stephens & Evans, 1971). Using the methods already described, we have since studied the effect of dietary proteins of varying biological value.

The chicks were fed, for 3 d prior to blood sampling, on a basal protein-free diet, supplemented by the test protein. The proteins were introduced at four levels (nominally 2·5, 5·0, 7·5, 10·0 g/d) direct into the crop to avoid loss by scattering.

In Table 1 is shown the effect of diet upon aspartic and glutamic acids, which are the amino acids most affected. It can be seen that increasing the intake of protein from the lowest to the highest level had little or no effect upon the plasma concentrations of aspartic acid and glutamic acid. With egg protein, which has the highest biological value (FAO, 1970), there was similarly little change in the blood cell content. However, with the other proteins, increased protein intake led to an accumulation of both amino acids within the cells. This accumulation was greatest at the highest levels of feeding and for the protein of lowest biological value.

Other amino acids, such as alanine, did not show this response to dietary protein. It is suggested that the phenomenon described may be developed into a rapid test for protein quality.

Table 1. *Amino acids in plasma and cells of chicks fed on different proteins*

	Aspartic acid				
Amount of protein supplement (g/d)	0·0	2·5	5·0	7·5	10·0
Test protein					
Egg (BV=94%). Amino acids:					
intake (mg/d)	0	248	486	743	963
in plasma (mg/100 ml blood)	2·0	3·1	3·5	3·5	3·1
in cells (mg/100 ml blood)	1·1	1·4	1·8	1·6	2·2
Casein (BV=80%). Amino acids:					
intake (mg/d)	0	142	298	432	591
in plasma (mg/100 ml blood)	2·0	3·7	3·4	3·4	4·2
in cells (mg/100 ml blood)	1·1	2·2	4·6	7·7	11·9
Gluten (BV=58%). Amino acids:					
intake (mg/d)	0	99	196	292	383
in plasma (mg/100 ml blood)	2·0	3·3	2·9	3·2	3·4
in cells (mg/100 ml blood)	1·1	2·1	9·0	22·8	35·7
	Glutamic acid				
Amount of protein supplement (g/d)	0·0	2·5	5·0	7·5	10·0
Test protein					
Egg (BV=94%). Amino acids:					
intake (mg/d)	0	324	636	971	1259
in plasma (mg/100 ml blood)	4·8	5·9	6·5	5·7	5·2
in cells (mg/100 ml blood)	9·9	11·7	13·9	11·3	12·3
Casein (BV=80%). Amino acids:					
intake (mg/d)	0	466	918	1332	1822
in plasma (mg/100 ml blood)	4·8	9·2	8·5	8·1	10·1
in cells (mg/100 ml blood)	9·9	11·6	17·8	20·7	25·5
Gluten (BV=58%). Amino acids:					
intake (mg/d)	0	1080	2131	3175	4166
in plasma (mg/100 ml blood)	4·8	16·8	16·9	16·9	23·6
in cells (mg/100 ml blood)	9·9	10·3	21·7	33·5	49·0

BV, biological value.

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Stephens, A. G. & Evans, R. A. (1971). *Proc. Nutr. Soc.* **30**, 49A.

The free amino acids in the blood of a variety of warm-blooded animals. By LUCYNA BURACZEWSKA*, M. V. TAS, R. F. E. AXFORD and R. A. EVANS, *Department of Biochemistry and Soil Science*, and A. G. CHAMBERLAIN, *Department of Agriculture, University College of North Wales, Bangor, Caernarvonshire*

The previous communication has shown that the avian blood cell may play a significant part in the transport of amino acids (Stephens & Evans, 1972).

We have examined samples of blood taken from humans, cattle, guinea-pigs, horses, mice, pigs, pigeons, rabbits, rats and sheep to see if the blood cells contained appreciable amounts of amino acids, and to see if these were distributed in the same proportion as in plasma. No control over food intake or composition was attempted.

The free amino acids were determined in plasma and lysed whole blood after deproteinization with sulphosalicylic acid and hydrolysis of amides to the free acids. The percentage plasma volume was determined by Evans blue dilution in a sample of the whole blood.

Of the total amount of amino acid found in whole blood, roughly a half was carried within the cells. The concentrations of individual amino acids in cells and plasma differed in all species, indicating selective mechanisms in the cell walls or disparate rates of metabolism within and without these cells. In particular, glutamic acid appears to be concentrated in the cell whereas the branched chain amino acids were found to be carried mostly in plasma.

It is suggested that studies of plasma free amino acids should be extended to include measurements on the cellular components of blood. These may yield significant information of the quality of dietary protein for other species as well as for the chick.

REFERENCE

Stephens, A. G. & Evans, R. A. (1972). *Proc. Nutr. Soc.* **31**, 50A.

Blood flow, oxygen consumption and thermogenesis in the portal-drained viscera of conscious sheep. By A. J. F. WEBSTER and F. WHITE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The continuous thermal dilution for measurement of blood flow (Linzell, 1966) has been applied to the measurement of portal venous flow in the conscious sheep.

The temperature of portal venous blood was measured using an individually calibrated copper–constantan thermocouple embedded in a polyvinyl cathether and

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inserted via a tributary of the anterior mesenteric vein. A Rikadenki potentiometric recording apparatus was calibrated to give a pen movement of 5 cm per 1° temperature change. Another catheter was inserted via a tributary of the right rumen vein into the gastrosplenic vein. Physiological saline at room temperature was infused for 30 s into the gastrosplenic vein at a rate of 0·5–2·0 ml/s to cool the mixed portal venous blood.

Eleven preparations have been made to date, of which seven have yielded successful results for periods of about 1 month after surgery. In three of the four unsuccessful preparations the portal vein was very short and did not retain the thermocouple.

In the most recent preparations double-bore catheters, one bore containing a thermocouple, have been inserted into the portal vein and via a branch of the carotid artery into the aortic arch. These preparations permit the simultaneous measurement of portal blood flow (ml/min) and its temperature, and oxygen concentration (ml/100 ml) of arterial and portal venous blood. From these measurements estimates may be made of the oxygen consumption ($\dot{V}o_2$) and the total heat production of the portal drained viscera (Durotoye & Grayson, 1971).

Table 1. *Portal blood flow and temperature, visceral oxygen consumption, aerobic and total thermogenesis in sheep before, during and after eating a meal of dried grass*

	Portal blood flow (ml/min)	Portal venous temperature (°C)	Visceral Vo_2 (ml/min)	Visceral thermogenesis, cal/min, (J/h)	
				Aerobic	Total
No. of experiments	8	8	4	4	6
Before eating	2329	39·73	59·7	290 (73)	348 (87)
During eating*	2286	40·34	55·5	271 (68)	523 (131)
After eating (2–6 h)	2642	40·36	58·1	284 (71)	709 (178)

*Mean consumption of dried grass was 480 g. Time during eating refers to the first 2 h after food was offered.

Portal blood flow before eating was about 2300 ml/min (Table 1). Blood flow did not alter significantly during eating but increased substantially thereafter to a peak 4–6 h after eating commenced. Portal venous temperature increased sharply at the beginning of eating and remained at an elevated level for about 6 h thereafter. Aerobic metabolic rate (cal/min) was taken as $\dot{V}o_2 \times 4{\cdot}89$. Before eating, aerobic metabolism accounted for about 80% of total visceral thermogenesis. During and after eating, $\dot{V}o_2$ did not alter significantly. Total thermogenesis, however, increased from about 350 cal/min to over 700 cal/min about 6 h after the meal. The difference between total and aerobic thermogenesis can be attributed to the heat of fermentation by the rumen micro-organisms, which increased from about 60 cal/min, 24 h after the previous feed, to about 420 cal/min at the time of maximum fermentation rate.

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Evaluation of a rumen fermentation balance, corrected for cell synthesis.

By D. DEMEYER, H. HENDERICKX and C. VAN NEVEL, *Department of Nutrition and Hygiene, State University, 9000 Gent, Belgium*

The amino acid composition, protein content and DNA content of mixed rumen micro-organisms were determined and found to be relatively constant. From these and other results (Keeney, Katz & Allison, 1962; Baldwin, 1970; Mason & Palmer, 1971) it was calculated that rumen microbial dry matter (DM) contains 10·72% nitrogen (83·5% being protein N), 46·16% carbon, 6·32% hydrogen and 30·66% oxygen. The CHO fraction can be represented by the formula $C_6H_{9\cdot85}O_{2\cdot99}$. Rumen samples, obtained from fistulated wethers, were incubated with glucose and NH_4HCO_3. The amounts of end-products that were produced were reported earlier (Demeyer, Van Nevel, Henderickx & Martin, 1970). From the amounts of inorganic N incorporated into cellular material, which are similar for rumen contents with a high- or low-propionate fermentation pattern, it was calculated that 40 g of cellular DM were formed per mol of substrate digested (digested=fermented+incorporated into cells). It was assumed that 50% of the cellular CHO fraction was formed from the substrate, using hydrogen derived from the fermentation following:

$$C_6H_{12}O_6 + 1{\cdot}93\ (2H) \rightarrow C_6H_{9\cdot85}O_{2\cdot99} + 3{\cdot}01\ H_2O.$$

It can then be calculated that synthesis of 1 g of cellular DM requires 0·0061 mol of 2H. The discrepancy observed in hydrogen recovery, calculated from fermentation balances as described earlier (Demeyer *et al.* 1970), can thus be accounted for: 95–99% of the theoretical amount of hydrogen was recovered in methane, propionate, butyrate and cell material.

A fermentation balance, accounting for cell synthesis (40 g of cellular DM per mol of hexose digested) and hydrogen used in cell synthesis (0·0061 mol of 2H per g of cellular DM) can be evaluated using values for carbohydrate intake, volatile fatty acid proportions in the rumen and methane production, obtained in whole-animal experiments with diets completely digested in the rumen. Methane production as determined by various authors (Coppock, Flatt, Moore & Stewart, 1964; Seeley, Armstrong & MacRae, 1969; Leng & Murray, 1971) was compared with values calculated from this stoichiometry: a mean value of 101·5% was obtained for (methane calculated ÷ methane observed) × 100. (Extreme values were 89% and 116%.)

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The energy metabolism of sheep—the effect of infusing small amounts of chloroform into the rumen. By J. L. CLAPPERTON and J. W. CZERKAWSKI, *The Hannah Research Institute, Ayr KA6 5HL*

Bauchop (1967) showed that when chloroform was added to rumen contents incubated in vitro, a concentration of 8 μmol/l caused the complete inhibition of methane production and resulted in an increase in hydrogen production. Further experiments in vitro and preliminary experiments (Harfoot & Czerkawski, 1971) in which the methane production of sheep given chloroform into the rumen was estimated indirectly by incubating their rumen contents confirmed the results of Bauchop. In the experiments reported here, the effect of chloroform on the energy metabolism of sheep was measured in vivo.

Two Down-Cross castrated male sheep were given daily 500 g medium-quality hay and 500 g long, dried grass in two equal feeds. The animals were confined in a respiration calorimeter and their energy metabolism was measured. Each experiment consisted of an initial control period of 5 d, an infusion period of about 10 d, during which 440 mg chloroform dissolved in 600 ml water were infused through a rumen cannula while the sheep was eating its food, and a final control period of about 10 d. The mean of the results obtained in the last 5 d of the control and of the experimental periods are shown in Table 1.

There was no effect on the faecal energy or urinary energy loss of the sheep. Because of the reduction in the loss of energy as methane and hydrogen, the metabolizable energy intake of the sheep increased by 680 kJ/d. The heat production rose by 450 kJ/d, thus the energy stored by the animals increased by 230 kJ/d as a result of infusing chloroform into the rumen.

Methane is produced in the rumen from 1 mol of carbon dioxide and 4 mol of hydrogen. Thus, a decrease in methane production of 23 l/d should make 92 l/d hydrogen available for other metabolic processes. Since the hydrogen production was only 18 l/d, there are some 74 l/d hydrogen unaccounted for. The increase of 29 l/d in the oxygen consumption suggests that most of the hydrogen is oxidized but whether this takes place in the rumen or in the body is not known.

Table 1. *The consumption of oxygen and the production of carbon dioxide, methane, hydrogen and heat in two sheep*

	Control	Infusion of chloroform	Change
O_2 consumption (l/d)	357±14	386±9	+ 29
CO_2 production (l/d)	364±1·0	372±5	+ 8
CH_4 production (l/d)	23·8±1·4	0·9±0·4	− 23
H_2 production (l/d)	0	17·7±2·9	+ 18
Heat production (kJ/d)	7457±234	7909±238	+452

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The effect of processing barley on its digestion in the rumen of growing steers. By A. PAVLIČEVIĆ, N. A. MACLEOD and M. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Experiments made with growing steers (Whitelaw and MacLeod, unpublished) showed that the digestibility of pelleted diets containing rolled or whole barley was similar whereas MacLeod, Macdearmid & Kay (1971) demonstrated that the digestibility of the diet was 10% lower when whole barley containing 86% dry matter was given in an unpelleted mixture. The purpose of this experiment was to measure the extent of the starch breakdown in the rumen of growing steers when given diets containing barley presented in different physical forms.

Two Friesian steers with permanent cannulas in their abomasums were given diets containing: (1) rolled barley in a loose mixture, (2) rolled barley in a pellet, (3) whole barley in a loose mixture, (4) whole barley in a pellet. All diets were supplemented with 10% soya-bean meal and a mineral–vitamin supplement and were given twice daily in amounts calculated to supply 80 g dry matter per $(kg)^{0.75}$ live weight. The diets were each given for 3 weeks and samples of digesta were taken from the abomasum at 2 h intervals over 24 h during the 18th and 21st day of each period. A sample of rumen liquor was withdrawn by stomach-tube 3 h after feeding on the last day of each period for the measurement of rumen pH and the molar proportion of volatile fatty acids. Chromic oxide impregnated in paper, given in capsules, was used as an indigestible marker. The main results are given in Table 1.

Table 1. *Dry matter and starch in the feed, and passing through the abomasum of steers given barley*

			Passing abomasum		
Treatment	Dry-matter intake (g/d)	Starch intake (g/d)	Dry-matter (g/d)	Starch (g/d)	Abomasal starch ÷ feed starch (%)
Rolled barley, loose	3360	1630	2815	105	6·4
Rolled barley, pellets	2750	1265	2440	150	11·8
Whole barley, loose	4030	1850	2870	680	36·7
Whole barley, pellets	2940	1195	2345	340	26·4

The amount of starch passing through the abomasum was increased when whole barley was given in either a loose mixture or a pellet. Pelleting the whole barley reduced the amount of starch reaching the abomasum whereas pelleting the rolled barley had the opposite effect. There were no significant differences in either rumen pH or in the molar proportions of volatile fatty acids in the rumen liquor; although rumen pH tended to be lower with the pelleted diets.

REFERENCE

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The value of different cereals in diets for growing steers. By M. KAY, N. A. MACLEOD and A. PAVLIČEVIĆ, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Thirty-two Friesian steers were grown from 100 to 400 kg live weight on pelleted diets containing whole wheat (W), whole maize (M), whole barley (B) and whole oats (O). Diets W and M contained 15% oats to minimize the risk of digestive disturbances. In addition, four Friesian steers were used in a trial of Latin square design to measure digestibility with the above diets at an equalized food intake. In another two steers, fitted with permanent cannulas in their abomasums, measurements were made of the extent of starch breakdown in the rumen when pelleted diets containing only a single cereal were given instead of a mixture of wheat:oats and maize:oats as in the growth and digestibility trials. Chromic oxide impregnated in paper, given in capsules, was used as an indigestible marker.

Dry-matter intakes and rates of gain (kg/d) were: W, 5·83 and 1·12; M, 5·96 and 1·27; B, 6·32 and 1·22; O, 6·49 and 1·18. The dry-matter digestibility coefficients for the diets were: W, 81·3; M, 79·5; B, 77·3; O, 70·4. The main results obtained with the cannulated steers are given in Table 1.

Table 1. *Amounts of dry matter, starch and nitrogen eaten and flowing through the abomasum of young steers*

Dietary treatment	Dry-matter intake (g/d)	Starch intake (g/d)	N intake (g/d)	Components passing abomasum (g/d)		Proportion of bacterial N in abomasal N
				Starch	N	%
Wheat	3910	2253	75	395	94	74
Maize	3930	2564	68	1008	77	54
Barley	4510	2098	73	398	93	72
Oats	3240	1566	61	417	80	76

Approximately 40% of the maize starch escaped breakdown in the rumen and the proportion of bacterial nitrogen, estimated from the diaminopimelic acid content of the digesta, in the nitrogen passing through the abomasum was lower with maize than with the other cereals. The proportion of dietary starch escaping rumen fermentation with oats was greater than with either wheat or barley.

The chemical composition and dilution rate of rumen fluid in sheep receiving a diet of barley, hay and flaked maize. By J. C. HODGSON and P. C. THOMAS, *The Hannah Research Institute, Ayr KA6 5HL*

Four wether sheep fitted with ruminal cannulas were used. Each animal received a standard diet (22·5 g/kg body-weight per d) of a mixture of ground barley, ground hay and flaked maize (56:24:20) during two 16 d periods; samples of rumen liquor were taken for analysis during days 12–16 of each period. One period was preceded by an introductory period when the animal was given a diet of chopped hay, the second by an introductory period when the animal was given a mixture of barley, flaked maize and hay. The feeding procedure was as described by Ishaque, Thomas

& Rook (1971) and was designed to establish in each sheep fermentation patterns that were characterized either by a high molar percentage of propionic acid, or a high molar percentage of acetic plus butyric acids.

One sheep was withdrawn before the end of the experiment and thus the digestion of the standard diet was studied on seven occasions. On four occasions the mixture of short-chain fatty acids in the rumen fluid was characterized by a high molar percentage of propionic acid (average composition: acetic acid, $52{\cdot}3 \pm 3{\cdot}9$; propionic acid, $32{\cdot}3 \pm 2{\cdot}4$; butyric acid, $10{\cdot}6 \pm 1{\cdot}5$) and on three occasions by a low molar percentage (average composition: acetic acid, $59{\cdot}9 \pm 1{\cdot}2$; propionic acid, $18{\cdot}4 \pm 1{\cdot}4$; butyric acid, $17{\cdot}5 \pm 0{\cdot}6$). There were no significant differences between the two fermentation pattern groups in rumen pH or in the concentration of sodium, potassium, calcium, magnesium, phosphorus, chloride and bicarbonate in rumen fluid but the concentrations of ammonia, $5{\cdot}5 \pm 1{\cdot}6$ and $21{\cdot}6 \pm 5{\cdot}4$ respectively, were significantly different.

Rumen volume, calculated using polyethylene glycol as a marker, varied between treatments and between animals irrespective of fermentation pattern but the turnover rate of the rumen liquid phase (volumes/d) differed significantly ($P<0{\cdot}05$) between the two fermentation pattern groups; mean values were $0{\cdot}9 \pm 0{\cdot}2$ and $2{\cdot}0 \pm 0{\cdot}4$. For all values there was a significant ($P<0{\cdot}05$) regression of the molar percentage of propionic acid in the rumen fluid on the turnover rate ($y=37{\cdot}27 - 8{\cdot}05x$; $r=-0{\cdot}72$, $n=7$). However, the relationship varied with the introductory diet. For animals introduced to the standard diet from hay, the regression was $y=29{\cdot}27-4{\cdot}51x$ ($r=-0{\cdot}99$, $n=3$), and for animals introduced from the diet of barley, hay and flaked maize the regression was $y=58{\cdot}21-23{\cdot}98x$ ($r=-0{\cdot}95$, $n=4$), although the number of observations was small.

The results indicate that the pattern of ruminal fermentation is closely associated with the rate of the dilution of the rumen liquid phase. However, the relationship between a single characteristic of rumen fermentation, such as the molar percentage of propionic acid and the turnover rate, varies with the previous diet and presumably with the microbial population of the rumen.

One of us (J.C.H.) is supported by a Scholarship from the Meat and Livestock Commission.

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Ishaque, M., Thomas, P. C. & Rook, J. A. F. (1971). *Nature (New Biology)* **231**, 253.

Partition of energy and nitrogen digestion in sheep given fresh pasture species. By J. C. MACRAE* and M. J. ULYATT, *Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand*

Three pasture species (*Lolium perenne* L., *cv.* Grasslands Ruanui perennial rye-grass (P); *L. perenne* × *L. multiflorum* Lam., *cv.* Grasslands Manawa short-rotation rye-grass (S); and *Trifolium repens* L., *cv.* Grasslands 4700 white clover (C)) were

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given twice daily as fresh herbage to Romney Marsh wether sheep prepared with rumen cannulas and with re-entrant duodenal and ileal cannulas. Chromic oxide-impregnated paper was administered, via the rumen fistula, at each feeding time as a digesta marker. The sheep were given various intakes between 500 and 1000 g dry matter daily, and the quantities of energy and nitrogen flowing past the duodenum, ileum and being excreted in the faeces were regressed against energy or nitrogen intake. All regressions were significant ($P<0{\cdot}01$).

Apparent digestibilities of energy and nitrogen in the stomach region, the small intestine and the large intestine were calculated from the regression equations at energy intakes (kcal/24 h) of maintenance (M) and twice maintenance (2M) for a 40 kg sheep and are given in Table 1. Similarly, partitions of nitrogen digestion at nitrogen intakes (g/24 h) corresponding to M and 2M energy intakes are also presented in the table.

Table 1. *Apparent digestibilities of energy and nitrogen in the stomach region, small intestine and large intestine, and partition of N digestion at N intakes corresponding to maintenance (M) and twice maintenance (2M) energy intakes in sheep given three pasture species, P, S, and C**

	Energy					
	P		S		C	
	M	2M	M	2M	M	2M
Intake (kcal/24 h)	2068	4152	2049	4082	1927	4078
Apparent digestibility (%)	77	76	77	78	82	78
% of digestion occurring in:						
Stomachs	51	62	43	48	58	60
Small intestine	35	24	43	37	24	31
Large intestine	14	14	14	15	18	9
	Nitrogen					
Intake (g/24 h)	18	39	16	35	16	35
Apparent digestibility (%)	82	85	84	82	83	82
% of digestion occurring in:						
Stomachs	35	43	1	24	36	32
Small intestine	46	46	81	67	53	59
Large intestine	19	11	18	9	11	9

*For explanation of P, S, and C, see text.

Although there was little difference between species in the apparent digestibilities of energy and nitrogen, there were considerable differences in the sites at which the digested energy and nitrogen disappeared. Apparent digestion in the stomach region was lower in sheep given S than those given P or C. Conversely, apparent digestion in the small intestine of energy and nitrogen was greatest at both intake levels in sheep given S.

Partition of digestion changed as intake increased from M to 2M. With the grasses, more energy and nitrogen were digested in the stomachs at 2M but, with the exception of P nitrogen, there were decreased amounts digested in the small intestine.

However, in sheep given C there was little change in stomach digestion but an increase in both energy and nitrogen digested in the small intestine, and a decrease of energy digested in the large intestine.

Estimation of food and microbial protein in duodenal digesta. By D. G. HARRISON, D. E. BEEVER and D. J. THOMSON, *Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR*

Studies on protein digestion within the alimentary tract of ruminants have been limited by the inability to distinguish quantitatively between the food protein and microbial protein in duodenal digesta. The infusion of $Na_2{}^{35}SO_4$ into the sheep rumen will yield a duodenal digesta containing labelled sulphur amino acids derived from protozoa and bacteria (Henderickx, 1961). If food protein is absent from the duodenal digesta the specific activity of the methionine of the whole labelled digesta (D) will approximately equal the specific activity of the methionine of the microbial protein (M). Thus the ratio of these two specific activities (M:D) will approximately equal 1. The presence of food S amino acids in the digesta will decrease the specific activity of the whole digesta and thus increase the value for M:D; this increased value (M^F:D^F) can be used to estimate the relative proportions of microbial and food protein in the duodenal digesta,

$$\text{i.e. proportion of microbial protein in duodenal digesta} = \frac{M \div D}{M^F \div D^F}.$$

The proportion of food protein will be obtained as the complement of the above ratio. The practical use of this theoretical method requires that the value for M:D, obtained when no food protein is present in duodenal digesta, should be constant (with a value of approximately 1) regardless of the relative proportions of bacterial and protozoal protein in the digesta. This was established in the following manner.

A sheep was given a purified, cellulose-based diet containing 7·5% urea as the sole nitrogen source, and $Na_2{}^{35}SO_4$ was infused intraruminally at a rate of 10 μCi/h for 24 h. The post-infusion rumen protozoal count was approximately 500 000/ml. After infusion, three samples of duodenal digesta were collected and a portion of each centrifuged at 3000$\times g$ for 5 min; the resultant supernatant was centrifuged at 20 000$\times g$ to yield a 'microbial' precipitate. The specific activities of the 'microbial' samples ('M') and the whole digesta (D) were determined, giving a mean 'M':D ratio of 1·02. The animal was then defaunated with dioctyl sulphosuccinate, giving rumen protozoal count of zero, and the above procedure repeated. The 'M':D ratio was 0·99. Thus the over-all value for 'M':D with duodenal digesta containing no food protein was established as being approximately 1 regardless of the presence or absence of protozoa in the rumen; this indicated that the 'microbial' fraction isolated by centrifugation was representative of the whole microbial protein in the digesta. Thus the infusion of $Na_2{}^{35}SO_4$ coupled with the use of the above equation appears to offer a convenient method for estimating the proportion of food protein in duodenal digesta.

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Determination of the quantities of food and microbial nitrogen in duodenal digesta. By D. E. BEEVER, D. G. HARRISON and D. J. THOMSON, *Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR*

In a preliminary study, the method proposed by Harrison, Beever & Thomson (1972) for the estimation of food and microbial nitrogen in duodenal digesta was examined with a sheep fitted with a rumen cannula and a re-entrant cannula at the proximal duodenum. A dried sainfoin diet (900 g dry matter/24h) was given either hourly in twenty-four equal feeds (H) or twice daily, at 09·00 hours and 17·00 hours, in two equal feeds (T).

In experiment (H), ^{35}S-inorganic sulphate was infused continuously into the rumen for 120 h and samples of duodenal digesta were removed every 12 h. The maximum incorporation of ^{35}S was achieved between 36 and 48 h after infusion commenced, and the mean ratio obtained was $3{\cdot}27 \pm 0{\cdot}07$, indicating that 69·4% of the duodenal N was from undegraded food. The quantities of total N, food N and microbial N at the duodenum are shown in Table 1.

Comparison of the ratio obtained during the first 48 h ($3{\cdot}29 \pm 0{\cdot}11$) and the last 72 h ($3{\cdot}26 \pm 0{\cdot}10$) indicated proportions of microbial N in the duodenal N of 30·4% and 30·6% respectively. This suggested that use of the ratio method would be valid with short infusion periods and before the point of maximum incorporation was achieved.

In the second experiment (T), ^{35}S was infused continuously into the rumen for only 34 h, with an initial 10 h 'priming' period followed by total collection of duodenal digesta every 2 h for 24 h. Separate analysis and summation of the results from the twelve 2-hourly samples gave an estimated flow rate of 18·02 g food N/24 h at the duodenum, or 63·2% of the total N entering the duodenum. During this period the percentage of food N in the total duodenal N varied from 86 immediately post-feeding to 44 at 8–10 h post-feeding.

Table 1. *The quantities (g nitrogen/24 h) of food N and microbial N entering the duodenum of a sheep given dried sainfoin hourly (H) or twice daily (T)*

	Food N	Microbial N	Total N
H	21·94	9·68	31·62
T	18·02	10·49	28·50

The ratio determined on a bulked sample derived from samples of the individual 2 hourly samples was 2·62, indicating that 61·8% of the duodenal N was of food origin (i.e. 17·61 g); this was in good agreement with the mean value based on the individual samples.

The use of ^{35}S and the ratio technique to distinguish food and microbial N in the duodenal digesta of sheep appears satisfactory. Samples of duodenal digesta taken from a sheep fed twice daily showed a wide variation in the ratio of food N to

microbial N, but a composite sample based on 2-h samples gave a valid estimate of the food:bacterial N ration.

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Changing patterns of fermentation and mineral absorption in the large intestine of lambs weaned from milk to concentrates. By M. G. ROBSON* and R. N. B. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The large intestine of adult ruminants seems unimportant as a site for absorption of calcium, magnesium and phosphorus (Smith, 1969). However, Smith (1962) has shown that milk-fed calves absorb large amounts of Mg from the large intestine during the 1st month of life. We have therefore made a pilot study in lambs to see if changes in absorption can be related to changing patterns of fermentation (Robson, 1971).

Three lambs were provided with cannulas in the terminal ileum and caecum, and the experiment began when they were 4 weeks old. During weeks 1–3 of the experiment (5–6 weeks of age) the lambs received raw cows' milk, 1·1 l (2 pints), then 1·7 l (3 pints) per d, at 08·00, 14·00 and 20·00 hours. This was supplemented with pelleted barley, 200 g/d, during weeks 4 and 5, and the lambs received the barley diet only, 400 then 600 g/d, during weeks 6–9. Chromium-EDTA was included in both milk and pelleted diets as an indigestible reference substance. For 24 h in each week faeces were collected and samples were drawn from the cannulas at 2 h intervals for analysis. Separate samples were drawn for bacteriological examination, kindly made by Mr S. O. Mann.

The caecal contents were quite acid during weeks 1–4, often falling to pH 5–6 after feeding. During this period the concentration of total volatile fatty acid was about 55 (range 40–90) mmol/l and that of lactic acid about 20 (0–60) mmol/l. Lactobacilli were present in abundance and sometimes formed the predominant organisms. After milk was withdrawn from the diet (week 6) caecal reaction rose to pH 6–7, volatile fatty acid concentration increased to about 80 (60–100) mmol/l, lactic acid virtually disappeared and lactobacilli became unimportant.

Net mineral absorption from the large intestine was assessed by comparing mineral:Cr-EDTA ratios in ileal contents and faeces. Absorption of P remained close to zero throughout the experiment. Substantial amounts of Ca and Mg were absorbed during the first 2 weeks of the experiment, averaging 30 and 44% respectively of the amounts consumed in the milk. From week 3 onwards this absorption became irregular and small, Ca absorption averaging 0% and Mg absorption 12% of intake.

The acid condition of the contents of the large intestine of the milk-fed lamb might be expected to render Ca and Mg more available for absorption and so

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contribute to the marked absorption observed in the first 2 weeks of the experiment. However, this absorption declined 3 weeks before milk was withdrawn and the lactate fermentation subsided, suggesting that the permeability of the epithelium of the lamb's large intestine may decrease at about 6 weeks of age, whatever the diet.

One of us (M.G.R.) was supported by a Postgraduate Studentship from the Department of Agriculture and Fisheries for Scotland.

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Factors influencing the total time spent chewing by sheep given diets containing long, dried forages. By S. B. CAMMELL and D. F. OSBOURN, *Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR*

The total time spent chewing during eating and ruminating, per kg of dry matter (DM) eaten, has been proposed by Balch (1969) as an index of the fibrousness of diets for ruminant animals. Change-over trials using mature sheep given diets based upon long, dried forages have been undertaken to investigate the factors influencing this index. Two forages containing the same concentrations of digestible DM but different concentrations (67·9 and 53·8%) of cell-wall constituents (Van Soest & Wine, 1967) were fed to rumen-fistulated sheep at the rate of 20 g DM/kg live weight. The mean indices observed were 558 and 450±21 min/kg DM for the forages of high- and low-fibre content respectively. When these two forages were given in mixture with a barley-based concentrate (18·1%, cell-wall constituents) the index declined linearly with decreasing concentration of cell-wall constituents in the total diet:

index of fibrousness = 9·33 cell-wall constituents − 61·9; r = 0·99, RSD = ±7·7.

In a second study, herbage of two varieties of perennial rye-grass, (D and T) having the same concentrations of both digestible DM and cell-wall constituents in the DM, were fed *ad lib.* Previous laboratory studies (Osbourn, 1970) had indicated that variety T was more resistant to comminution than variety D. The voluntary consumption of the two varieties by non-fistulated sheep was the same but the index of time spent chewing was higher ($P<0·05$) for variety T than for variety D (335 and 302±17·7 min/kg DM eaten).

Both fistulated and non-fistulated sheep consuming equal restricted quantities of a hay diet spent similar periods of time chewing (675 and 664 min/kg DM). When given the same diet *ad lib.* the non-fistulated animals ate more hay and spent less time chewing than the fistulated sheep (23·7 and 16·4 g DM/kg live weight/d and 505 and 631±21·3 min/kg DM eaten). It was concluded that for diets containing long forages this index is influenced primarily by the concentration of cell-wall

constituents in the diet (see also Welch & Smith, 1969*b*). In addition, the index is influenced also by the resistance of the forage to comminution. However, in line with the observations made by Welch & Smith (1969*a*) on time spent ruminating, these results suggest that the index of total time spent chewing does not remain constant for a given diet when consumption approaches *ad lib.* levels.

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The utilization of starch in the small intestine of sheep and the possible long-term adaptation. By R. W. MAYES* and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In an attempt to study long-term adaptation to the utilization of starch by the ruminant small intestine, a solution of gelled maize starch (7%, w/v) was continuously infused into the abomasum over a 4 week period into two fistulated lambs, 5–9 weeks (27 g starch/kg live weight$^{0.75}$ per d) and 13–17 weeks of age (21 g starch/kg live weight$^{0.75}$ per d), and into two mature fistulated sheep (15 g/kg live weight$^{0.75}$ per d), the infusion rate in each case being such that the faeces were soft but pelleted. The starch solution contained chromium-EDTA complex (0·7 g/l as chromium sesquioxide) as a water-soluble indigestible marker. The animals were offered dried grass to appetite.

Samples of digesta removed from the terminal ileum at 3 d intervals throughout the infusion period were analysed for total α-glucose polymers, α-glucose polymers not precipitated by hot 90% (v/v) ethanol (both determined by a modification of the enzymic method of MacRae & Armstrong (1968)), unpolymerized glucose and chromium. Hence by difference the contents in ileal digesta of starch (ethanol-precipitable α-glucose polymers) and α-glucose oligosaccharides (non-precipitable α-glucose polymers) could be estimated. Further separation of the non-precipitable glucoside fraction into individual sugars was achieved by paper-chromatography.

The relative proportions of the individual oligosaccharide glucosides (the oligosaccharide of longest chain-length separated, being maltotetraose) were similar and did not change throughout the infusion period. The percentage net disappearance of total α-linked glucose polymers in the small intestine was rather low in all animals (58%). The percentage disappearance of starch (representing conversion to oligosaccharide) was high (92%), whereas the percentage disappearance of oligosaccharide (the conversion of oligosaccharide released from starch digestion to unpolymerized glucose) was lower (69%). There were no tendencies for these percentage disappearance values to change with time.

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These results suggest that on the whole there was no adaptation in the utilization of starch in the small intestine as a response to large quantities of gelled maize starch being administered into the abomasum over long periods. They also suggest that the factor limiting in the intestinal utilization of gelled starch is probably the digestion of oligosaccharide, presumably by intestinal maltase.

The finding that the gelled starch was very effectively digested to short-chain oligosaccharides may suggest that pancreatic α-amylase was not limiting its digestion. The necessity for amylase to be present was investigated using a lamb with its pancreatic duct exteriorized. When pancreatic juice was not returned to the animal and gelled starch was infused into the abomasum, the α-linked glucose polymer fraction from lamb ileal fluid consisted almost entirely of starch. When pancreatic juice was returned to the animal the fluid contained large amounts of short-chain oligosaccharides.

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Faecal magnesium output and plasma Mg concentration in sheep.

By T. F. Allsop and J. A. F. Rook*, *School of Agricultural Sciences, University of Leeds, Leeds LS2 9JT*

In an earlier communication (Allsop & Rook, 1970), a technique, for the study of the relationship between faecal magnesium output and plasma Mg concentration, was described in which sheep were given artificial, Mg-free diets and supplied with Mg in a continuous intravenous infusion. A total of thirty-two experiments, involving eight sheep and three artificial diets, have now been completed and a small, but statistically significant increase in faecal Mg output (g/d) with increase in plasma Mg concentration (mg/100 ml) has been demonstrated ($y=0{\cdot}028x+0{\cdot}070$).

In a further, similar study, two sheep fitted with duodenal and ileal cannulas were given an artificial diet (washed straw, 27%; cellulose, 27%; maize starch 27%; casein, minerals and vitamins, 19%) with added polyethylene glycol (PEG) as a marker. The relative concentrations of Mg to PEG in food, faeces and duodenal and ileal contents were:

Sheep no.	Plasma Mg concentration (mg/100 ml)	Diet	Duodenal contents	Ileal contents	Faeces
5	0·32	0·63	2·41	2·14	0·80
	4·18	0·70	6·70	7·14	2·79
7	0·56	0·64	2·53	3·49	0·81
	3·13	0·67	3·43	4·53	2·16
	5·13	0·69	5·42	7·22	2·76

Thus, there was a net entry of Mg prior to the duodenum and, to a smaller extent, between the duodenum and ileum, and a considerable net resorption of Mg after the ileum.

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In sheep receiving natural diets, the increase in faecal Mg output (g/d) in response to an increase in plasma Mg concentration (mg/100 ml), over the range of about 3-4 mg/100 ml, was much greater (frozen grass, $y=0{\cdot}157x+0{\cdot}01$; hay and barley, $y=0{\cdot}186x+0{\cdot}22$) than for artificial diets. It appears that the increase in plasma Mg concentration reduces the absorption of dietary Mg.

One of us (T.F.A.) is in receipt of a grant from the New Zealand Department of Agriculture.

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The digestion and utilization of grass processed by wafering or by grinding and pelleting. By D. J. THOMSON and D. E. BEEVER, *Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR*

The utilization of the dietary energy contained in a dried grass (cock's-foot) (*Dactylis glomerata* L. var. S37) processed into wafers or pellets was measured in a comparative slaughter experiment using growing lambs. The digestion of these diets was also studied using sheep fitted with re-entrant cannulas. The chopped dried cock's-foot was processed through a Glomera piston press into wafers, 50 mm in diameter, which were split radially into halves. This process was designed to package the dried grass with the minimal effect on particle-size distribution compared with the original chopped diet. The pelleted diet was prepared by grinding the dried crop through a 2 mm screen prior to pelleting. The lambs were given the wafered and pelleted diets at three planes of nutrition for a period of 72 d. The initial energy content of the lambs was estimated with the use of previously derived relationships between fasted (18 h) live weight and total body energy (Thomson & Cammell, 1972). The final energy content of the lambs was determined after slaughter.

The metabolizable energy of the dried grass was utilized more efficiently ($P<0{\cdot}05$) when given in the ground, pelleted form (35%) than in the wafered form (14%). There was a marked depression in the metabolizable energy content of the grass diet due to grinding and pelleting, and the resultant utilization of the gross energy was similar for both processed diets ($P>0{\cdot}05$).

Digestion studies with the same wafered and pelleted cock's-foot diets showed that grinding and pelleting depressed the digestion of energy in the fore-stomach, reduced the production of volatile fatty acids in the rumen, and increased the digestion of energy (40% increase) and protein in the small intestine compared with the grass in the wafered form.

The results of this experiment with grass are in agreement with previous work which compared chopped and ground, pelleted lucerne diets. An increased efficiency of utilization of metabolizable energy for the pelleted lucerne (Thomson & Cammell

1971) was associated with a change in the site of energy digestion within the alimentary tract (Thomson, Beever, Coelho da Silva & Armstrong, 1969).

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Measurement of volatile fatty acid production in cattle: distribution of infusates in the rumen. By J. D. SUTTON, G. K. MACLEOD*, J. W. SISSONS and V. W. JOHNSON, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

A technique being used increasingly for the measurement of the rate of volatile fatty acid (VFA) production in the rumen of sheep requires the continuous infusion for some 24 h of radioactive VFA and, often, an indigestible marker (Weston & Hogan, 1968). In sheep it appears to be adequate to sample the rumen contents from one site only (Weller, Gray, Pilgrim & Jones, 1967). In cattle the rumen digesta are more heterogeneous than in sheep and greater care is needed in the choice of infusion and sampling sites.

We have infused simultaneously polyethylene glycol (PEG) (5·6–8·4 g/h) and [1–C^{14}]acetic acid (0·02–0·04 mCi/h) for 22 h into the rumen of cows given various rations at hourly intervals. The solutions were infused at two points about 30 cm apart in the midline of the rumen contents just below the hay layer. Samples were taken for the last 12 h of the infusion by two methods. In the 'Manual' method, rumen contents were stirred *in situ* by hand and samples were taken by means of a glass jar from three separate areas in the middle of the contents. In the 'Sites' method, samples were taken with as little disturbance as possible by suction through strainers kept throughout the experiment in four sites in the reticulo-rumen and by hand from the hay layer of the contents.

In two dry cows given 4 kg hay and 4 kg dairy concentrate daily, the concentration of PEG and the specific activity of the total VFA were usually considerably higher in samples taken from the mid-point between the two infusion points than in any other samples and these values were discarded. In six infusions in these cows, and in a milking cow given 0·9 kg hay, 5·4 kg flaked maize and 5·4 kg dairy concentrate daily, the mean specific activity for the other four points was 90–99% (mean 97%) of that for the 'Manual' samples. For PEG, the mean value for 'Sites' samples was 99–108% (mean 104%) of that for 'Manual' samples.

If it is assumed that, in these cows, the relation between the net rate of production and concentration is similar for all VFA, as is the situation in sheep given roughages (Weller *et al.* 1967), it can be estimated that VFA production in the rumen provided about 64% of the digestible energy of the food in the two dry cows and 61% in the milking cow given the high-concentrate ration.

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Utilization of salts of acetic, propionic and butyric acids by growing lambs. By F. D. DEB. HOVELL and J. F. D. GREENHALGH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The utilization of salts of volatile fatty acids (VFA) was investigated in a comparative slaughter trial in which the barley of a barley, soya-bean meal, fish meal control diet was partly replaced by a mixture of sodium and calcium salts of VFA (60 parts Na:40 parts Ca, anhydrous, dry-matter basis). The relative concentrations of crude protein and metabolizable energy (ME) were kept constant in all diets, the control diet contiaining 17% crude protein in dry matter. The ME of the salts was calculated as the heat of combustion of their acid equivalents.

Eight groups, each of seven entire male lambs were allocated to initial slaughter, or to one of the following diets: (1) control (two groups); (2), (3) and (4) as control, but with 7, 15 or 22% acetate ME; (5) 22% propionate ME; (6) 22% butyrate ME. Lambs were individually fed and were grown from 23 to 51 kg during 137 d (group means). Evaluation was by chemical analysis of the half-carcass and all remaining components.

The main results were as follows:

	Diet						
	1	2	3	4	5	6	SE†
ME* intake (kcal/d)	2392	2445	2469	2495	2560	2553	22
Gain (g/d) in:							
Live weight	216	219	208	207	212	211	6·6
Carcass weight	117	130	125	116	124	126	5·3
Remainder of body	66	69	69	65	69	68	3·9
Wool fibre	5·3	4·5	5·6	5·6	4·3	4·3	0·8
Whole body N × 6·25	30·4	33·2	33·2	31·4	33·7	33·9	1·9
Whole body fat‡	55·6	61·7	55·0	50·6	52·7	54·6	3·8
Whole body energy gain (kcal/d)§	711	780	722	671	696	714	34
Utilization of ME above maintenance (efficiency %)	58·4	60·8	55·2	49·7	53·5	49·5	3·2

*ME corrected for heat of fermentation.
†SE of difference between diet 1 and diets 2–6.
‡Excluding wool grease.
§Excluding wool grease, but including wool fibre.

It is concluded that high levels of acetate and butyrate were inefficiently utilized for energy retention, but that low levels of acetate were efficiently utilized. The poor utilization of the propionate diet relative to that of the control may have been due to the gluconeogenic capacity of the liver being exceeded. Increases in protein deposition were associated with increased digestibility of dietary protein.

The Two Hundred and Forty-third Scientific Meeting of the Nutrition Society was held in the Edward Lewis Lecture Theatre, The Middlesex Hospital Medical School, Cleveland Street, London on Friday, 17 March 1972, at 14.15 hours, when the following papers were read:

Energy and protein digestion of spring- and autumn-harvested rye-grass by sheep. By D. E. Beever, C. R. Lonsdale and D. J. Thomson, *Grassland Research Institute, Hurley, Berkshire SL6 5LR*

Corbett, Langlands, McDonald & Pullar (1966) and Blaxter, Wainman, Dewey, Davidson, Denerley & Gunn (1971) in calorimetric studies, and Lonsdale & Tayler (1971) with the comparative slaughter technique have indicated that the digestible energy contained in autumn regrowth herbage is utilized less efficiently for growth and for fattening than that contained in a primary growth of similar digestibility harvested in the spring. The reasons for this difference in the utilization of herbage of the same digestibility are not clear.

The spring (S) and autumn (A) cuts of S24 rye-grass used by Lonsdale & Tayler (1971) were pelleted without grinding and offered at approximately 900 g dry matter/24 h to three sheep equipped with intestinal re-entrant cannulas, and the digestion of energy and protein within the alimentary tract was studied.

The apparent digestibility of the grass energy was similar (S 66·6%; A 65·5%; $P>0.05$) and the digestion of energy before the duodenum, within the small intestine and in the caecum and colon was also similar ($P>0.05$); 1534 kcal/24 h and 1431 kcal/24 h were digested in the fore-stomachs of sheep fed on the spring and autumn herbage; total rumen volatile fatty acid (VFA) production rates, measured by isotope dilution technique, were 4·58 (S) and 4·72 (A) mol/24 h. There was, however, a 25% increase in the production of propionic acid and a lower production of acetic acid in the rumen of sheep given the spring grass than in those given the autumn grass (Table 1). The efficiencies of conversion of ruminally digested energy into VFA energy were 82 and 87% respectively.

The higher nitrogen content of the autumn-cut grass resulted in a difference in the quantities of N consumed by the sheep given the two diets (S 21·7; A 29·0 g/24 h). Higher concentrations of ammonia were measured in the rumen of sheep fed on autumn grass, the mean concentrations throughout one feeding cycle being 7·9 (S) and 16·1 (A) mg NH_3/100 ml. The amounts of N entering the small intestine were

Table 1. *Individual and total volatile fatty acid production rates (mol/24 h) in sheep*

	Acetic	Propionic	Butyric	Total
Spring	3·09	1·10	0·39	4·58
Autumn	3·44	0·87	0·41	4·72

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24·9 (S) and 29·6 (A) g/24 h, indicating an increase of 3·2 (S) and 0·6 (A) g N/24 h compared with the amounts ingested. There was a significantly higher ($P<0·05$) loss of N within the small intestine of sheep fed on the autumn grass (S 15·4; A 19·5 g/24 h). The results of this study were discussed in terms of the digestion of the energy and protein, and the nature of the absorbed energy in relation to the efficiency of utilization of spring and autumn grass for growth.

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Intake and absorption of essential fatty acids by the sheep. By W. M. F. LEAT and F. A. HARRISON, *ARC Institute of Animal Physiology, Babraham, Cambridge*

The ruminant animal is born with little reserve of essential fatty acids and throughout its life the dietary supply is limited (see Leat, 1970). After the rumen becomes functional, dietary unsaturated fatty acids are hydrogenated in the rumen and little linoleic acid will reach the site of absorption in the small intestine.

To estimate more accurately the amount of linoleic acid absorbed daily by the sheep, animals were prepared with re-entrant cannulas in the duodenum, anterior to the common bile duct, and with re-entrant fistulation of the thoracic duct. The sheep were fed once daily on a diet of 850 g hay, 200 g crushed oats and 100 g concentrates, which provided 4·6 g palmitic acid (16:0), 0·5 g stearic (18:0), 5·3 g oleic (18:1), 7·9 g linoleic (18:2) and 4·0 g linolenic acid (18:3) daily. Digesta were collected for periods of 6 h after feeding, and lymph for 4–9 h.

Of the fatty acids in digesta leaving the abomasum, 70–80% was non-esterified and 20–30% esterified; the major part (60–80%) of the $C_{18:2}$ acid was associated with the esterified lipids. It was calculated that up to 1·5 g $C_{18:2}$ and up to 0·3 g $C_{18:3}$ acid passed daily into the duodenum; this amount of linoleic acid was equivalent to 0·3% of the total energy.

The major fatty acids of lymph lipids were $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ acids with $C_{18:2}$ accounting for 5–7% and $C_{18:3}$ for 2–3% of the fatty acids. It was calculated that up to 1·2 g $C_{18:2}$ ($\equiv$0·25% of total energy) and 0·5 g $C_{18:3}$ acid were transported daily in the lymph; the major part (70%) of the $C_{18:2}$ acid was located in the lymph phospholipids.

When [1-^{14}C]linoleic acid and [1-^{14}C]linolenic acid were injected separately into the duodenum, 57% of the linoleic acid and 63% of the linolenic acid were recovered in thoracic duct lymph. The major part (60%) of the linoleate radioactivity was located in the phospholipid fraction.

Thus, estimates of $C_{18:2}$ acid entering the small intestine and absorbed into the lymphatics are well below the 1% of the energy which is the minimum recommended allowance for non-ruminants, suggesting that the ruminant animal conserves and utilizes its essential fatty acids more efficiently than the non-ruminant.

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The effect of fasting on plasma insulin after repeated glucose loading in pigs. By D. M. ANDERSON, *Biochemistry Department, ARC Institute of Animal Physiology, Babraham, Cambridge*

Pigs weighing 15–45 kg were fasted 18 h (six experiments), 39 h (five experiments) and 72 h (three experiments). Blood samples were taken and 0·3 g glucose/kg was infused over 4 min. After about 40 min a second identical infusion was made.

Resulting glucose and insulin concentrations are shown in Table 1. Initial values in infusion 2 are the values 38 min after the beginning of the first infusion. Plasma glucose concentration was depressed after 39 and 72 h fasting ($P<0·05$), as was plasma insulin ($P<0·01$) and ($P<0·02$). Plasma free fatty acids (FFA) were approximately 170 μequiv./l after 18 h fasting and 700 μequiv./l after 39 and 72 h fasting. The first glucose infusion consistently produced an increase in plasma insulin, but the second infusion increased insulin only after 18 and 39 h fasting.

After 18 h fasting plasma insulin increased sharply and then declined rapidly after both infusions, with a smaller response to the second ($P=0·02$). Plasma FFA were unaltered throughout. The rate of glucose removal from plasma (K, %/min) was the same after both infusions. In pigs fasted 39 h the first glucose infusion produced an immediate rise in plasma insulin concentration, which then continued to rise very slowly to a maximum at 30 min. Plasma FFA declined rapidly after the first infusion. The second infusion produced an increment in plasma insulin, but thereafter insulin concentration declined sharply, FFA were low throughout and K was greater ($P<0·02$). After the 72 h fast, the insulin response to increased plasma glucose was small after the first infusion, and very small after the second. Insulin concentration varied little after the initial increase. Plasma FFA declined rapidly after the first infusion and remained low after the second, but K was the

Table 1. *Mean values with their standard errors for plasma glucose and insulin concentrations in pigs and the time after glucose injection at which they occurred*

Period of fasting (h)	Infusion	Plasma glucose (mg/100 ml)			Plasma insulin (μ units/ml)				
		Initial	After 5 min	K (%/min)	Initial	Maximum	Time (min)	Minimum	Time (min)
18	1	102± 2	251± 7	5·1±0·6	16·2±1·9	60·7±7	10·0	14·5	30
	2	66± 8	239±24	4·7±0·4	16·8±7·9	38·9±3	10·0	9·9	38
39	1	79± 5	253±20	2·1±0·2	9·0±0·8	37·0±5	30·0	26·7	38
	2	151±10	304±13	3·7±0·4	26·7±5·0	50·3±6	5·0	15·7	38
72	1	89± 5	267±13	1·7±0·4	8·9±0·9	28·7±3	12·5	22·2	38
	2	149±21	287±18	1·7±0·4	22·6±7·0	29·2±6	5·0	19·2	38

same after both infusions. When 0·05 units/kg 'regular' ox insulin was injected intravenously immediately after glucose infusion in animals fasted 18 and 72 h the half-life of this insulin was the same at both times.

These results suggest that, in the pig with this load of glucose, insulin secretion is related to plasma glucose concentration or the rate of change in it, but this relationship is modified by fasting. When K is high and plasma glucose declines rapidly, after infusion, insulin concentration declines rapidly; when K is low and glucose concentration declines slowly, the decline in insulin concentration is slow or absent. However, the initial glucose increment is similar in all experiments but the insulin response becomes progressively smaller, i.e. at 18 h>39 h>72 h. In pigs, however, 'fasting diabetes' is not simply an impairment of insulin secretion, since plasma insulin values do not correlate well with K values and the inhibition of glucose removal can be reversed partly after a 39 h fast by prior glucose loading.

Mammary and whole-body metabolism of glucose, acetate and palmitate in the lactating horse. By J. L. LINZELL, *Institute of Animal Physiology, Babraham, Cambridge*, E. F. ANNISON and R. BICKERSTAFFE, *Unilever Research Laboratory, Sharnbrook, Bedford* and L. B. JEFFCOTT, *Animal Health Trust, Equine Research Station, Newmarket*

Little is known about lactation or metabolism in the horse. Pigs (Linzell, Mepham, Annison & West, 1969) derive much energy from glucose and synthesize some milk fatty acids from it, but ruminants do not. However Popják, Hunter & French (1953) reported that rabbits, which have a high blood acetate, readily form milk fatty acids from glucose. The horse resembles the rabbit in being a herbivore but not a ruminant.

Two ponies, weighing 242 and 312 kg, which had been lactating for 35 and 18 d, were infused, one with [U-^{14}C]glucose and the other with [U-^{14}C]acetate plus [9:10^{3}H]palmitate, each for 5 h; arterial and mammary venous blood were taken during the last hour. The foals were allowed to suck one-half of the udder and the other was milked hourly, after intravenous injection of 0·5 units oxytocin. The average yields were 157 and 146 ml/h. Entry rates were calculated from steady-state blood specific radioactivities and udder blood flow from milk yield:blood flow values for cows and goats.

Entry rates were acetate 3, glucose 2 and palmitate 0·19 mg/min per kg body-weight. Very little glucose was oxidized by the animal or its udder, but both derived 20% of CO_2 from acetate. Negligible carbon from glucose was transferred to milk fatty acids, but 95% of lactose and 20% of triglyceride glycerol were formed from glucose. Acetate and palmitate were important precursors of milk fatty acids. As in ruminants, C_{12} and C_{14} were exclusively formed from acetate and C_{16}, half from acetate and half from palmitate. In the horses, unlike ruminants, C_{18} (44%) and $C_{18:1}$ (7%) were formed from acetate.

Udder blood flow was about 2 l/min and there were significant extractions (20–40%) of O_2, glucose, acetate, β-hydroxybutyrate, triglycerides and α-amino

nitrogen, but not of free fatty acids, phospholipids, cholesterol esters or lactate. There was a reasonable balance between the uptake of glucose, fatty acids and amino acids, and the output in milk of lactose plus glycerol, fatty acids and protein respectively.

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The influence of dietary cadmium concentration on liver copper in ewes and their lambs. By C. F. MILLS and A. C. DALGARNO, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Recent work indicates that appreciable contamination of pasture herbage with cadmium can occur in areas downwind of some industrial complexes. Thus in such situations Goodman & Roberts (1971) reported Cd contents of *Festuca* L. spp. within the range 0·7–40 μg/g, whereas our own analyses of mixed herbage from similar areas have shown Cd contents between 1·4 and 40·5 μg/g. With higher (100 μg/g) concentrations of dietary Cd, Bunn & Matrone (1966) and Hill & Matrone (1970) have demonstrated significant antagonistic effects of Cd upon copper and zinc metabolism in chicks, mice and rats. The present communication presents brief details of an experiment to investigate the possible effects of lower concentrations of Cd in diets of cubed, dried grass upon Cu metabolism in ewes and lambs.

Twenty-four pregnant Cheviot ewes were randomized by weight into four treatment groups which, for the last half of pregnancy and 7–8·5 weeks after lambing were offered a ration of cubed, dried grass either untreated or supplemented with cadmium sulphate to give the following final Cd concentrations: A, no supplement (basal Cd concentration 0·7 μg/g); B, 3·5 μg/g; C, 7·1 μg/g; D, 12·3 μg/g. Deionized water was offered *ad lib.* and animals were housed in pens from which metal-work was excluded.

Several lambs were slaughtered at birth to gain information on tissue trace-element composition at this stage of development; the remaining twenty-one

Table 1. *Influence of diets differing in cadmium content offered during the last half of pregnancy and up to 8·5 weeks after lambing on liver copper in ewes and their lambs (μg/g dry matter ± SEM; numbers of observations in parentheses)*

Treatment group	A	B	C	D	Over-all significance of treatment effect
Dietary Cd concentration (μg/g)	0·7	3·5	7·1	12·3	
Lamb liver Cu	100±31 (5)	22± 3 (5)	61±35 (4)	12± 2 (7)	$P<0·01$
Ewe liver Cu	185±50 (4)	174±32 (4)	131±55 (4)	59±19 (4)	$P<0·05$

lambs were slaughtered at the termination of the experiment together with four ewes selected at random from each experimental group. Results of liver Cu analyses on lambs and ewes at the termination of the experiment are presented in Table 1.

Despite an apparently anomalous value for liver Cu (161 μg/g) in one lamb of group C (which we can nevertheless find no just cause to exclude), over-all treatment effects were significant and indicated that dietary Cd concentrations well within the range encountered in contaminated herbage had marked effects on liver Cu stores. Preliminary evidence obtained from some lambs killed at birth leads us to suggest that the greater part of the effects on liver Cu in lambs aged 7–8·5 weeks may have arisen from brief exposure to the Cd-supplemented rations of their dams to which they were not denied access.

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The effect of zinc deficiency on the activity of the adrenal glands.

By J. QUARTERMAN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

An excess of dietary zinc causes changes in the adrenal glands, and steroid hormones influence Zn metabolism (Roventa, Pora, Sāhleanu & Vadūva, 1968).

The effect of Zn deficiency was examined in rats given a semi-purified diet low in Zn (Williams & Mills, 1970). Control rats were given the diet supplemented with 40 parts/10^6 Zn and pair-fed with deficient rats. The food intake of Zn-deficient rats varies greatly (Chesters & Quarterman, 1970) and in these experiments rats were used after 1 or 2 d of high (*c.* 8 g) food intake.

The adrenal glands of Zn-deficient rats always contained more cholesterol than did those of controls and they tended to have more ascorbic acid, but the concentration of 11-hydroxysteroids in pooled plasma samples was the same (30·0 μg/100 ml) for both groups.

Table 1 shows the results of an experiment in which anaesthetized rats were given either 3 units ACTH i.p. or no injection and the adrenals were removed for

Table 1. *Mean values with their standard errors for composition of pairs of adrenal glands from zinc-deficient and Zn-treated rats with or without an injection of ACTH*

	No. of rats	Fresh wt (mg)	Total protein (mg)	Cholesterol		Ascorbic acid	
				Total (μg)	μg/mg protein	Total (μg)	μg/mg protein
Without ACTH:							
Zn-treated	5	27·0±0·6	4·6±0·1	605± 8	130± 4	128± 2	27·7±0·7
Zn-deficient	4	24·4±0·8	3·8±0·3	650±23	171± 4	137±15	36·5±1·6
With ACTH:							
Zn-treated	4	23·1±1·1	3·3±0·1	530±15	161± 3	44± 1	13·7±0·5
Zn-deficient	7	20·7±0·4	2·7±0·3	473±13	172±15	68± 5	25·7±1·6

analysis 3 h later. As a result of the ACTH injection there were decreases of adrenal weight, protein content and ascorbic acid content which were similar in Zn-deficient and control rats, but the decrease in cholesterol was about 2·5 times greater in the deficient than in the control group. In a similar experiment the plasma 11-hydroxysteroids rose to 43 μg/100 ml in the controls but to 65 μg/100 ml in deficient rats as a result of an injection of 2 units ACTH. Preliminary experiments have indicated that corticosterone clearance is not altered in Zn deficiency.

These experiments suggest that the Zn-deficient adrenal gland is hypersensitive to ACTH and therefore probably to any stress condition, and it is possible to speculate that some signs of Zn deficiency may be brought about by adrenal hyperfunction.

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Availability of some iron compounds to the veal calf. By I. BREMNER and A. C. DALGARNO, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It was recommended in the Brambell Report (Command Paper, 1965) that the rations for veal calves should be supplemented with sufficient iron, in a suitable form, to prevent the occurrence of anaemia. However, little is known of the Fe requirements of calves maintained on fat-supplemented skim-milk diets, such as are used commercially for veal production, or of the availability to these calves of different Fe sources. We have studied the haematological status of calves maintained on these rations, with no additional Fe, and have also compared the availabilities of $FeSO_4$, ferric citrate, ferric EDTA and iron phytate to the calves.

Twenty Ayrshire bull calves were used in a randomized block experiment of 11 weeks duration. The diet, which contained 24% fat and 26·5% protein, was supplemented with minerals and vitamins. The Fe concentration of the basal diet was 9·8 μg/g and Fe supplements were added where appropriate to provide an additional 30 μg Fe/g.

There were no apparent effects of treatment on weight gain (general mean 87·5 ± 9·7 kg) or on feed conversion ratio (1·48 ± 0·09) but both these measurements were related to the final blood haemoglobin concentrations of the calves. The regression equations were: $y = 0{\cdot}826 + 0{\cdot}039x$ (SE = 0·014), where y = weight gain (kg/d) and x = haemoglobin concentration (g/100 ml), and $y = 1{\cdot}70 - 0{\cdot}0269x$ (SE = 0·015), where y = feed conversion ratio and x = haemoglobin concentration (g/100 ml).

All calves showed an initial rapid fall in blood haemoglobin concentrations and packed cell volumes. The fall in haemoglobin concentrations for the unsupplemented calves was significantly greater than for the others within 2 weeks of the start of

Table 1. *Effect of treatment on haematological status of calves at end of the experiment*

	No supplement	$FeSO_4$	Ferric citrate	Ferric EDTA	Iron phytate	SE of difference of mean
Haemoglobin (g/100 ml)	6·1	8·8	9·1	8·5	7·2	0·74
Packed cell volume (%)	21·0	29·0	30·5	31·5	25·5	3·0
Red cell count (10^{-6}/mm^3)	6·90	8·49	8·83	7·92	7·18	0·80

the experiment. After 6–8 weeks it was found that haemoglobin concentrations and packed cell volumes for the calves given iron phytate were significantly less than for the other supplemented calves. These differences persisted to the end of the experiment (Table 1). After 6–8 weeks the haematological status of the supplemented calves generally improved.

The pattern of changes in plasma Fe concentrations was similar to that for haemoglobin. It was concluded from the experiment that the soluble Fe salts were more available to the calves than was iron phytate but no significant differences were detected between the soluble salts. It would appear that a dietary intake of 40 μg Fe/g in soluble form is sufficient to prevent the occurrence of all but a very mild anaemia.

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Copper status of the veal calf. By I. BREMNER and A. C. DALGARNO, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In a previous paper (Bremner & Dalgarno, 1972) the haematological status of veal calves and the availability of different iron compounds to these calves were examined. The diet used was typical of that used commercially, consisting of fat-supplemented skim-milk with added vitamins and minerals. It contained no added copper, the Cu concentration in the ration being only 0·8 μg/g, which is very much less than a calf's normal requirement. Consequently the Cu status of the calves used in that experiment was determined and, in addition, the effect of supplementing the diet of one calf in each treatment with Cu as $CuSO_4$ (15 mg Cu/feed) from week 6 to the end of the experiment, at week 11, was investigated.

The concentrations of Cu in blood and in various tissues are shown in Table 1. Although blood Cu concentrations were not abnormally low, liver Cu levels were indicative of a state of Cu deficiency. In seven of the fifteen calves, liver Cu concentrations were $\leqslant$12·5 μg Cu/g dry matter. About 50% of the supplementary Cu was retained in the livers of the Cu-treated calves. Although other tissues also showed increased Cu concentrations on treatment, the effects were less dramatic.

There were no effects of Cu treatment on either blood Cu or ceruloplasmin levels at the end of the experiment, but transient increases, lasting at most 3 weeks,

Table 1. *Effect of copper treatment on blood and tissue Cu concentrations of calves*

	No extra Cu	Cu-treated	SEM
Blood (μg/ml)	1·15	1·36	0·17
Liver (μg/g DM)	25·3	452	4·7 (−Cu)
			54 (+Cu)
Spleen (μg/g DM)	4·50	6·51	0·43
Kidney (μg/g DM)	19·9	26·4	2·20
Muscle (μg/g DM)	2·22	2·33	0·41

DM, dry matter.

were noted when Cu was first introduced into the ration at week 6. The increase in ceruloplasmin level was the more persistent and more marked; at week 9 the levels were 12·8 in the calves not receiving extra Cu, and 32·8 i.u./ml in the Cu-treated calves.

There was also an increase in plasma Fe concentrations (by 100% at week 8), suggesting an effect of Cu on Fe metabolism. This was confirmed by examination of the concentrations of storage (non-haem) Fe in the livers of the calves. Dietary Cu supplementation caused a 35% reduction in Fe stores from 23·7 to 15·4 μg Fe/g dry matter.

These findings are in accord with the view that in Cu deficiency it is the reduction in ceruloplasmin concentration, with resultant decrease in plasma ferroxidase activity, which is at least partly responsible for the decreased mobilization of Fe from storage depots (Lee, Nacht, Lukens & Cartwright, 1968).

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Seven generations of protein–calorie deficiency in rats. By R. J. C. STEWART, R. F. PREECE and HILDA G. SHEPPARD, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, WC1E 7HT*

The difficulties of maintaining rat colonies on powdered diets of known protein value (Stewart & Sheppard, 1971) have been largely overcome by the production of cubed diets having protein values of NDpCal%=6·8 and 10 (Payne & Stewart, 1972).

Black-and-white hooded rats have been maintained on these cubes for seven generations and the average birth weight within the malnourished community is now 4·4 g against 5·5 g in the well-fed colony. All the animals were born at full term but the birth weights varied considerably. However, whereas over 20% of those born into the malnourished community could be regarded as small-for-dates only 3% of the controls were in this category.

There are significant differences in the average values for individual and litter weights in the later generations but not for numbers per litter (Table 1). During

Table 1. *Birth weights (g) and litter sizes of rats maintained on diets of different protein values*

	Diet of NDpCal% = 6·8			Diet of NDpCal% = 10		
Generation	Individual weight	Litter weight	No. in litter	Individual weight	Litter weight	No. in litter
0	5·6	51·1	9·2	5·6	51·1	9·2
1	4·8	50·1	10·4	5·3	48·8	9·2
2	4·6	48·4	10·5	5·5	49·9	9·0
3	5·0	43·6	8·7	5·4	53·9	9·9
4	4·6	39·8	8·6	5·6	56·0	10·0
5	4·7	44·3	9·4	5·6	67·0	12·1
6	4·8	44·9	9·4	5·6	62·4	11·2
7	4·3	45·6	10·6	5·5	62·8	11·5

suckling the disparity in body-weight increases and at 4 weeks of age the malnourished young weigh about half as much as the controls. When mated, at 3 months of age, the deficient females weigh 124 g and the controls 219 g. Even when 6 months old, body-weights, body lengths and brain weights are below those of the controls. Males are more severely affected than females.

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A further observation on urea-cycle enzymes in rats during alteration in dietary protein intake. By T. K. DAS, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

It is well established that the activity of the urea-cycle enzymes of the liver varies according to the protein content of the diet (Schimke, 1962), but the time-course of these changes has not been fully worked out. In a previous communication (Das, 1971) it was shown that when rats are transferred from a 14% to a 5% casein diet, adaptation of three urea-cycle enzymes—arginase, argininosuccinase, and argininosuccinate synthetase is complete in 30 h. This is also the time needed for the urinary nitrogen excretion to reach its new level. The results are therefore consistent with the hypothesis that the enzyme changes are closely related to the reduced N excretion.

The present communication is concerned with the opposite effect—the time-course of adaptation of the urea-cycle enzymes when the protein content of the diet is reversed from low to high. Adaptation again appears to be complete in about the same time. The results suggest that argininosuccinate synthetase was the rate-limiting step, in agreement with the findings of McLean & Novello (1965).

Preliminary experiments show that, when rats are fed on a diet containing 14% gelatin, the enzyme levels are the same as on 14% casein, although the urinary N output is much higher. The significance of these results was discussed.

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Progesterone – the hormone of protein anabolism in early pregnancy.

By D. J. NAISMITH and R. B. FEARS, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

After the 6th day of pregnancy, the rat experiences a marked improvement in the efficiency of protein utilization (Naismith & Fears, 1972). Pregnant rats, pair-fed with non-pregnant litter-mates, retained progressively more nitrogen, and had a much reduced capacity to form urea. Plasma concentrations of amino acids were raised, and the activity of argininosuccinate synthetase, the enzyme which controls the rate of urea synthesis in the liver, was greatly reduced. The coincidence of the reduction in N excretion with the rise in excretion of metabolites of progesterone suggested that this hormone might have anabolic properties. It has also been observed that the weight of the adrenal glands is significantly reduced in mid-pregnancy (Naismith, unpublished results). A fall in the secretion of corticosterone, a hormone known to have catabolic properties (Goodlad & Munro, 1959), might therefore achieve the same results.

The role of progesterone in the metabolism of protein was studied in eleven litter-mate pairs of adult female rats which were maintained on a diet containing 25% casein. One of each pair was given, by subcutaneous injection, 5 mg progesterone in 0·25 ml maize oil for 10 consecutive d. Control rats received maize oil alone. The rats were killed, and blood was drawn for analysis for plasma corticosteroids and for amino acids. The adrenal glands were weighed, and the activities of argininosuccinate synthetase and alanine aminotransferase were measured in the livers. The summarized results were:

	Wt of adrenals (mg)	Plasma corticosteroids (μg/100 ml)	Plasma amino acids (μmol/ml)	Argininosuccinate synthetase (i.u./liver)
Controls	70·5	61·2	6·55	190
Treated with progesterone	61·6	46·8	7·07	167

Rats treated with progesterone showed a significant reduction in the weight of the adrenal glands ($P<0·01$) and in the plasma concentration of corticosteroids ($P<0·01$). A significant correlation was found between the changes in these measurements induced by progesterone ($r=0·9126$; $P<0·01$), indicating that the secretion of corticosteroids had been reduced. The activity of both hepatic enzymes fell significantly ($P=0·01$), whereas the plasma concentration of amino acids rose ($P=0·02$).

Adaptations in the metabolism of protein observed in the pregnant rat can therefore be reproduced by the administration of progesterone. This hormone appears to exert its anabolic effect by suppressing the activity of the adrenal cortex.

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Nutritional value of a protein isolate from the Throws M.S. variety of field beans (*Vicia faba* L.) grown in the UK. By I. F. DUTHIE, P. D. PORTER and B. GADSBY, *The Lord Rank Research Centre, High Wycombe, Bucks.*

Increasing production of field beans (FB), *Vicia faba* L., in the UK has made the crop available in substantial tonnages and warrants its investigation as a protein source for human nutrition.

A protein isolate powder, containing 91% protein (N×6·25) on a dry-matter basis was prepared by extracting flour prepared from dehulled and ground beans, Throws M.S. variety, in dilute aqueous alkali, followed by acid precipitation, neutralization and spray-drying. The net protein utilization (NPU) based on body N analysis, and protein efficiency ratio (PER) assays, based on the methods recommended by Miller (1963) and the Association of Official Agricultural Chemists (1960), respectively, were used as indices of protein quality. Weanling SPF status male rats (CD. Charles River France SA) were given feeds incorporating the following materials as sole protein sources to provide 10% crude protein (1·6% N× 6·25): FB isolate and commercial soya-bean (SB) isolate, with and without 0·25% DL-methionine, lactic casein, accelerated freeze-dried (AFD) whole egg. There were eight individually caged rats per treatment in each experiment.

The results of the 10 d NPU (Table 1) and 28 d PER (Table 2) assays indicate that FB isolate is an inherently useful protein source which compares well with SB isolate, both being considerably improved by the addition of a small amount of DL-methionine: its digestibility based on a 5 d collection period was also satisfactory (Table 1).

Table 1.

Treatment	NPU %	Wt gain (g/d)	Feed intake (g/d)	N digestibility (%)
Casein	70·0± 4·7	3·58±1·05	12·03±1·81	95·4±1·8
AFD egg	76·7± 4·7	5·87±0·86	14·65±1·63	90·9±3·0
FB isolate	42·1± 4·8	0·07±0·30	7·52±0·94	93·5±3·2
FB isolate+0·25% DL-methionine	68·0± 5·1	4·19±0·72	13·74±1·45	94·2±1·3
SB isolate	37·4±13·9	0·19±0·33	8·33±1·03	94·8±2·3
SB isolate+0·25% DL-methionine	57·8± 4·3	5·37±1·21	13·13±2·59	93·9±1·8

NPU, net protein utilization.

Table 2.

Treatment	PER	Weight gain (g/d)	Feed intake (g/d)
Casein	3·06±0·30	4·32±0·93	15·14±2·23
FB isolate	0·99±0·48	0·96±0·83	8·43±3·30
FB isolate+0·25% DL-methionine	3·08±0·24	5·56±1·63	17·16±2·02
SB isolate	1·19±0·48	1·21±0·62	9·18±1·82
SB isolate+0·25% DL-methionine	2·77±0·13	5·20±0·66	17·46±2·89

PER, protein efficiency ratio.

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Nutrient intake of students in various types of accommodation.

By B. JACQUELINE STORDY, *Department of Biochemistry, University of Surrey, Guildford*, and J. R. COWHIG, *Orbis Publishing Ltd, 49 Russell Square, London WC1*

There are about 250 000 full-time university students in the UK, and of these only 37% are in residential accommodation. Usually, but not always, such accommodation includes the provision of breakfast and an evening meal. For the majority, however, meals during term time are provided at best by parents or landladies, or by the students themselves in flats or bed-sitters. Very little information is available on the food habits and nutrient intakes of students in any of these groups.

During a 5 d period in winter, including one weekend day, 221 male and female students in four categories of accommodation recorded everything that they ate. Quantities were recorded in 'homely' measures, except when weights were clearly indicated on packaging. Portion sizes in residences and university restaurants were weighed; other weights, including all those in meals taken at home, were estimated from portion sizes.

The average nutrient content of the diets was calculated by means of a computer programme, kindly provided by Erica Wheeler of the Department of Human

Table 1. *Average daily intake of energy and nutrients by students in various places*

	Energy (kcal)	Protein (g)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
Women:						
Hall A	2552 (24)	77 (18)	15 (27)	1·5 (9)	1·6 (27)	59 (12)
Hall B	2416 (33)	70 (4)	15 (12)	1·3 (4)	1·6 (21)	83 (8)
Home and lodgings	2427 (53)	74 (13)	15 (27)	1·5 (7)	1·8 (27)	69 (13)
Flats and bed-sitters	2148 (61)	67 (27)	13 (50)	1·2 (20)	1·2 (35)	72 (9)
Men:						
Hall A	3015 (34)	90 (6)	17 (3)	1·8 (6)	2·0 (28)	46 (12)
Hall B	2986 (26)	81 (16)	17 (0)	1·5 (10)	2·0 (26)	68 (0)
Home and lodgings	3010 (27)	98 (0)	19 (0)	1·8 (0)	2·3 (10)	79 (0)
Flats and bed-sitters	2975 (34)	90 (21)	19 (0)	1·5 (10)	2·1 (24)	68 (10)

Figures in parentheses indicate the percentage of students receiving less than the recommended intakes.

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Nutrition, London School of Hygiene and Tropical Medicine, and based on standard food tables (Orr & Watt, 1957; McCance & Widdowson, 1960). The results are shown in Table 1.

The table also shows the percentage of students receiving less than the recommended intake of some nutrients (Department of Health and Social Security, 1969). This was highest among women catering for themselves—a result to be expected since the averages conceal a large scatter.

In view of these figures, the present trend towards self-catering in residential accommodation seems to be undesirable.

We are grateful to Mrs Susan Johnston de Tomas and Miss Hilda Currie for assistance.

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Consumption of major food groups by students over a 3-month period.

By SUSAN J. ATKINSON, P. NICHOLAS and CHRISTINE WYN-JONES (introduced by J. W. T. DICKERSON), *Beecham Group Research Unit, Walton Oaks, Tadworth, Surrey*

A 2-year longitudinal dietary and biochemical study of fifty student volunteers from the University of Surrey is being made. We felt that, as increasing numbers of students are catering for themselves, it would be an interesting population on which to study nutritional status. Findings for the first 3 months are reported.

For 1 week in each month during term time, students record in a book everything taken by mouth, and this book is collected at the end of the week. In each term one weighed and one estimated record of food eaten is completed. Random checks of accuracy are made by interview.

A computer programme has been developed using values from food tables (McCance & Widdowson, 1969) which permits the calculation of food and nutrient intake.

The consumption of the major food groups is summarized in Table 1. The ranges of intake of the major food groups were large for both sexes in each month;

Table 1. *Consumption of major food groups (kg/head per week) by male and female students in March, May and June, 1971 (group means and ranges)*

Month	Sex and no.	Meat	Citrus fruit	Bread	Potatoes	Milk and cream	Cheese	Egg and egg dishes
March	♀, 23	0·73	0·43	0·48	0·24	1·64	0·26	0·24
	♂, 14	0·94	0·28	0·79	0·59	2·10	0·14	0·45
May	♀, 23	0·60	0·43	0·49	0·32	1·64	0·33	0·24
	♂, 21	1·22	0·12	0·61	0·80	1·97	0·12	0·34
June	♀, 22	0·69	0·35	0·50	0·38	1·54	0·32	0·21
	♂, 19	1·10	0·13	0·71	0·93	1·42	0·20	0·30

in citrus fruits, for example, the range was from 0 to approximately 2 kg/head per week.

There were distinct differences between the sexes in the consumption of the major food groups. Thus, the females ate greater quantities of citrus fruit and cheese, but less meat, bread and potatoes.

The average consumption of meat, milk, potatoes and bread by this student group was at least 25% below the national household average consumption in 1968 (Ministry of Agriculture, Fisheries and Food: National Food Survey Committee, 1970), whereas their consumption of citrus fruit, eggs and cheese was at least double.

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Nutrient intake by students over a 3-month period. By CHRISTINE WYN-JONES, SUSAN J. ATKINSON and P. NICHOLAS (introduced by B. JACQUELINE STORDY), *Beecham Group Research Unit, Walton Oaks, Tadworth, Surrey*

As part of the longitudinal study of student nutritional status (Atkinson, Nicholas & Wyn-Jones, 1972), nutrient intakes were calculated from the students' dietary records and the results are summarized in Table 1.

There was a wide range of nutrient intakes within the group. The means and ranges of nutrient intake were, however, similar for each month studied. Most group mean intakes were above the recommended daily allowances (Department of Health and Social Security, 1969). The mean energy intakes of the women were marginally below the 2200 kcal recommended in each of the 3 months. Males generally had higher intakes of protein, energy and iron, whereas females had higher ascorbic acid intakes. In each month it was generally the same students who had intakes at the extreme ends of the range for a particular nutrient compared with the rest of the group.

Table 1. *Daily nutrient intakes of male and female students for 3 months of 1971 (group means and ranges)*

Month	Sex and no.	Protein (g)	Energy (kcal (MJ))	Iron (mg)	Thiamin (mg/1000 kcal)	Riboflavin (mg/1000 kcal)	Ascorbic acid (mg)
March	♀, 23	60	2030 (8·4)	14	0·58	0·67	55
		31–103	970–3790	4–18	0·35–1·06	0·45–1·06	12–110
	♂, 14	83	2850 (12·2)	16	0·51	0·73	47
		56–103	2070–3730	9–23	0·36–0·89	0·46–1·13	8–78
May	♀, 23	62	2150 (9·2)	11	0·47	0·72	59
		41–83	1370–2760	5–19	0·31–0·85	0·41–1·35	20–149
	♂, 21	81	2600 (10·9)	17	0·43	0·78	43
		48–113	1870–3690	9–25	0·27–0·74	0·43–1·09	17–101
June	♀, 22	67	2110 (8·8)	13	0·47	0·72	53
		37–99	1200–3140	6–18	0·36–1·0	0·42–1·17	9–140
	♂, 19	85	2750 (11·8)	16	0·49	0·74	57
		58–117	1690–3510	11–22	0·29–0·73	0·37–1·07	17–73

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Observations on ascorbic acid and iron concentrations in the blood of students. By P. NICHOLAS, SUSAN J. ATKINSON and CHRISTINE WYN-JONES (introduced by M. BROOK), *Beecham Group Research Unit, Walton Oaks, Tadworth, Surrey*

Fasting blood samples were obtained from students taking part in a longitudinal study of nutritional status (Atkinson, Nicholas & Wyn-Jones, 1972) at the end of each week of the dietary record. Plasma and buffy layer ascorbic acid concentrations (Denson & Bowers, 1961), haemoglobin (British Standards Institution, 1966) and serum iron (Caraway, 1963) were determined.

The low mean buffy layer ascorbic acid concentration in March 1971 was similar to that found in old people living at home during the same period of the year (Andrews, Brook & Allen, 1966). No significant differences in buffy layer ascorbic acid concentrations were found between males and females. Plasma ascorbic acid concentration in females were, however, significantly higher than those in males, and this difference was maintained from March to June, though the difference in June was barely significant.

Haemoglobin concentrations in males were significantly higher than those of females, but no difference was found in serum iron concentrations.

Table 1. *Blood nutrient concentrations in male and female students during 3 months of 1971 (group means and ranges)*

	March		May		June	
	Males (14)	Females (23)	Males (23)	Females (21)	Males (22)	Females (19)
Buffy layer ascorbic acid (μg/10^8 cells)	20·5 (8–37)	18·9 (8–40)	28·9 (12–41)	29·0 (12–52)	29·0 (16–41)	28·0 (16–41)
Plasma ascorbic acid (mg/100 ml)	0·55 (0·10–0·95)	0·80 (0·2–1·7)	0·56 (0·12–1·10)	0·77 (0·21–1·35)	0·72 (0·23–1·0)	0·78 (0·22–1·55)
		†		†		*
Haemoglobin (g/100 ml)	16·1 (13·1–20)	14·1 (9·1–15·8)	15·8 (13·1–19·8)	14·2 (7·2–16·6)	15·7 (13·2–18·8)	14·0 (7·3–15·7)
		†		†		†
Serum iron (μg/100 ml)	165 (95–196)	155 (101–188)	143 (87–260)	140 (85–180)	160 (105–198)	162 (71–246)

*P=0·05. †P<0·02.

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Although there was no increase in ascorbic acid intake between March and June, there was a significant increase in the buffy layer ascorbic acid concentration of the males. This observation suggests either a change in efficiency of ascorbic acid absorption, which seems unlikely, or a reduction in rate of utilization.

Statistically significant correlations between certain indices of nutritional status were observed irregularly. For example, ascorbic acid intake and plasma ascorbic acid concentration for twenty-two males had a barely significant correlation coefficient of 0·4 ($P=0·05$) in June only; serum iron and haemoglobin in twenty-one females had a significant correlation coefficient of 0·4 ($P<0·02$) in May only. The explanation of such irregularities may emerge as the study continues, and this is one reason why we think that a longitudinal study relating dietary intake and blood biochemistry will prove valuable.

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A survey of school meals. By A. E. Bender, Pamela Magee and A. H. Nash, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

A survey was carried out of 772 meals in forty-eight schools of four age-groups in south-east England. In order to include variables due to menu, appetite variation, and so on, twenty meals were selected at random (this figure was not always reached) in each school, roughly spaced throughout the serving period and including some samples of each dish where there was a choice. Each school was visited once.

Food served on the plate and the uneaten remainder were weighed, and energy and nutrient intakes were calculated from food composition tables. The results are shown in Table 1.

Table 1. *Energy and protein consumption (median and range)*

Type of school	No. of schools	Energy			Protein		
		Recommended (kcal)	Consumed (kcal)	% recommended	Recommended (g)	Consumed (g)	% recommended
Infant	8	600	420 (360–640)	70	23	13 (10–16)	57
Infant/junior	18	700	480 (330–790)	69	25	15 (11–30)	60
Junior	12	750	475 (280–660)	63	29	16 (8–26)	55
Senior	10	870	650 (420–1340)	75	33	20 (11–31)	61

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The school meal is intended to supply one-third of the daily energy requirements of a child (880 kcal) and 29 g protein (Department of Education and Science, 1965). It is stated that these figures will vary with the size of portions served to children of different age and sex. In Table 1 the recommended daily intake figures of the Department of Health and Social Security (1969) have been used, taking the average age of the children in each type of school.

The average meal reached only 70% of the target figure for energy and 60% for protein. Thus, even had the quantity of food been sufficient to reach the energy target, the meal still would not have reached the protein target.

Plate waste averaged 10% of the food offered but there was a wastage of 20% by half the children. Waste bore no relation to the size of the meal.

Average daily intake of iron was 3·6 mg and of calcium, 190 mg/head. Average sugar intake was 22 g/head which was 17% of the energy intake (and reached 33% in one school).

Food taken from store plus food purchased was divided by the number of meals served to provide a figure for available food, and the difference between this and the food on the plate yielded a figure of apparent waste during preparation. While this was 27–35% in the various types of school, this average masks a loss exceeding 45% of the available energy in five schools and exceeding 45% of available protein in fifteen schools. Moreover, it appears that insufficient food was purchased, since in the four types of school to achieve the targets of 600, 700, 750 and 870 kcal, food purchased and taken from store (before preparation losses) had energy values of 800, 750, 710 and 1000 kcal respectively.

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