

The Two Hundred and Twenty-eighth Scientific Meeting of the Nutrition Society, was held in the School of Agriculture, University of Newcastle upon Tyne, on Thursday 3 December, 1970, at 14.45 hours, when the following papers were read:

The effect of leuco-anthocyanins in sainfoin (*Onobrychis viciifolia* Scop.) on the availability of protein to sheep and upon the determination of the acid detergent fibre and lignin fractions. By D. F. OSBOURN, R. A. TERRY, S. B. CAMMELL and G. E. OUTEN, *Grassland Research Institute, Hurley, Berkshire*

Herbage from nine harvests of each of sainfoin, perennial and Italian ryegrasses, and timothy was frozen and the thirty-six herbage fed to sheep at levels of intake equivalent to maintenance. Total faecal output was determined. The linear regressions describing the relationship between the contents of apparently digested crude protein (DCP) and crude protein (CP) were similar for the three grass species but these differed significantly from the regression derived for sainfoin ($P < 0.01$):

$$\left. \begin{array}{l} \text{Grasses} \quad \text{DCP} = 0.960 \text{ CP} - 4.210 \\ \text{Sainfoin} \quad \text{DCP} = 0.960 \text{ CP} - 6.398 \end{array} \right\} \begin{array}{l} \text{standard error of } b = \pm 0.0126; \\ \text{residual means square} = 0.1751 \end{array}$$

The crude protein of the sainfoin diets was apparently less digestible as a result of more crude protein, equivalent to 2.19% (6.398-4.210) of the dry matter consumed, being lost in the faeces when sainfoin was fed than when grasses were fed.

Using the methods of Van Soest (1963), it was found that from 25 to 140% more lignin was recovered in the faeces than was present in the sainfoin herbage fed, confirming the observations of Allinson & Osbourn (1970). The mean content of nitrogen $\times 6.25$ isolated from the faecal lignin fraction of the sainfoin diets was 2.68 ± 0.102 , expressed as a percentage of the dry matter fed. The crude lignin recovered from untreated sainfoin herbage and the same herbage subjected to hydrolysis with normal HCl or extraction with neutral detergent solution (Van Soest, 1967) comprised 6.85, 11.49 and $5.44 \pm 0.366\%$ of the herbage dry matter. The residues of sainfoin herbage after digestion in vitro with rumen micro-organisms contained considerably more crude lignin than the untreated herbage.

The presence of leuco-anthocyanins in the sainfoin was demonstrated by the intense red colour produced upon hydrolysis, by paper chromatographic separation of cyanidin and delphinidin, and by the presence of hippuric acid crystals in the urine from the sheep (Kay, 1969). No anthocyanidins were detected in the grasses, confirming the observations of Bate-Smith & Lerner (1954). These two anthocyanidins were also identified as present when the crude lignin fraction from the faeces of sheep fed sainfoin was digested with concentrated sulphuric acid.

It is postulated that the leuco-anthocyanins present in the sainfoin herbage are hydrolysed in the digestive tract to produce anthocyanidins which react with

protein to produce an insoluble phenolic complex, thereby reducing the digestibility of the protein and interfering with the determination of both the acid detergent fibre and lignin fractions in herbage and faecal samples from sainfoin.

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The digestion of dried lucerne (*Medicago sativa* L.) and dried sainfoin (*Onobrychis viciifolia* Scop.) by sheep. By D. J. THOMSON, D. E. BEEVER, D. G. HARRISON, I. W. HILL and D. F. OSBOURN, *Grassland Research Institute, Hurley, Berkshire*

In the previous communication, Osbourn, Terry, Cammell & Outen (1971) showed that the protein of sainfoin was less digestible than the protein of grasses. Further experiments demonstrated that the protein in dried sainfoin was apparently less digestible than the protein of another legume, dried lucerne (lucerne (apparently) digested crude protein (DCP) = 0.960 crude protein (CP) - 5.83; sainfoin DCP = 0.960 CP - 8.32). The increased faecal excretion of nitrogen was attributed to the presence of leuco-anthocyanins in the sainfoin herbage, but could have been caused by increased microbial synthesis of protein in the caecum (Ørskov, Fraser, Mason & Mann, 1970).

The digestion of dried lucerne and dried sainfoin herbages was examined with three sheep fitted with re-entrant duodenal and ileal cannulas. The sheep were fed 902 g dry matter/head per d of the dried chopped herbages in two equal feeds. The procedures for digesta collection were similar to those described by MacRae & Armstrong (1969) and flow values were corrected to 100% recovery of chromic oxide.

Table 1. *Digestion of cellulose in the alimentary tract of sheep fed dried lucerne and dried sainfoin diets*

	Lucerne	Sainfoin	SE of mean
Digestibility of cellulose (%)	67.0	78.1	1.08
Disappearance of digestible cellulose (%)			
Before duodenum	92.9	96.5	2.15
In small intestine	7.1	1.6	1.69
In caecum and colon	0.0	1.8	1.69

The digestibility of the cellulose in sainfoin was higher than that of lucerne ($P < 0.01$), but there was no difference between the diets in the site of cellulose digestion (Table 1). Digestion in the caecum and colon was negligible, suggesting that the elevated faecal excretion of N on the sainfoin diet was not due to microbial synthesis in the large intestine.

Table 2. *Flow of nitrogen (g/24 h) through the alimentary tract of sheep fed dried lucerne and dried sainfoin diets*

	Lucerne	Sainfoin	SE of mean
Feed	27.9	25.6	—
At duodenum	24.8	32.6	2.33
At ileum	12.3	14.6	0.46
In faeces	8.9	12.6	0.50
Apparent N digestibility (%)	68.1	50.8	1.96

The higher faecal excretion of N observed with the sainfoin diet would seem to be attributable to the presence of leuco-anthocyanins. Despite this increased faecal N excretion, the flow of N into the duodenum and the disappearance from the small intestine (Table 2) were much greater on the sainfoin diet (32.6 and 18.0 g/24 h) than on the lucerne diet (24.8 and 12.5 g/24 h), suggesting that more protein was available to the animals from the sainfoin than the lucerne diet.

The authors wish to thank Drs A. T. Cowie and H. Buttle for the surgical preparations.

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 MacRae, J. C. & Armstrong, D. G. (1969). *Br. J. Nutr.* **23**, 15.

The supplementation of a low-nitrogen hay with urea and its effect on nitrogen transformations within the alimentary tract of adult sheep.

By D. E. BEEVER, D. G. HARRISON, D. J. THOMSON and D. F. OSBOURN,
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Barn-dried S24 ryegrass, the energy of which was 67% digestible and which contained 1.1% nitrogen, was ground through a 1.96 mm sieve and pelleted. In the first period of the experiment this basal diet was fed at the rate of 360 g dry matter (DM)/d, in twenty-four equal feeds at hourly intervals. In the second period, the same diet was again fed hourly at 360 g DM intake/d and urea was infused continuously into the rumen at the rate of 12.6 g/d, so elevating the level in the DM to 2.5%. During storage, the water-soluble carbohydrate content of the dried ryegrass fell from an original 16% to 11%. Sucrose was infused continuously into the rumen during the feeding of both the unsupplemented and urea-supplemented diets in order to restore the water-soluble carbohydrate intake to the equivalent of the original dietary content of 16% (in the DM).

The two diets were fed to two Welsh × Half-bred wethers (25–30 kg live weight) which had been equipped with rumen fistulas and re-entrant cannulas at the proximal duodenum and terminal ileum. The methods for the collection of digesta were similar to those described by MacRae & Armstrong (1969).

Despite large differences in total intakes of N, 4.26 and 9.86 g/d, the flow of N into the duodenum was similar, at 11.5 and 12.2 g N/d, including 54.7 and 57.0 g amino-acid/d for the control and urea-supplemented diets respectively (Table 1).

Table 1. Mean quantities (g/24 h) of total α -amino acids present in the feed and faeces and passing at the proximal duodenum and terminal ileum of the two sheep fed low-nitrogen hay pellets with and without urea

	Feed	Duodenum	Ileum	Faeces
Control	21.7	54.7	20.9	12.8
Treatment	20.5	57.0	19.0	11.1

There was no change in the site of digestion of energy or cellulose or in the amino acid composition of the digesta samples in response to the infusion or urea.

Frequent feeding of a ground, pelleted forage of low N content with the provision of readily available energy (water-soluble carbohydrate) led to marked synthesis of microbial protein. Continuous urea supplementation of the low-N forage diet increased only slightly the flow of total amino acids into the duodenum, 57.0 v. 54.7 (control) g/d, and the quantity disappearing in the small intestine, 38.0 v. 33.8 (control) g/d, suggesting that available energy rather than N limited the further synthesis of microbial protein.

The authors wish to thank Drs A. T. Cowie and H. Buttle for the surgical preparations.

REFERENCE

MacRae, J. C. & Armstrong, D. G. (1969). *Br. J. Nutr.* **23**, 15.

The occurrence of α -amino-isobutyrate in sheep digesta. By D. G. HARRISON, D. E. BEEVER and D. J. THOMSON, *Grassland Research Institute, Hurley, Berkshire*

The amino acid composition of the digesta from sheep fed low-nitrogen hay (diet A) has been described previously (Beever, Harrison, Thomson & Osbourn, 1971). With the exception of α -amino-isobutyric acid (AIB) and diaminopimelic acid (DAP), the mean quantities of each amino acid (g/24 h) decreased between the duodenal and ileal cannulas. The DAP value remained essentially constant, whereas the AIB value rose markedly, indicating a net synthesis of the amino acid between the duodenum and ileum. Similar results were obtained with three sheep fed fresh clover (diet B) at the rate of 880 g/24 h. Neither feed contained AIB nor DAP. The values for AIB are shown in Table 1.

Table 1. Mean quantities (g/24 h) of α -amino-isobutyrate found in the digesta of sheep fed on diets A and B

Diet	Duodenum	Ileum	Faeces
A	0.617	1.160	0.289
B	Trace	1.494	Trace

The net increase in AIB in the small intestine could be due either to bacterial activity or to secretion from the gut mucosa. The following experiment was designed to examine the two possibilities.

The duodenal cannula of one sheep fed diet B was opened and the contents collected. Acidified Ringer's solution (pH 2.3) was returned into the duodenum until no solid digesta was appearing at the ileal cannula. The duodenal cannula was reconnected, and collections were made at the ileum. The protein concentration in the ileal digesta rose by over 50% following the arrival of solid material at the cannula, but the AIB : protein ratio remained constant, suggesting that AIB is present in digesta and not in any endogenous secretion. Further, AIB is not metabolized by mammals (Briggs & Walker, 1960); consequently its synthesis by the sheep is unlikely.

Bacteria isolated from rumen and ileal digesta by sedimentation at 25 000 g contained 1.6 and 3.5 g AIB/100 g protein respectively. This represented only 40% of the AIB in ileal digesta; the remainder was concentrated in the supernatant fraction which contained only 18% of the total protein.

The presence of AIB in rumen and ileal bacteria, and its increase within the ileum, provide further evidence to support the suggestion made by Kay (1969) of significant bacterial activity occurring in the ileum of sheep.

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Some observations on the metabolism of α - ϵ -diaminopimelic acid in sheep. By V. C. MASON and F. WHITE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

α - ϵ -Diaminopimelic acid (DAPA) is a constituent amino acid of the cell wall mucopeptide of many species of bacteria found in the alimentary canal of ruminants.

In a series of experiments with cannulated sheep, the quantities of this amino acid passing daily through the abomasum, terminal ileum and rectum were measured. Although there was a very significant net absorption of α -amino-nitrogen between the abomasum and terminal ileum, there was no absorption of DAPA between these points. Indeed, when concentrate rations were given, there was a tendency for more DAPA to leave the terminal ileum daily than entered the abomasum, suggesting appreciable bacterial growth in the small intestine. In all instances a smaller amount of DAPA passed out of the rectum, indicating considerable removal or destruction of this amino acid in the hind-gut, probably as a result of microbial activity. No DAPA was detected in blood plasma and only trace amounts were measured in the urine.

When synthetic DAPA was introduced into the blood, abomasum, rumen or caecum in physiological amounts, however, approximately 80, 55, 8 and 0% of the dose was recovered in the urine. Furthermore, when introduced into the abomasum, DAPA could be measured in plasma from the anterior mesenteric and jugular veins.

It was concluded that the digestive enzymes of the sheep are unable to digest the DAPA-containing mucopeptides of bacteria originating in the rumen. The micro-organisms of the hind-gut can, however, degrade this bacterial fraction.

Methods of measuring flow of duodenal digesta in sheep. By D. A. CORSE and J. D. SUTTON, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Three yearling sheep were fitted with duodenal re-entrant cannulas and three others with a T-piece cannula immediately caudal to the pylorus. All six had a rumen cannula. Total collections from re-entrant cannulas were made from 12.00 hours to 24.00 hours by the technique of Nicholson & Sutton (1969). On other occasions, spot samples of 150–200 g were accumulated from the proximal part of the re-entrant cannulas with the distal part pinched to prevent backflow. Six samples were combined over 3 d to represent every 2 h of the 12 h period. Spot samples from the T-pieces were collected according to the above sampling regimen to coincide with periods of both total and spot collections from the re-entrants. Each collection procedure was carried out on two occasions.

Feeds were offered in equal quantities twice daily at 06.00 and 17.00 hours. Chromic oxide (Cr_2O_3), impregnated into paper, was placed in the rumen at feeding time and polyethyleneglycol (PEG), in solution, was infused into the rumen at a constant rate. Rations fed at 1.5 times maintenance were composed of 70:30 hay-concentrates (940 g/d, ration 1) and 20:80 hay-concentrates (790 g/d, ration 2). The concentrates contained a conventional dairy mix (21% crude protein) and flaked maize in equal proportions.

Amounts of dry matter reaching the duodenum in 12 h, based on ratio of marker input to concentration in digesta, are shown in Table 1.

Table 1. *Mean values, with standard errors, for digesta dry-matter flow at the duodenum (g/12 h)*

		Total	Spot (re-entrant)	Spot (T-piece)
Ration 1	Cr_2O_3 -corrected	284.1 ± 16.6	281.1 ± 8.2	300.2 ± 7.4
	PEG-corrected	302.0 ± 12.2	330.3 ± 19.1	357.1 ± 7.4
Ration 2	Cr_2O_3 -corrected	184.3 ± 8.6	171.6 ± 4.3	189.7 ± 6.4
	PEG-corrected	191.6 ± 12.2	209.0 ± 23.1	201.5 ± 6.9

Dry-matter flow corrected for PEG was consistently higher than that corrected for Cr_2O_3 , indicating lower recovery of PEG at the duodenum. Caution is obviously

needed in comparing quantitative results from experiments in which the individual different markers have been used.

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The effect of nematode parasitism on intake, growth and mineral metabolism of growing lambs. By A. E. REVERON, J. H. TOPPS and G. PRATT, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Twenty-four cross-bred wether lambs, reared under worm-free conditions, were divided at random into two groups of six (control) and eighteen (treated) animals. All animals were started on the experiment when they reached 23 kg in weight and were given a concentrate diet of malt distillers' grains and dried grass *ad lib*. Each of the treated lambs was infested with 40 000 larvae of *Trichostrongylus colubriformis*. Measurements were made of intake, growth, digestibility of the diet, levels of certain blood constituents and mineralization of the skeleton.

Table 1. *Voluntary intake of metabolizable energy (Mcal/24 h) and growth of lambs (kg/24 h) infested with Trichostrongylus colubriformis*

	Control lambs (mean for six)	Infested lambs (mean for eighteen)	Significance of difference (<i>P</i>)
Intake	3.11	2.49	< 0.001
Growth	0.223	0.105	< 0.001

There was no significant difference in digestibility of dietary energy, protein or crude fibre between the control and infested lambs. The poorer growth appeared to be entirely or mainly due to a lower food intake. Within the infested group, half the animals reached the approximate slaughter weight of 40 kg and appeared to be less affected by the parasite than the other half whose growth was severely retarded since they failed to attain weights greater than 30 kg. Two lambs from each of these two subgroups and two control lambs, all chosen at random, were used for measurement of bone growth and mineralization and of certain blood constituents.

Table 2. *Percentage composition of the right femur from control lambs and lambs infested with Trichostrongylus colubriformis*

	Cortex	Ash	Calcium	Phos- phorus	Mag- nesium	Ash organic matter	Ratio, Ca:Mg
Control	40.0	59	36	18	0.70	1.40	51
Infested, growth slightly retarded	40.0	56	38	18	0.64	1.25	59
Infested, growth severely retarded	19.3	47	36	18	0.53	0.89	68

Concentrations of serum calcium and magnesium and of alkaline phosphatase

were in the normal range for all six lambs throughout the experiment, but concentrations of serum inorganic phosphorus and albumin of the severely affected animals gradually declined. These and other results indicate that poor absorption and losses of certain nutrients may be associated with the inappetence of the parasitized lambs.

Effects of high levels of dietary sulphate on voluntary feed intake of sheep.

By P. K. UPTON and J. L. L'ESTRANGE, *Department of Agricultural Chemistry, University College Dublin, Glasnevin, Dublin 9*

Previously, it was shown (L'Estrange, Clarke & McAleese, 1969) that sulphate added to grass-meal pellets to provide 1% sulphur in the feed dry matter (DM), either as H_2SO_4 or as the salts Na_2SO_4 , $NaHSO_4$, NH_4HSO_4 and $(NH_4)_2SO_4$, reduced the voluntary feed intake (VFI) of sheep. Intake was about 78% of the control value (grass pellets only) with Na_2SO_4 and $NaHSO_4$, and about 56% of the control value with the other compounds. The relationship between dietary sulphate and VFI is further examined here.

In Expt 1, Na_2SO_4 in solution was added to grass-meal pellets to provide 0.5, 1.0 and 1.5% S in the feed DM. For a fourth treatment, NaCl was added to provide the same level of sodium as the 1.5% S treatment. In each treatment, along with the control (grass pellets alone), food was given *ad lib.* for 14 d to five sheep in a 5×5 Latin square design.

Table 1. Mean values (day 0-14) for five sheep/treatment

Treatment	Intake			Plasma SO ₄ -S (mg/100 ml)	Rumen fluid*		
	DM (kg/d)	S (g/d)	Na (g/d)		Total S (mg/100 ml)	SO ₄ -S (mg/100 ml)	SH-S (mg/100 ml)
Control	1.37	6.4	0.94	6.5	16.2	5.4	0.21
0.5% S	1.11	10.8	7.8	9.7	23.4	13.8	0.41
1% S	0.71	10.2	9.5	9.0	30.0	19.7	0.59
1.5% S	0.56	10.4	9.3	9.6	32.9	16.0	0.75
NaCl	1.22	5.2	22.8	6.4	16.7	6.8	0.16
SE of treatment means	0.066	(-)	(-)	(-)	2.61	2.31	0.058

*Mean values for samples taken twice daily on 3 d.

Results are given in the Table. Feed intake on the three sulphate treatments decreased with increasing dietary sulphate level so that S intake and plasma sulphate concentration were very similar for each treatment. The concentration in rumen fluid of total-S, sulphate-S and sulphide-S increased with increasing dietary sulphate levels. VFI was only slightly reduced by the NaCl treatment showing that excess dietary sodium was not responsible for reduced intake on the sulphate treatments.

In Expt 2, the effects of Na_2SO_4 and H_2SO_4 at a level of 1% S in the feed DM were compared. Each was given either as part of the pelleted grass diet or intraruminally while the sheep consumed the grass pellets. In each treatment, along

with the control (grass pellets alone), the diet was offered *ad lib.* for 14 d to five sheep in a 5 × 5 Latin square design. DM intakes on the treatments, Na₂SO₄ in the diet, H₂SO₄ in the diet, Na₂SO₄ intraruminally and H₂SO₄ intraruminally, were 75%, 26%, 66% and 55% respectively of that on the control. Thus, H₂SO₄ decreased VFI more than Na₂SO₄ did, but the difference was much less when both were given intraruminally. Taste influenced intake of H₂SO₄, presumably because of the low pH of the treated pellets.

REFERENCE

L'Estrange, J. L., Clarke, J. J. & McAleese, D. M. (1969). *Irish J. agric. Res.* **9**, 133.

Effects of dietary hydrochloric and sulphuric acids on voluntary feed intake and metabolism of sheep. By J. L. L'ESTRANGE and F. MURPHY, *Department of Agricultural Chemistry, University College Dublin, Glasnevin, Dublin 9*

A. I. Virtanen in several publications, e.g. Virtanen (1933), claimed that silage preserved with H₂SO₄ was much less palatable than silage preserved with a mixture of HCl and H₂SO₄. This implies that excess dietary sulphate adversely affects the voluntary feed intake (VFI) of ruminants. Recent experiments on ammonium bisulphate and other sulphate salts with cattle (McCarrick, Gardiner, Poole, Spillane & Maguire, 1966) and sheep (L'Estrange, Clarke & McAleese, 1969) indicated that metabolic acidosis induced by the acid sulphate salts was more important in reducing VFI than sulphate *per se*.

The effects of dietary HCl and H₂SO₄ have now been compared direct in sheep. The acids, either singly or as a 50:50 mixture, were added to a pelleted grass diet to reduce the pH to 3.8 (80 ml 4 N-acid/kg grass pellets). Each diet, as well as the grass pellets alone, was offered *ad lib.* to eight sheep in a double 4 × 4 Latin square design for 20 d. The results are summarized in Table 1.

Table 1.

	DM-intake (kg/d) (day 0-20)	Wt gain (kg/d) (day 0-20)	Digestibility of DM (day 8-20) (%)	pH of rumen fluid*
Grass pellets only	1.46	0.168	54.5	6.51
Grass pellets + HCl	1.18	0.082	56.0	6.36
Grass pellets + HCl-H ₂ SO ₄	1.18	0.052	56.0	6.44
Grass pellets + H ₂ SO ₄	1.02	0.022	58.2	6.43
SE of treatment means	0.033	0.033	0.41	0.034

	Mean of samples taken from day 2-20				Urine pH
	Blood pH	Plasma CO ₂ (m-moles/l)	Plasma SO ₄ -S (mg/100 ml)	Plasma Cl (mg/100 ml)	
Grass pellets only	7.46	26.4	6.52	372	8.07
Grass pellets + HCl	7.38	22.4	6.84	388	5.89
Grass pellets + HCl-H ₂ SO ₄	7.40	23.0	7.58	394	5.78
Grass pellets + H ₂ SO ₄	7.42	23.4	8.22	386	5.69
SE of treatment means	0.009	0.78	0.21	5.0	(-)

Values for DM-intake and wt gain are the means for eight sheep whereas the other values are the means for four sheep/treatment.

DM, dry matter.

*Mean value for samples taken four times daily on 2 d.

Dry-matter intake was reduced to 70% of that in the control treatment by H_2SO_4 and to 80% on both the HCl and the HCl- H_2SO_4 treatments. Live-weight gain of the sheep was correspondingly reduced. Rumen fluid pH was slightly lower but the digestibility of the diets slightly higher on each acid treatment. The acids caused a similar degree of metabolic acidosis but, whereas plasma sulphate was increased by dietary H_2SO_4 , plasma Cl was not affected by dietary HCl.

The effect of HCl on VFI is ascribed to metabolic acidosis rather than to taste as intake decreased gradually up to day 4 on the treatment. The level of dietary sulphate was sufficiently high on the H_2SO_4 treatment but not on the HCl- H_2SO_4 treatment to reduce intake further.

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Transfer of metabolites between sheep by cross-circulation dialysis as a means of studying chemostatic control of voluntary food intake in ruminants. By J. M. FORBES, *Department of Agricultural Sciences, University of Leeds*

A survey of the literature suggests that 'chemostatic regulation of voluntary food intake is a distinct possibility' (Thye, Warner & Miller, 1970). Despite many attempts to elucidate such a control mechanism, both by infusion of substances intraruminally, intravenously or intracranially and by measurement of changes in rumen and plasma metabolites during a meal, chemostatic control has not yet been fully explained.

Parabiosis is a technique which has proved useful in the study of the regulation of food intake in rats (Fleming, 1969). It was not thought possible to join ruminants parabiotically and direct cross-circulation would involve the use either of identical twins, whose occurrence in sheep is not proved, or of immunosuppressive drugs which might affect energy metabolism.

These considerations have led to a development of a technique of cross-circulation dialysis (CCD) in which the blood of two animals is circulated independently through the two layers of a Kiil dialyser ('kidney machine'). Warm saline is recirculated over the cellophane membrane surfaces of the blood chambers and carries dialysed molecules along the concentration gradient between the blood-streams.

At first, sheep were equipped with semi-permanent carotid artery-jugular vein shunts but circulation through these could not be maintained for more than a few days, even when the animals were continuously infused with anticoagulants. Temporary polytetrafluoroethylene cannulas are now introduced into both jugular veins of each sheep and circulation is induced by peristaltic pumps. Heparin is used during CCD to prevent coagulation, and flow rates of 150 ml/min from each sheep are achieved. The duration of each CCD has so far been restricted to 4 h but runs of 24 h or more would appear to be quite feasible.

Work so far has concentrated on measuring rate of exchange of metabolites between animals and thiocyanate has been used as a marker. Blood samples are taken at regular intervals before and during CCD, and the plasma is analysed for thiocyanate, glucose, free fatty acids and ketone bodies. The results show that plasma concentrations do not completely equilibrate during 4 h of CCD, presumably because of the buffering action of extracellular fluid; however, the rate of transfer is sufficient to show that a considerable interchange of metabolites would occur between animals, particularly with longer periods of CCD.

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Voluntary intake and energy metabolism of sheep fed chopped dried grass and the same material milled and pelleted. By F. W. WAINMAN, J. S. SMITH and K. L. BLAXTER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9BS*

A general-purpose sward of perennial ryegrass, cocksfoot, timothy and clover, sown in 1967, was grazed and then harvested twice for drying in 1968. About 100 units of nitrogen were applied as pig sludge before each crop.

The grass that had been dried in August was fed both chopped (5–8 cm), and milled (3 mm screen) and pelleted (10 mm diam., 13–36 mm long) to each of four sheep in two amounts, 800 g/d and *ad lib.* The sheep were Suffolk × Half-bred wethers 2.5 years old. For 21 d prior to the measurements of energy exchange, when the sheep were fed *ad lib.*, they were fed such that the amount offered was equal to 110% of the amount eaten the previous day.

The measurements of respiratory exchange were for 16 consecutive d in the *ad lib.* fed sheep and for 5 d when the 800 g ration was given, excreta collections lasting 16 d and 6 d respectively. The composition of the feeds and the mean values of data obtained with the four sheep are shown in Table 1.

When fed *ad lib.*, the sheep consumed 22% more dry matter as pellets than as chopped grass. The digestibility of organic matter decreased from 64.8% for the chopped grass to 59.7% for the pellets. The mean energy retention was almost doubled by the pelleting process.

The net availability of metabolizable energy of the chopped grass for growth and fattening was 31.2%, and this accorded with the values previously reported for third-harvest grass (Blaxter, Wainman, Dewey, Davidson, Denerley & Gunn, 1970). The utilization of metabolizable energy of the pelleted grass 45.5% was in agreement with the values associated with first harvest material. Thus, the increase in intake associated with pelleting was accompanied by a more efficient utilization of meta-

bolizable energy for fattening and together these factors more than offset the decline in digestibility when the pelleted grass was fed.

Table 1. *Energy metabolism of sheep fed chopped dried grass and the same material milled and pelleted (means for four sheep)*

Ration	800 g chopped	800 g pellets	<i>ad lib.</i> chopped	<i>ad lib.</i> pellets
Crude protein (%)	10.0	11.0	10.1	11.2
Crude fat (%)	2.2	3.0	2.7	3.4
N-free extract (by difference) (%)	52.8	53.3	52.1	51.8
Crude fibre (%)	27.0	24.2	27.0	25.1
Ash (%)	8.0	8.5	8.1	8.5
Intake dry matter (g/d)	711	717	1829	2332
Apparent digestibility of organic matter (%)	71.6	69.7	64.8	59.7
Apparent digestibility of energy (%)	68.0	66.0	61.3	56.5
Percentage losses of energy:				
in faeces	32.0	34.0	38.7	43.5
in urine	5.0	5.2	3.5	3.4
as methane	8.3	8.4	6.8	6.3
ME/g organic matter ingested (kcal)	2.63	2.52	2.44	2.25
Energy retention (kcal/24 h)	-300	-349	673	1293
Intake (g dry matter/kg W ^{0.75})			76.6	96.9
Net availability of ME			31.2	45.5

ME, metabolizable energy,

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The oesophageal groove reflex and the performance of individually versus group-fed lambs. By M. J. LAWLOR, J. K. KEALY* and S. P. HOPKINS, *Animal Nutrition and Biochemistry Department, Agricultural Institute, Dunsinea, Castleknock, Co. Dublin*

Two experiments were conducted to study the extent to which the oesophageal groove reflex functions in lambs sucking from individual versus group feeders and throughout the fattening period. The performance of lambs fed combinations of liquid and solid diets has also been studied. In the first experiment, ten lambs weaned at 48 h were trained to suck from individual bottles. They were fed a reconstituted ewe's milk substitute, Ewelac, *ad lib.* to slaughter weight. A further twenty-two lambs weaned between 6 and 14 d were trained to suck from a bulk dispenser. They were fed the milk replacer *ad lib.* and a pelleted roughage-concentrate feed from approximately 14 kg live weight. At 20 kg live weight, eleven were weaned on to solid food only but were subsequently offered the milk substitute on two occasions. The remaining eleven were fed the milk substitute on a restricted basis and the pelleted feed *ad lib.* All lambs were x-rayed periodically.

Seventy-four lambs weaned at 48 h were used in the second experiment. Twenty-four were individually fed the milk substitute and the remaining fifty were group-fed. All were fed the milk substitute *ad lib.* to 17 kg live weight. Both individually fed

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and group-fed lambs were then placed on the following four treatments: (1) a pelleted roughage-concentrate ration containing 15% crude protein, (2) milk substitute plus the same feed as in (1), (3) milk substitute plus a roughage-concentrate containing 7.5% crude protein, (4) a homogenate of calcium caseinate (Casilan) and the same feed as in (3). The solid food was offered *ad lib.* whereas the milk substitute and Casilan were restricted to 2 l/animal daily. All lambs were x-rayed at 3-week intervals.

In both experiments, the effective sucking behaviour of the lambs was closely related to the functioning of the oesophageal groove reflex and to lamb performance. In the first experiment, the milk substitute consistently entered the abomasum in 80% of individually fed and 55% of the group-fed lambs. The corresponding values for the second experiment were 92% for individually fed and 68% for the group-fed lambs. In all instances where the milk substitute entered the abomasum, it continued to do so right up to slaughter even when it was withdrawn and offered to the lambs after some weeks. Poor sucking, low milk consumption and the entry of the milk into the rumen resulted in very poor lamb performance. Such lambs behaved like 'tail enders' which sometimes occur among lambs reared with ewes. In the second experiment, the best growth rate was obtained with the lambs fed the combination of milk substitute and low-protein roughage-concentrate ration. The growth rates for the four treatments were 0.26, 0.28, 0.33 and 0.21 kg/d respectively.

We thank MacCormac Products Limited, Killeshandra, Co. Cavan for supplies of Ewelac and a financial contribution towards this work. We thank Glaxo Laboratories Limited, Greenford, Middlesex for supplying the Casilan.

The effect of protein supplementation via the abomasum on the voluntary intake of concentrate by young growing sheep. By E. R. ØRSKOV, C. FRASER and ELIZABETH L. CORSE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Egan & Moir (1965) and Egan (1965) have shown that the voluntary intake of low-nitrogen roughage diets is increased as a result of infusing casein into the duodenum of mature sheep. This effect could not be explained in terms of a greater quantity of endogenous N being recycled to the rumen since urea infusion had little or no effect, and Egan (1965) pointed out a possible effect of the protein status of the animal in controlling voluntary intake of roughage diets.

With concentrate diets, we have noted an increase in voluntary intake by young lambs when the protein concentration of the diet was raised. This could, however, be due to an alteration in the rate of rumen digestion as discussed by Balch & Campling (1962).

The use of the oesophageal groove for giving protein supplements (Ørskov & Benzie, 1969) had made it possible to separate these effects, and the influence of protein supplements given in this manner on the voluntary intake of a barley diet has been investigated. A rolled barley diet which included urea, vitamin and mineral supplements, and which contained 13% crude protein in dry matter was given *ad lib.*

to ten male and ten female Suffolk × North Country Cheviot lambs which had been trained to receive milk from a bottle. Water was always available from a trough. The lambs were randomized within each sex to receive one of five treatments. In about 400 ml of water, the animals were given daily in four equal feeds either 0, 17, 34 or 51 g of a fish-protein concentrate (Astra Nutrition, Sweden) to give 2.3, 4.6 or 6.9 g N/d. For the fifth treatment, urea was added to the water to give 4.6 g N/d.

There were no differences between the lambs receiving only water and those receiving the urea in the water. The increase in intake due to protein supplementation amounted to about 15%. At each live weight, however, intake was significantly lower for these two treatments than for those in which protein supplements were given. There were no differences that were significant between the levels of fish supplementation.

The results show that, regardless of rumen fermentation, the protein intake of young, fast growing lambs has an effect on voluntary intake. In this respect the ruminant does not appear to behave differently to the non-ruminant animal, results for which were reported by Osborne & Mendel (1918).

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The subsequent effects of removing the large intestine from newborn pigs. By A. W. WILKINSON and R. A. McCANCE, *Institute of Child Health, London and Sidney Sussex College, Cambridge*

Thirteen sucking pigs, about 10 d old, were taken from the mother at 08.00 hours and, 3–6 h later, they were anaesthetized with ether and the large gut, including the caecum and the ileo-caecal valve, was removed. The terminal ileum was joined to the lower descending colon by an end-to-end anastomosis. The piglets were returned to the mother on the following morning. Litter-mates were removed from the mother for the same length of time but not interfered with surgically.

After the operation, all the animals passed voluminous watery faeces and eight of them died within 10 d from surgical or nursing troubles. Five of them survived and were weaned gradually at 6–8 weeks on to a combination of Amvilac No. 2 (Glaxo Laboratories Ltd) and a barley–fish meal mixture (McCance, 1960). On the latter, the animals grew to maturity although not so rapidly as their litter-mates and other normal animals on the same diet. Their mean weight at 1 year was $127 \pm \text{SD } 19.8$ kg (controls 198 ± 25) and at 2 years $229 \pm \text{SD } 31$ kg (controls 268 ± 22).

With time, the diarrhoea subsided and, as the animals got older, the stools gradually became almost normal. In adult life the stools contained about 83% of water and those of the controls 75%. The percentage of fat was normal and not consistently raised by making up the diet to contain 12% and then 20% of fat.

Two of the animals had litters. One of them had ten, and the other five, piglets; and four in each litter survived, were suckled successfully and grew very well.

One Sunday evening, one adult died unexpectedly of acute strangulation and intestinal obstruction originating among adhesions. The remainder were killed and found to be without adhesions. The carcasses were all as fat as those of the controls and in equally good condition, but measurements of the carcasses (McCance & Wilkinson, 1967) and bones showed them to be somewhat smaller animals.

The evidence for any hypertrophy of the small intestine as an adaptation to the removal of the caecum and colon is being carefully examined.

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Activity of drug-metabolizing enzymes in the liver of growing rats fed on diets high in sucrose, glucose, fructose or an equimolar mixture of glucose and fructose. By J. W. T. DICKERSON, T. K. BASU and D. V. PARKE, *Department of Biochemistry, University of Surrey, Guildford*

Previous work has shown that the pattern of drug-metabolizing enzymes in the liver responds in different ways to the amount of protein in the diet and to the ratio of carbohydrate to protein (Dickerson, Basu & Parke, 1971). The present study is concerned with the response of these enzymes to various carbohydrates.

Male Wistar rats of similar body-weight were divided at 24 d of age into five groups. Group A was maintained on a diet containing 60% starch, and groups B, C, D and E on diets that were similar in every respect except that the starch was replaced by sucrose, glucose, fructose, or an equimolar mixture of glucose+fructose respectively. In the first experiment, ten animals were used in each group and these were killed after 14 d on the diet.

Since phenobarbitone in suitable doses is known to induce the drug-metabolizing enzymes, a second experiment was performed in which five animals on each of the five dietary regimens were given intraperitoneally a dose of 100 mg/kg body-weight of sodium phenobarbitone 24 h before killing.

The activities of biphenyl 4-hydroxylase, *p*-nitrobenzoate reductase and cytochrome P-450 were determined in the liver as previously described (Basu, Dickerson & Parke, 1970).

In the first experiment, the liver weights tended to be higher in rats fed sucrose and the monosaccharides than in those fed starch. However, liver weights were significantly increased after phenobarbitone in all groups except the group fed sucrose. The specific activity of biphenyl 4-hydroxylase was lower in those animals fed sucrose or glucose+fructose than in those fed starch or glucose or fructose. After phenobarbitone, the activity in the starch-fed animals was higher than that in all other groups and the differences previously noted between the other groups had disappeared.

The specific activity of *p*-nitrobenzoate reductase was increased by fructose and glucose alone but not by sucrose or by the equimolar mixture of fructose and glucose. Phenobarbitone did not alter the pattern.

The effect of the various diets on cytochrome P-450 tended to be similar to that on biphenyl 4-hydroxylase in that sucrose and the monosaccharides tended to result in a decrease in specific activity. This pattern was enhanced by giving phenobarbitone.

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The effects of duodenal infusions of sunflower oil on the yield and fatty acid composition of milk fat in the cow. By R. BICKERSTAFFE and E. F.

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The extensive biohydrogenation of dietary fats in the rumen (see Dawson & Kemp, 1970) severely limits the amounts of polyunsaturated fatty acids (including the essential fatty acids) which become available to the animal, and accounts for the low levels of those fatty acids in the carcass and in milk. Recent studies in the lactating goat given diets rich in linoleic acid have shown that the amounts reaching the small intestine were 1.0–1.5% of the total dietary calories (Bickerstaffe, Noakes & Annison, 1970), a value which is marginally low in relation to the established requirement for pigs (Sewell & McDowell, 1966) and chickens (Hopkins & Nesheim, 1967). The effects of increasing the availability of unsaturated fatty acids to the mammary gland of the cow have been investigated by the intravenous infusion of emulsified fats (Tove & Mochrie, 1963; Storry, Hall, Tuckley & Millard, 1969; Stewart & Irvine, 1970) and by feeding unsaturated fats protected from ruminal hydrogenation (Scott, Cook, Ferguson, McDonald, Buchanan & Loftus-Hills, 1970). In all instances the proportions of polyunsaturated fatty acids in milk fat were raised, but in these short-term experiments the effects on milk yield and milk fat output were not investigated.

We have infused emulsions of sunflower oil into the duodenum of a lactating cow given a conventional ration (including 380 g fat/d) and prepared with a duodenal fistula. The amounts of sunflower oil were increased at each successive infusion period (4–5 d) to a maximum of 650 g/d. Milk yield remained unchanged at 22 l/d over each infusion period, but fat output was increased from 750 g to a maximum of 1050 g/d and the linoleic acid content of milk fat from 3.0 to 27.0 moles/100 moles. The increased milk-fat output and greatly increased linoleic acid content of milk indicated the absence of adverse effects when the mammary gland was supplied with increased amounts of linoleic acid.

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The digestion and absorption of carbohydrates in chicks infected with intestinal coccidiosis. By A. H. SYKES and J. WALTERS, *Wye College (University of London), Ashford, Kent*

It has been shown in the chick that heavy infections of *Eimeria acervulina* cause a severe reduction in the uptake of glucose by isolated sacs of the infected intestine and it has been suggested that this malabsorption may contribute to the loss of body-weight which normally occurs (Preston-Mafham & Sykes, 1970). It has since been observed, however, that absorption from the intestine distal to the infection is not affected and it was necessary, therefore, to determine whether or not there was any net reduction in absorption in the intact, infected bird.

Experiments were carried out on 6-week-old chicks infected with 8 million oocysts of *E. acervulina*. Absorption was measured by the method of Reynell & Spray (1956) using an oral dose of glucose or maltose, 2 g, and polyethylene glycol, 50 mg, as marker. The chicks were killed on day 5 at intervals of 0.5–4 h after intubation and the rates of gastric emptying (proventriculus and gizzard), intestinal transit and absorption were determined.

The rate of gastric emptying was reduced by the disease but intestinal transit was not significantly affected. Glucose absorption in the duodenum and jejunum was depressed but not in the ileum. In the control birds, 96% of the intestinal glucose load was absorbed within 0.5 h compared with 35% in the infected birds. At 1, 2, 3 and 4 h the amount of glucose absorbed by the infected birds increased to 64%, 77%, 93% and 99% respectively. With maltose, there was little or no absorption in the duodenum and jejunum and the rate of hydrolysis was reduced, but after 4 h practically all the maltose load had disappeared.

These results suggest that, despite extensive malabsorption, the gastro-intestinal contribution to body-weight loss, as distinct from alterations in feed intake and utilization (Sykes, 1970), arises more from changes in the rate of passage of feed than from changes in the total absorptive capacity.

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Gaseous nitrogen evolution by animals? A study of nitrogen retention by rats and chicks. By M. LEWIS and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns*

Nitrogen retention by animals may be measured indirectly by the balance technique or direct by carcass analysis. In the nitrogen balance technique, the difference between input and output is assumed to be retained in the body. In the carcass analysis method, the final nitrogen content of the animals is compared with their initial nitrogen content, predicted from analyses of a control group killed at the start of the trial.

The two methods have given results agreeing within experimental error (Becker & Harnisch, 1958*a,b*; Sanslone & Squibb, 1962; Nehring, Knabe & Bock, 1964) but discrepancies have been reported which cannot be explained (Henry, 1965; Davidson & Williams, 1968). Costa, Ullrich, Kantor & Holland (1968) have suggested that the discrepancy may be explained if some of the nitrogen fed is eliminated as nitrogen gas. We have tested this hypothesis and the need for it by determining nitrogen retention by both methods using rats and chicks in a closed circuit respiration chamber in an argon-oxygen atmosphere; the nitrogen in the chamber was monitored by mass spectrometry. The results show that neither rats nor chicks liberate gaseous nitrogen from dietary proteins, even when presented with amounts far in excess of requirement. There is no consistent discrepancy between balance and carcass analysis methods of estimating nitrogen retention. In six experiments, lasting on average 262 h, the mean discrepancy was $+0.017 \pm 0.297$ g nitrogen for twenty-two rats which retained a total of 20.5 g nitrogen. In a seventh experiment, lasting 190 h, four chicks retained 9.45 g nitrogen and the discrepancy was $+0.041 \pm 0.157$ g nitrogen.

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Body-weight and its adipose tissue component. By J. V. G. A. DURNIN and J. WOMERSLEY, *Institute of Physiology, The University, Glasgow, W2*

Infant feeding patterns and social class. By ALISON E. BLACK, *MRC Reproduction and Growth Unit, Princess Mary Maternity Hospital, Newcastle upon Tyne NE2 3BD*

Some dietary findings, obtained during a small-scale study of the growth and health of preschool children in Newcastle, are presented. Sixty-four families from

three general medical practices, representing a wide socio-economic range and containing babies initially aged 2–4 months, were visited monthly and notes were made of the infant feeding habits. For present purposes, the families are divided into white collar (NM, non-manual) and manual (M) groups, according to the fathers' occupations.

Breast-feeding was started with twenty-five infants but was abandoned for twenty of these within 4 months. Five infants that were fully breast-fed for 6 or 7 months were all in the NM group.

Full or partial bottle-feeding was used with fifty-nine infants. Two infants were completely weaned from the bottle at 6 months and nineteen were still receiving at least one bottle daily at over 18 months. In many M families, the bottle was used to provide a bed-time drink for toddlers. No NM mother continued to provide a bottle at night for longer than 4 months after stopping day-time feeds. The duration of bottle-feeding, defined as two or more bottles daily, also tended to be longer among M families (Table 1).

Table 1. *Duration of feeding two or more bottles/d in fifty-nine infants who were entirely or partly bottle-fed (five fully breast-fed infants excluded)*

Period (months)	Non-manual (26)		Manual (33)		Total (59)	
	No.	%	No.	%	No.	%
Under 6	7	26.9	5	15.2	12	20.3
6–9	11	42.3	7	21.2	18	30.5
9–12	4	15.4	10	30.3	14	23.7
12–15	2	7.7	6	18.2	8	13.6
15–18	2	7.7	1	3.0	3	5.1
Over 18	0	0	4	12.1	4	6.8
Median	8 months		11 months		9 months	

The patterns of early feeding with solids revealed a high dependence on convenience foods in both classes. Only five families used no special baby food. A proprietary baby cereal was usually introduced between 1 week and 3 months, and was soon accompanied by canned, sieved 'baby dinners'. These gave place to canned 'junior dinners' which in turn were replaced by shares of the family diet. The timing of the sequence was variable. Of the fifty-nine mothers using special baby foods, three gave them up before 6 months; all three were in the NM group and used liquidizers at home. Five mothers continued using them after 18 months. All the mothers were offering 'family' foods to their babies by 15 months; a few began before 3 months.

Although wide variations of detail existed from family to family, with the exceptions noted above, these seemed to be unrelated to the socio-economic background. Preliminary quantitative results (not reported here) show no evidence of a 'poverty gradient'. The propaganda of Child Welfare agencies and commercial advertising may have eliminated important social class differences in infant feeding patterns,

and this may help to explain why social class differences in growth rates during infancy are small or absent (Ministry of Health, 1959; and the present study).

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A comparison of the calcium and phosphorus availability in a diet fed to both the rat and the pig. By C. T. WHITTEMORE,* A. THOMPSON and W. C. SMITH, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

A simplified radioisotopic procedure (Whittemore & Thompson, 1969) has been used in experiments with pigs and rats to study the availability of calcium and phosphorus contained in a standard pig diet (M.L.C. No. 1 Sib diet). In the pig experiment, 25 kg animals were used; four pairs were fed 600 g/24 h (maintenance level) and five pairs were fed 1200 g/24 h (Agricultural Research Council recommended production level). In the rat experiments, 50-d-old animals of approximately 125 g live weight were used; six pairs were fed 10 g/24 h (maintenance) and six pairs were fed 15 g/24 h. The essential procedure was the same for both species. ^{45}Ca and ^{32}P were administered 7 d after commencement of feeding, orally to one animal of a pair and intramuscularly to the other. The animals were slaughtered after a further 7 d. With the pigs, the recoveries of radionuclides from the metacarpal and metatarsal bones were determined; with the rats the recoveries were determined for the entire carcasses.

The present results (Table 1) are in agreement with those reported by Besancon & Gueguen (1969) and Gueguen, Besancon & Rerat (1968) for 30–50 kg pigs fed at production levels, 45.3% for calcium and 71.0% for phosphorus.

The availability of calcium in the present studies is appreciably lower than the value of 67% suggested by Agricultural Research Council (1967) and that of phosphorus higher than the suggested value of 50% (Agricultural Research Council, 1967).

Thus, in the present study for a calcium intake of 9.3 g/24 h the amount absorbed was 3.9 g/24 h; the Agricultural Research Council recommend a net requirement of

Table 1. *Availability of calcium and phosphorus in M.L.C. No. 1 Sib diet fed at each of two levels to young pigs and rats*

Species	Food intake (g/24 h, air-dry basis)	Intake		Availability (%)	
		Ca	P	Ca	P
Pig	600	5.1	4.9	39.6 ± 1.6	82.5 ± 2.6
	1200	9.3	9.6	41.5 ± 2.4	78.1 ± 1.5
Rat		mg/24 h			
	10	97	81	38.2 ± 1.9	60.6 ± 2.1
	15	145	121	31.8 ± 1.4	56.8 ± 2.2

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6.3 g/24 h. For a phosphorus intake of 9.6 g/24 h, the amount absorbed was 7.5 g/24 h; the Agricultural Research Council recommend a net requirement of 4.6 g/24 h. It appears that, at the production level of feeding, the pigs were absorbing an inadequate amount of calcium and excess amount of phosphorus.

It can be seen from the table that availability of calcium for the rat, though identical to that for the pig at the maintenance level, is significantly lower at the higher level of intake. At both levels of feeding, the values for phosphorus are lower than those found for the pigs. Clearly, further data are needed to assess the value of using the rat as an assay animal for studies on pig diets.

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Energy retention in the pig at several environmental temperatures and levels of feeding. By W. H. CLOSE and L. E. MOUNT (Introduced by W. F. M. LEAT), *ARC Institute of Animal Physiology, Babraham, Cambridge*

Twelve groups of pigs, fed either 34, 39, 45 or 52 g food/kg body-weight per d, were kept for periods of 4 weeks in a large calorimeter equipped as an animal pen (Mount, Holmes, Start & Legge, 1967). The calorimeter was maintained at 7, 12, 20 or 30° in order to determine the extent to which feeding level influenced the heat loss of the animals at various ambient temperatures. The rate of heat loss from the pigs increased as the plane of nutrition increased at environmental temperatures of 12, 20 and 30°; at 7° the mean heat loss was not related to the plane of nutrition and was higher than at the other temperatures (Close & Mount, 1971).

Mean values of the energy retained by the animals were calculated as the differences between the rates of heat loss and rates of intake of metabolizable energy at each temperature and feeding level. Energy retention was depressed at 7° relative to 12, 20 and 30°, but it varied little between the three higher temperatures at any one level of feed intake.

Table 1. *Relative amounts of protein and fat deposited by pigs fed 45 g/kg body-weight per d*

Ambient temperature (°C)	Mean body-wt (kg)	Protein deposition (g/d)	Fat deposition (g/d)
7	23.3	33	95
12	33.7	55	178
20	31.0	57	154
30	32.3	52	158

The mean daily depositions of protein and fat at each temperature and level of feeding were calculated from the energy retention and the gain in weight (Close,

1970). Values for the 45 g/kg per d feeding level are given in Table 1. At all temperatures and feeding levels, the deposition of fat occurred at a greater rate than that of protein.

From the collected results at 12, 20 and 30°, the energy costs of formation of protein and fat were estimated statistically as 12.1 and 13.7 kcal/g respectively. These estimates are similar to those of Thorbek (1970) and Kielanowski & Kotarbinska (1970) for the pig, and Ørskov & McDonald (1970) for the young sheep.

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The effect of ligating the pancreatic duct on digestion in the pig. By D. M. ANDERSON and R. W. ASH, *ARC Institute of Animal Physiology, Babraham, Cambridge*

Wass (1965) reported that pigs which were totally deprived of exocrine pancreatic secretion either by ligation or blockage of the pancreatic duct survived but failed to maintain a good nutritional state. This contrasts with observations which suggested that administration of pancreatic enzymes to weaned and pancreatectomized pigs appeared unnecessary (Anderson & Ash, 1970).

In order to investigate the role of exocrine pancreatic secretion in digestion, the apparent digestibilities of dry matter, protein, fat and nitrogen-free extract (NFE) of two diets were estimated before and at regular intervals during 10-22 weeks after ligation of the pancreatic duct. The operations were performed on six pigs 71-116 d old weighing 23-37 kg; recovery was rapid and there were no clinical signs of digestive dysfunction. Food consumption was restricted to 27 or 40 g/kg body-weight per d.

The digestibilities of dry matter and NFE were not affected appreciably by duct ligation but those of fat and protein were reduced. The effect on fat digestion varied more than the effect on protein digestion both within and between animals; further, there was no obvious relation between the digestibilities of fat and protein in the same animal. When an extract of pig pancreas (Cotazym) was added to the diets, the digestibility of fat returned to normal whereas that of protein increased slightly or not at all. The amount of food consumed appeared to have no effect on the digestibility of the nutrients. The mean results from three pigs on diet A and two on diet B are summarized in Table 1. The losses of nutrients attributable to the absence of pancreatic secretion in an animal receiving 2 kg food/d were approximately 50 g protein and 7-25 g fat/d. The animals were in positive nitrogen balance and body-

weight increased uniformly before and after ligation of the duct; glycosuria was never observed.

Table 1. *Apparent digestibilities (%)*

	Diet*	Intact animal	Ligated duct preparation	Ligated duct preparation + pancreatic extract
Protein	A	87.4	74.8	79.2
	B	84.0	69.8	72.2
Fat	A	74.2	54.5	71.9
	B	58.7	50.9	58.0
NFE	A	90.3	90.5	90.4
	B	89.2	89.5	89.5
Dry matter	A	83.8	79.3	81.6
	B	79.9	78.5	79.0

NFE, nitrogen-free extract.

*Diet A: crude protein 20.33%, fat (Folch, Lees & Stanley, 1957) 6.30%, ash 5.41%, crude fibre 2.80%, NFE 55.70%. Diet B: crude protein 16.60%, fat (Folch *et al.* 1957) 4.16%, ash 4.59%, crude fibre 4.06%, NFE 58.11%.

When the pigs were killed, the pancreatic ducts were found to be markedly dilated, tortuous and filled with clear fluid; the parenchyma appeared shrunken. Histologically, the acini were involuted, atrophied and surrounded by much connective tissue; the acinar cells were flattened, faintly stained and zymogen granules were absent but the islets of Langerhans appeared normal.

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The effect on glucose entry rate of abomasal protein infusion in sheep.

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The great dependence of fed ruminants on gluconeogenesis is now well understood (Ballard, Hanson & Kronfeld, 1969). It is usually supposed that the main exogenous sources for glucose synthesis are propionate and protein. The contribution of propionate is well documented (Leng, Steel & Luick, 1967; Bergman, Roe & Kon, 1966). However, the significance of protein has proved more difficult to evaluate. Ford (1965) and Judson & Leng (1968) have shown that glucose entry rate is increased when dietary protein is increased. The contribution of absorbed amino acids is difficult to assess in this way because the extent of degradation of protein in the rumen is not known. We have, therefore, determined the effect on glucose entry rate of infusion of protein into the abomasum.

Sheep were fed hourly with an automatic feeding device for 8-9 d. For the last 3 d, protein (100 g casein/d) was infused through a catheter inserted into the abomasum. In initial experiments, glucose entry rate was determined by means of a

single intravenous injection of [6-³H]glucose just before and at the end of the protein infusion. In later experiments, the isotope was infused continuously from 18 h before, until the end of the protein infusion. Similar results were obtained with both techniques, but the continuous infusion technique had the advantage of showing that the effect of protein infusion on the specific activity of circulating glucose was first shown after about 24 h.

The effect of protein on glucose entry rate is shown in Table 1. In all experiments, protein infusion increased glucose entry rate, the effect being least in a sheep starved 5 d before infusion of protein when mobilization of tissue protein should be considerable. The size of the increase in fed animals is similar, irrespective of whether the feed was predominantly hay or grain, and is approximately that expected if most of the glycolytic amino acids of casein were used for gluconeogenesis.

Table 1. *Effect on glucose entry rate of infusion of casein into the abomasum of sheep*

Ration (Starved)	Glucose entry rate (g/24 h)	
	Control	+100 g casein
450 g hay + 50 g wheat	35.2	46.8
450 g hay + 50 g maize	69.2	99.4
250 g hay + 250 g maize	51.1	80.1
450 g wheat + 50 g hay	83.7	115.6
400 g wheat + 100 g hay	59.6	86.7
	57.1	111.9

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An assessment of the nutritional status of the lactating beef cow. By S.

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Eleven Hereford × Shorthorn beef cows in their first lactation were divided into three groups of four, four and three animals. Each animal in the two larger groups was given a diet which provided sufficient energy for maintenance only whereas the third group was given enough dietary energy for maintenance and the daily production of 9 kg milk. This group of cows (HM) and one of the maintenance-fed groups (LM) were machine-milked whereas the other group (LS) were allowed to suckle their calves. Measurements were made at regular intervals of milk yield, body-weight and levels of certain blood constituents over a period of 72 d which began in the 2nd or 3rd month of lactation. One cow in group LS died from hypomagnesaemia on the 37th day of the experiment; another in group LM lost milk between milkings and responded differently to different stockmen. For these reasons the results for nine cows only are considered.

The mean daily yields of the cows in the three groups, HM, LM and LS were 9.3, 6.8 and 5.5 kg and mean changes in body-weight over the experiment were +19, -30 and -18 kg respectively. Concentration of serum calcium and inorganic phosphorus and of plasma glucose and urea were in the normal range for all cows throughout the experiment. Marked changes occurred in serum magnesium and plasma free fatty acids; these are summarized in Table 1.

Table 1. Concentrations of magnesium (mg/100 ml) in serum and free fatty acids (µequiv./l) in plasma of lactating beef cows either adequately fed or underfed (mean values for three animals)

Group of cows	Time on experiment (d)							
	0	15	30	40*	41	50	65	75
	Mg							
Adequately fed, milked	2.41	2.82	2.81	1.87	2.69	2.37	2.57	2.33
Underfed, milked	1.77	1.87	1.58	1.03	1.51	2.68	3.87	1.51
Underfed, suckled	2.51†	2.13†	1.23†	1.35	1.84	2.74	3.28	2.04
	Free fatty acid							
Adequately fed, milked	231	297	210	—	—	285	139	168
Underfed, milked	220	517	848	—	—	725	449	272
Underfed, suckled	97	349	301	—	—	372	264	177

*Mg supplement of 50 g calcined magnesite given daily to each animal after this sampling.

†Mean values for four animals.

The likelihood of hypomagnesaemia appeared to be related to a low dietary intake, but the possibility of an interaction between plane of nutrition and Mg intake could exist.

Milk-fat synthesis in cows fed high-roughage and low-roughage diets.

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The low milk-fat syndrome in dairy cows is frequently associated with low-roughage rations which result in decreased acetate concentrations and raised propionate concentrations in the rumen (see Davis & Brown, 1970). Many workers have suggested that the increased production of propionate is the critical factor, and that its effect is mediated through the increased availability of glucose which leads direct (or indirectly, via increased insulin secretion) to a change in the balance of fatty acid uptake and release in adipose tissue in favour of triglyceride synthesis. This results in the reduced availability of fatty acids for the synthesis in the liver of the lipoproteins which account for 50–60% of milk fat. A concurrent reduction in the availability of acetate, a major fatty acid precursor in adipose tissue and mammary tissue in the ruminant, acts in the same direction. A major difficulty to date in the evaluation of these hypotheses has been the absence of quantitative information on the availability and mammary uptake of the precursors of milk fat. We have used

lactating cows prepared with exteriorized mammary veins and carotid arteries to obtain this information, using techniques originally developed for the lactating goat (Annison & Linzell, 1964).

When the cow was transferred from a high-roughage to a low-roughage diet the fall in milk output (from 245 to 170 mg/min) was accompanied by a reduction in the mammary uptake of acetate (from 330 to 170 mg/min), β -hydroxybutyrate (from 119 to 60 mg/min) and triglyceride (from 203 to 110 mg/min). These reduced uptakes largely stemmed from the lower circulating levels of the milk precursors when the low-roughage diet was fed—i.e. blood acetate; high-roughage, 7.5 mg/100 ml, low-roughage, 3.6 mg/100 ml: corresponding values for β -hydroxybutyrate were 7.0 and 3.2 mg/100 ml respectively, and for plasma triglyceride, 10.7 and 7.7 mg/100 ml respectively. Corresponding levels of ruminal acetate and propionate were 44.4 and 21.3 m-equiv./l (high-roughage) and 38.7 and 46.3 m-equiv./l (low-roughage).

The reported increased proportion of unsaturated fatty acids (Storry & Sutton, 1969) and *trans*-fatty acids (Steele & Moore, 1968) in the milk fat of cows fed low-roughage diets was confirmed, but no differences were noted in the mammary uptake and secretion of *cis*- and *trans*-octadecenoic acids.

The present results suggest that the reduced milk-fat output associated with low-roughage diets is due to the reduced availability of the precursors of milk fatty acids.

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