The Two Hundred and Seventy-sixth Scientific Meeting of the Nutrition Society (One Hundred and Tenth of the Scottish Group) was held in Room M406, James Weir Building, Montrose Street, Glasgow C1, on Friday, 14 February 1975, at 11.00 hours, when the following papers were read:

Composition of microbial matter in the rumen. By J. W. CZERKAWSKI, *Hannah Research Institute, Ayr KA6 5HL*

Rumen contents were obtained just before and 2 h after feeding, from sheep that had been given a ration (1 kg/d) consisting of hay only, 400 g concentrate/kg or 820 g concentrate/kg (goat mix), for at least 3 weeks. The samples of rumen contents were strained through gauze and fractionated by sedimentation and centrifugation to give preparations of mixed protozoa (PR), large bacteria (LB) and small bacteria (SB). Whole particulate matter was also isolated by centrifugation at 20 000 *g*. All preparations (twenty-six including feeds) were freeze-dried.

Lipids were determined by extraction with chloroform–methanol and weighing, and the non-lipid residues were used for the determination of nucleic acids by a simplified procedure of McAllan & Smith (1969). Protein content of original samples was determined by the method of Toennies & Feng (1965) and from the amino acid composition. Total carbohydrate was determined by the phenol–sulphuric acid method and the cell wall content was estimated from the determination of glucosamine by the method of Cessi & Piliego (1960). Total nitrogen and ash content were also determined.

The recoveries were calculated by adding the weight of lipid, nucleic acids, protein, carbohydrate (corrected for nucleic acid sugars), cell wall (10×glucosamine), nitrogenous compounds (corrected for protein and nucleic acid N) and ash. In general the recoveries in protozoa fractions were lower (85–95%) than in the bacterial fractions (95–105%).

The relative composition of microbial preparations varied more with diet than with time with respect to feeding, and some components varied less than others. Lipid content of microbial preparations increased with concentrate in the rations. It was largest in SB (138 mg/g) and smallest in PR (75 mg/g). The RNA content did not vary greatly, but the ratio of RNA:DNA was distinctly lower in SB than in LB and PR (2·1, 5·9 and 4·4 respectively). Protein concentrations were highest in PR, particularly in samples taken after feeding, and were lowest in SB, but the amino acid composition was similar in all preparations. The glucosamine content of PR was small; the concentration of glucosamine was highest in SB and increased with roughage in the rations. The SB preparation had distinctly more ash than the preparations LB and PR.

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The results showed certain regularities and relations. For instance, there was an inverse relationship between lipid and carbohydrate content. The results also suggested possibilities of estimating relative proportions of the three groups of micro-organisms, from relatively simple chemical analyses.

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The effect of feeding whole or pelleted barley to lambs on their rumen bacterial populations and pH. By S. O. MANN and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Ørskov, Fraser & Gordon (1974) found substantial differences in rumen fermentations and pH values when lambs were given diets based on whole or pelleted barley. Preliminary observations on rumen microbial populations in mature sheep fed on similar diets led us to monitor certain aspects of the rumen flora in four early-weaned Suffolk cross lambs through their fattening period from about 15 to 35 kg live weight. Two lambs were fed on whole barley and two on pelleted barley diets *ad lib.* At 2-week intervals samples of rumen digesta were obtained by stomach tube and the bacterial counts shown in Table 1 were determined by techniques described by Mann & Ørskov (1973) and Hobson (1969).

Table 1. *Effects on rumen bacterial counts and pH of giving diets based on whole or pelleted barley to lambs*

		Bacteria (/ml rumen digesta)			
Lamb	Diet	Total viable ($\times 10^9$)	Cellulolytic ($\times 10^4$)	Lactobacilli ($\times 10^7$)	Rumen pH
A	Whole barley	10·5	100·0	6·9	6·6
B	Whole barley	15·1	100·0	5·9	6·7
C	Pelleted barley	28·0	1·0	71·4	5·7
D	Pelleted barley	21·3	0·1	246·0	5·6

Stained smears of rumen digesta showed that for lambs fed on whole barley, Gram-negative cocci and coccobacilli were the predominant morphological forms. Gram-negative vibrios were also present but in limited numbers. Lambs fed on pelleted barley showed Gram-negative, wide, straight rods as the predominant morphological form, but compared with the lambs given whole barley, they had considerably fewer Gram-negative cocci and coccobacilli, higher numbers of non-sporing, Gram-positive rods and fewer Gram-negative vibrios.

There were no consistent differences with time, and the average counts and rumen pH for each lamb are given in Table 1. Total viable counts of bacteria were about 2 times greater, and Lactobacillus counts on average about 20–30 times greater in the lambs fed on pelleted barley than in those fed on whole barley.

In agreement with previous reports (Ørskov *et al.* 1974), the rumen pH was about 1 unit higher in the lambs fed on whole barley, and what may be more significant, the numbers of cellulolytic bacteria in the lambs fed on whole barley were 100–1000 times greater than in the lambs fed on pelleted barley.

We thank Carol Grant for valuable technical assistance.

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The effect of feeding with whole or pelleted barley on rate of digestion of dried grass in the rumen of sheep. By E. R. ØRSKOV and A. Z. MEHREZ, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Previous work on the effect of feeding whole, unpelleted cereals to sheep has shown that rumen pH was increased (Ørskov, Fraser & Gordon, 1974), and the cellulolytic bacteria were present in much greater numbers (Mann & Ørskov, 1975) than when the cereals were pelleted. This led us to examine the rate of disappearance of dried grass when it was incubated in the rumen of sheep receiving either whole unpelleted or pelleted barley.

Four mature rumen-cannulated sheep were used. Two were given whole, and two pelleted barley amounting to 1000 g/d in two equal meals. Four Dacron bags containing approximately 5 g dried grass were incubated in the rumen of each sheep during each of two consecutive days. The technique was similar to that described by Schoeman, De Wet & Burger (1972). A bag was withdrawn after 6, 12, 18 or 24 h of incubation and the rate of dry matter disappearance was assessed after drying at 100° for 48 h. The results are shown in Table 1.

Table 1. *The effect of feeding whole or pelleted barley on the rate of digestion (mg/g incubated) of dry matter from dried grass incubated for different periods in the rumen of sheep*

Incubation time (h)	Whole barley	Pelleted barley	SE of treatment differences
6	285	242	36
12	453	373	11
18	530	406	20
24	625	423	23

At each period of incubation the rate of disappearance was greater when the bags were incubated in the rumens of the sheep fed on whole barley. Except for the difference at 6 h of incubation, all the differences were highly significant ($P<0.001$). This effect of cereal processing on the rate of digestion of the dried grass is most

probably the cause of the greater reduction in *ad lib.* intake of dried grass when it was supplemented with pelleted rather than whole barley (Ørskov & Fraser, 1974).

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A method for estimating the quantities of microbial and dietary proteins flowing in the duodenal digesta of ruminants. By R. A. EVANS, R. F. E. AXFORD and N. W. OFFER, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

Methods have been proposed for estimating the flow of microbial protein in the digesta leaving the ruminant stomach, involving determination of a single chemical marker believed to characterize the microbial components. Diaminopimelic acid (DAPA), ribonucleic acid, and isotopes incorporated into protein in the rumen have been used for this purpose. Other workers approached the problem by determining food residues, but this depends on giving proteins containing distinctive groups, and is not generally applicable.

The present paper proposes a method based upon a consideration of amino acid profiles. The individual proteins passing to the duodenum are identified by their characteristic comprehensive amino acid profiles. It is assumed that the profile of the digesta is the weighted sum of the various profiles contributing to it. The method depends upon the generation, by computer, of a profile by mixing, in different proportions, the known profiles of the dietary and endogenous components which may be arriving at the duodenum. The best match to the composition of the duodenal digesta is obtained by an iterative optimizing process (N.A.G. Library procedure EO4CAA, based on the work of Powell (1964)), which continues until the sum of squares of residuals is minimal. The amounts of components passing are constrained to give real results, i.e. no dietary component of the digesta is permitted to exceed the dietary intake of that component.

Table 1. *Estimated composition of proteins in digesta (g/kg)*

(1) Sheep given barley plus urea (Ørskov, Fraser & McDonald, 1971)
Barley 140±40; pepsin 30±60; microbial 810±80 (820 by DAPA)

(2) Sheep given maize plus urea (Ørskov *et al.* 1971)
Maize 500±50; pepsin 10±60; microbial 490±80 (530 by DAPA)

(3) Sheep given clover (Hogan, 1973)
Clover 350±140; pepsin 80±80; microbial 550±140 (540 by DAPA)

(4) Sheep given lucerne (Coelho da Silva, Seeley, Thomson, Beever & Armstrong, 1972)
Chopped lucerne 200±100; pepsin 10±60; microbial 770±100 (460, author)
Cobbed lucerne 320±150; pepsin 0±90; microbial 660±150 (440, author)
Pelleted lucerne 290±130; pepsin 30±70; microbial 660±130 (320, author)

DAPA, diaminopimelic acid.

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The method assumes a constant amino acid composition for microbial protein, and that the protein in each dietary component behaves as a single entity. Little information is available about endogenous protein secretion, so a profile of bovine pepsin has been used to represent this ill-defined contribution. Table 1 shows some results obtained by applying this method to published results, together with estimates of microbial total amino acids obtained by independent methods.

We have investigated the relationship between microbial total amino acids (AA), estimated by the present method, and the DAPA passing through the duodenum and find that:

microbial AA (g) = $42(\pm 5) + 36{\cdot}5(\pm 3{\cdot}6)$ DAPA (g),

with $r = +0{\cdot}92$, based upon twenty-one samples each of 3 d duration.

The method is capable of refinement by including profiles which represent more accurately the dietary and endogenous proteins involved.

We thank T. Williams and J. Parkinson for assistance in developing the program.

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The protection of dietary casein from degradation in the rumen. By R. F. E. AXFORD, N. W. OFFER and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

It is well established that formaldehyde treatment of dietary casein leads to an increase in the amount of amino acids reaching the intestine (Offer, Evans & Axford, 1971; MacRae, Ulyatt, Pearce & Hendtlass, 1972; Faichney, 1974). The source of this increased flow of amino acids has not been clearly determined.

A further experiment is reported in which a Welsh mountain wether was given 600 g/d of a pelleted ration providing 11·5 g amino acid nitrogen daily. The ration consisted of (g/kg): hay 570, barley 340 and casein 90, providing respectively 20, 22 and 58% of the total amino acid intake. Digesta samples were collected automatically for 34 d during which the sheep received, in continuous sequence, untreated casein (8 d); casein pretreated with formaldehyde solution (10 g/l) (8 d); untreated casein (8 d); casein pretreated with a solution (10 g/l) of an extract of wattle bark (6 d) and untreated casein (4 d). The daily amino acid flow to the duodenum was determined and the contribution of dietary and microbial amino acids was estimated by the method of Evans, Axford & Offer (1975). The mean composition of the digesta for each experimental period is given in Table 1 and compared with that of the authors previously quoted.

The results indicate that in all instances the flow of casein was markedly increased by pretreatment with formaldehyde. The difference between experiments in the digestibility of casein in the rumen probably results from variation in the details of pretreatment.

Table 1. *Estimated flow of amino acids (g/d) from the rumen after feeding untreated and treated casein to sheep*

(Mean values with their standard errors where given; percentage of intake in parentheses)

Form of casein in diet	Source of amino acids				
	Casein	Hay	Barley	Pepsin	Microbial
Present experiment					
1. Untreated	1·1±0·4 (2·2)	12·5±0·9(74·3)	1·1±0·3 (6·0)	4·1±0·7	27·8±0·7
2. Formalin-treated	29·0±4·7(59·0)	6·2±1·6(37·0)	7·5±2·0(40·8)	3·2±0·9	31·4±4·8
3. Untreated	6·5±2·2(13·3)	16·1±0·5(95·2)	5·7±3·0(31·2)	1·2±0·4	18·5±3·3
4. Wattle-treated	1·7±0·6 (3·4)	11·4±1·8(67·4)	1·5±1·0 (8·0)	2·6±0·3	24·1±2·5
5. Untreated	0±1·1 (0)	12·3±3·5(72·6)	0·4±0·4 (2·0)	4·2±0·8	25·2±3·4
Offer, Evans & Axford (1971)					
Untreated	0·1±0·1 (0·2)	10·5±3·0(47·0)	8·3±0·8(28·8)	8·0±1·0	45·1±2·9
Formalin-treated	19·0±3·5(27·8)	2·2±2·2 (9·8)	21·7±1·5(75·9)	10·3±0·9	49·4±4·5

MacRae, Ulyatt, Pearce & Hendtlass (1972)	Casein	Dried grass	Pepsin	Microbial
Untreated	13·3 (25)	72·4 (62)	4·6	44·6
Formalin-treated	31·0 (58)	77·0 (66)	0·6	51·1

Faichney (1974)	Casein	Wheaten hay	Lucerne	Maize	Pepsin	Microbial
Untreated	9·0 (9)	0 (0)	44·2 (47)	19·1 (64)	3·5	56·2
Formalin-treated	101·1(100)	4·6 (69)	51·4 (55)	30·0(100)	3·5	36·1

Formalin, solution of 10 g formaldehyde/l; wattle, 10 g wattle bark/l.

There is no indication that wattle tannin protects casein from degradation in the rumen.

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The synergistic effect of dietary carbohydrates on the passage of amino acids to the small intestine of the sheep. By N. W. Offer, R. A. Evans and R. F. E. Axford, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

Investigations have suggested that dietary components act synergistically in promoting the flow of nitrogen from the ruminant stomach (Offer, Evans & Axford, 1972; Chamberlain & Thomas, 1974).

Seven diets were compounded containing a basal mixture (g/d: soya-bean meal 250, dried grass 150, molasses 80, mineral–vitamin mixture 30 and chromic oxide 2) providing 65 g N/3 d period, together with supplements of paper or starch, or both, as indicated in Table 1. The diets were given to seven adult Clun wether sheep,

fitted with re-entrant duodenal cannulas, in a balanced incomplete block design. Sheep were restrained in metabolism cages and fed every 2 h. Each sheep received a diet for a period of 9 d. On the 6th day the cannulas were connected to a sampler (Axford, Evans & Offer, 1971) and digesta were collected for 3 d.

Table 1. *Passage of nitrogenous materials to the duodenum of sheep*

Supplement (g/d)	Total AA nitrogen (g/3 d)	Microbial AA (g/3 d)	Microbial AA:E (g/MJ)	Total AA:E (g/MJ)
0 (basal)	18·3	73·6	5·2	8·9
300 starch	21·1	110·5	4·1	5·7
600 starch	41·3	196·5	5·6	8·8
300 paper	24·0	121·2	5·2	7·5
600 paper	23·6	122·2	3·8	5·6
150 starch+ 150 paper	32·6	162·8	8·3	12·0
300 starch+ 300 paper	44·2	232·9	9·7	13·5
SE of corrected means	2·6	26·6	1·3	1·0

AA, amino acid; E, food energy disappearing between the mouth and the duodenum (see text).

Recoveries of chromic oxide at the duodenum averaged 98·0±3·0%. Individual flow values were corrected for 100% recovery. Food intake was lower for four periods when high-paper diets were given. All results were normalized for a theoretical 65 g N intake.

Corrected mean values for each dietary treatment were calculated, independent of sheep variation, and some results for duodenal flow are given in Table 1. Energy supplementation resulted in increased flows of total amino acids to the duodenum. The microbial contribution to the flow was estimated by the method of Evans, Axford & Offer (1975). Although there were no significant differences in the microbial percentage (mean 67), supplementation increased the absolute amounts of microbial amino acids leaving the stomach.

A comparison of the efficiency with which each carbohydrate source was utilized for microbial protein synthesis was made by calculating the amounts of microbial total amino acids passing to the duodenum per MJ food energy (E) disappearing between the mouth and the duodenum. Allowance was made for the energy of the microbes passing to the duodenum using a value of 39·8 J/g microbial total amino acid, determined on a bulked sample of micro-organisms isolated from the rumen contents of sheep given a variety of diets.

The results presented in the last two columns of Table 1 support the contention that the amino acid supply to the duodenum is greatest, per unit of food energy disappearing in the rumen, for animals provided with a mixed energy source.

This work was carried out while N.W.O. held a Rank fellowship.

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Variations in the composition of blood and urine following the ingestion of a high-protein diet. By D. M. HEELEY, *The Alfred Chester Beatty Body Dynamics Laboratory, Brooksden, Cranbrook, Kent*, I. M. SHARMAN, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council, Milton Road, Cambridge CB4 1XJ*, and D. F. COOPER, *St. Mary's College of Education, University of London, Waldegrave Road, Twickenham, Middx. TW1 4SX*

The energy potential of athletes can be increased by building up their body stores of glycogen. Though this can be effected by the consumption of a high-carbohydrate diet, Astrand (1967) has recommended that a higher storage level can be achieved if the athlete first exhausts his existing stores by performing fairly heavy work and then remains on a high-protein diet for a few days before consuming the carbohydrate diet. It is not known how frequently this dietary regimen can be repeated with the same desired effects nor whether the procedure is entirely innocuous. As a step towards answering the latter question we have examined six healthy young men before and after receiving a high-protein diet for 4 d.

Blood specimens were collected at the start and finish of the investigation. Urine was collected for the 24 h period immediately preceding and during the last 24 h on the special diet. The subjects lost an average of 1·4 kg body-weight during the 4 d. Blood and urine specimens were analysed for a variety of elements and compounds.

In blood, significant differences were observed in a number of the indices examined. Thus there were significant average rises in urea (4·65–8·13 mmol/l), cholesterol (4·66–5·52 mmol/l), total phosphorus (2·87–3·16 mmol/l), phosphatides (2230–2440 mg/l) and in osmolality (282–293 mosmol/l). There were significant falls in triiodothyronine levels (83–31%) and in glucose (4·90–4·53 mmol/l).

In urine also, significant changes were observed. Thus average rises in urea from 22·5 to 38·7 g/24 h and in cyclic AMP from 15·7 to 31·5 μmol/24 h were found. There was also a significant rise in chromium from 6·01 to 11·68 μg/24 h. A significant average fall in uric acid from 2·89 to 0·62 mmol/24 h was also noted.

Various other indices measured both in blood and urine did not show any significant differences. Thus although the average fasting level of insulin in the blood fell from 9·9 to 7·1 μU/ml, this difference was not significant. However, when the values for an exceptional obese subject were removed, the values were 10·2 and 5·7 μU/ml and this difference was significant.

Some implications of the differences that were found will be considered.

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Metabolism of aromatic compounds in the rumen. By A. K. MARTIN, *Hannah Research Institute, Ayr KA6 5HL*

Although benzoic acid is the principal aromatic compound excreted in the urine of sheep, its origin is unknown. In this laboratory, in studies with sheep given a

variety of foods, the following ranges in urinary output per kg food intake have been observed: benzoic acid, 2·7–16·3 g; phenylacetic acid, 0·3–3·2 g; 3-phenylpropionic acid, up to 0·1 g; cinnamic acid, up to 0·2 g; phenol, 7–35 mg; *p*-cresol, 600–1750 mg; catechol, 100–200 mg.

Possible dietary precursors of these compounds include phenolic derivatives of benzoic, phenylacetic, 3-phenylpropionic and cinnamic acids. These acids have been administered as continuous drips to adult wether sheep fitted with ruminal or abomasal cannulas, and urinary increments in aromatic acids and phenol excretion have been determined. Extensive ruminal metabolism of the infused substances was only observed when they contained a 4-hydroxy substituent. The products obtained depended on the length of the aliphatic side chain; derivatives of benzoic and phenylacetic acid were decarboxylated to give phenols (11–61% yield), whereas those of 3-phenylpropionic acid or cinnamic acid were dehydroxylated to yield urinary benzoic acid increments equivalent to between 80 and 107% of the infused acids. Abomasal infusion of these compounds yielded no increments in urinary benzoic acid or phenols.

The ability of animals to aromatize quinic acid to benzoic acid has been shown to depend on the activity of their intestinal microflora (Adamson, Bridges, Evans & Williams, 1970). Surprisingly, ruminal infusion of quinic acid resulted in only small urinary increments in benzoic acid output equivalent to 26% of the infused acid.

In rumen liquor, benzoic acid was found to account for $<2\%$ of the total aromatic acids present; the major acid was 3-phenylpropionic (50–80%) with phenylacetic (13–50%) and cinnamic (0–7%) acids comprising the remainder.

The preponderance of 3-phenylpropionic acid in rumen liquor, the extensive metabolism of 4-hydroxy-containing derivatives of 3-phenylpropionic acid and cinnamic acid to benzoic acid and the presence of small amounts of 3-phenylpropionic or cinnamic acid in urine combine to suggest that the urinary benzoic acid excreted by ruminants is derived from rumen microbial metabolism of 4-hydroxy derivatives of 3-phenylpropionic or cinnamic acids. The latter are widely distributed in plants (Harborne & Simmonds, 1964).

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Urinary quinol and orcinol outputs as indices of voluntary intake of heather (*Calluna vulgaris* L. (Hull)) by sheep. By A. K. MARTIN, J. A. MILNE and P. MOBERLEY, *Hannah Research Institute, Ayr KA6 5HL*, and *Hill Farming Research Organisation, Bush Estate, Penicuik EH26 0PY*

Phenol, *p*-cresol and catechol are normal urinary constituents excreted by sheep. Urinary output of other phenols requires the consumption of foods containing the phenols themselves or their precursors. Simple phenols are of limited distribution

in plants but some Ericaceous plants may contain quinol (Thieme & Winkler, 1971) and orcinol (Harborne & Williams, 1971) glycosides. Experiments showed both of these phenols to be present in heather and in the urine of sheep consuming heather.

Urinary recoveries of *p*-cresol (1000 mg/d), catechol (500 mg/d), orcinol (300 mg/d) or quinol (300 mg/d) have been studied following ruminal infusions as continuous drips for 7 d periods to four wether sheep. The sheep were given rations of good quality hay. Recoveries of *p*-cresol (88–98%) and orcinol (87–101%) were high, but variable recoveries of quinol (55–88%) were obtained and those for catechol (22–44%) were low.

To study the relationship between urinary aromatic acid or phenol excretion and heather intake, two experiments were conducted. In one experiment twenty-five sheep were housed in metabolism cages and offered one of five levels of grass (mainly *Lolium perenne*), 0, 0·2, 0·4, 0·6 or 0·8 kg dry matter (DM)/d. After the grass had been eaten, heather was offered *ad lib.* Voluntary intakes of heather, in order of increasing grass intake, were 456 ± 89, 476 ± 114, 344 ± 128, 224 ± 82 and 127 ± 69 g DM/d (mean values and standard deviations).

In the second experiment the voluntary intake of grass of twelve wethers was measured. Subsequently, groups of three sheep were offered 40%, 60% and 80% of this intake as grass. After the grass had been eaten heather was offered *ad lib.* The three remaining sheep were offered grass *ad lib.* Voluntary intakes of heather in order of increasing grass intake were 789 ± 78, 626 ± 151 and 500 ± 36 g DM/d. The current season's shoots of heather used in the first experiment were harvested in September and in the second experiment in June.

In addition to those phenols normally present in the urine of grass-fed sheep, quinol, 4-methylcatechol, orcinol and pyrogallol were identified. Of these, quinol and orcinol outputs were linearly related ($P<0{\cdot}001$) to heather intake in both experiments. The regression coefficients and their 95% confidence limits showed that 365 ± 111 mg orcinol and 172 ± 62 mg quinol/kg were derived from heather in Expt 1, and 54 ± 15 mg orcinol and 689 ± 113 mg quinol/kg were derived from heather in Expt 2.

In Expt 1, urinary benzoic acid output was not related to either heather or grass intake, but urinary phenylacetic acid was linearly related ($P<0{\cdot}001$) to grass intake.

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An apparatus for the measurement and sampling of urine from grazing female sheep. By A. R. M. CHAMBERS, J. A. MILNE, A. J. F. RUSSEL and I. R. WHITE, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY*

Many types of nutritional and physiological investigations require the quantitative collection of urine from sheep and a representative sample for subsequent biochemical

analysis. One example of such a need is outlined by Martin, Milne & Moberley (1975). Urinary measuring and sampling equipment (UMASE), which is attached to the sheep and is suitable for use with either penned or grazing female sheep, is described here.

Urine, collected by an indwelling bladder catheter, drains into two glass reservoirs. The upper, smaller reservoir is joined by a narrow glass tube to the lower, larger reservoir. Light-activated switches (LAS) are located at the top of the smaller reservoir, at the narrow tube, and at the bottom of the lower reservoir. The upper and lower LAS control a relay through a bistable circuit and the middle LAS controls a second relay. These relays operate three solenoid valves, which control the flow of urine in the UMASE, and a counter. The counter records the number of draining cycles of the reservoir system. Urine from the small reservoir, comprising a representative sample, is stored in a sample collector and the urine from the large reservoir is voided. By changing the size of the small reservoir, different proportions of total urine output can be collected. Total urine output is calculated as the product of the value of the counter and the known volume of the reservoir system. The UMASE is operated by one 8 V and one 2×12 V rechargeable battery.

Experiments in which the UMASE was tested on twelve sheep in metabolism cages for several periods of 5 consecutive days indicated that: (a) catheterization of the bladder and attachment of the UMASE did not affect daily urine output; (b) the estimate of urine volume provided by the UMASE differed from total urine output by between $-1{\cdot}5$ and $+4{\cdot}3\%$; (c) the sample of urine collected by the UMASE was compositionally representative of total urine output; (d) without change of batteries UMASE can be used continuously for 19 h or with urinary outputs of up to 6 l.

The UMASE has also been shown to operate satisfactorily under rigorous field conditions.

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Fermentation products in the rumen of a gnotobiotic lamb dosed with *Bacteroides ruminicola*. By C. S. STEWART, *Department of Microbiology, Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*, and R. J. LYSONS, *Department of Animal Health, Royal (Dick) School of Veterinary Studies, Edinburgh University Veterinary Field Station, Easter Bush, Roslin, Midlothian* (introduced by E. R. ØRSKOV)

A number of studies have been made of the establishment of limited, defined, rumen bacterial floras, consisting of six to nine bacterial species, in gnotobiotic lambs, as an aid to understanding the complex interactions which occur between micro-organisms in the rumen (Lysons, Alexander, Hobson, Mann & Stewart, 1971; Mann & Stewart, 1974). In only one known instance, however, has a gnobotiotic lamb been reared with a rumen populated by a pure culture of a rumen bacterium, in this instance *Bacteroides ruminicola* 46/5(2) (Lysons, 1975).

The aim of the present study was to determine the concentration of fermentation products in the rumen liquor of this lamb, so that the contribution of *B. ruminicola* to the more complex rumen floras of gnotobiotic lambs which contained up to nine species of bacteria might be understood.

Table 1 shows the fermentation products present in the rumen liquor of the lamb at various intervals after inoculation with *B. ruminicola*. Details of the diet, sampling techniques and numbers of *B. ruminicola* present are given by Lysons (1975). No contaminating bacteria were found, so that acids present before inoculation with *B. ruminicola* were presumably derived partly from tissue metabolism and partly from the foodstuffs.

Table 1. *Fermentation products* (μ*mol/ml*) *detected in rumen liquor taken 2–2·5 h after feeding from a lamb*

Time after inoculation (d)	Lactate	Succinate	Acetate	Propionate
0	13·0	0·0	26·1	17·8
2	55·6	3·0	13·2	6·3
6	54·1	5·3	16·0	3·9
15	60·5	6·2	27·0	8·4
36	42·6	9·7	40·1	8·8
42	28·6	5·7	30·2	8·4

The initial decrease in acetate, and the permanent drop in propionate concentration, may indicate inhibition of a volatile fatty acid (VFA)-producing process or stimulation of VFA absorption by the introduction of *B. ruminicola*, since there was no evidence for the presence of contaminating micro-organisms, nor is *B. ruminicola* thought to be capable of the direct breakdown of these acids. The fermentation products of *B. ruminicola* growing in vivo (lactate, acetate and succinate) were essentially similar to those found in vitro after growth in batch culture on a range of mediums.

It is evident that, in gnotobiotic lambs containing this strain of *B. ruminicola* together with predominantly lactate-producing Lactobacilli or Streptococci, there must be a considerable production of lactic acid even though the presence of the lactate utilizers *Megasphaera* and *Veillonella* generally ensures that the measurable lactate pool is extremely small, or undetectable (Mann & Stewart, 1974).

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Mechanical processing of wet roughage. By J. F. D. GREENHALGH and G. W. REID, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Considerable comminution of wet roughages is achieved when they are passed through a screw-press to extract protein-rich juice (Houseman, Jones & Cadenhead, 1974), and it seemed likely that this would increase their acceptability to ruminants in a manner comparable to the grinding and pelleting of dry roughages. Herbage from ryegrass-dominant pastures was chopped (treatment C) or passed twice through a screw-press to give fractions of pulp (treatment P) and juice. For treatment R, pulp and juice were recombined to give a food equal in chemical composition to that for treatment C. All three foods were frozen and then given to six castrate male sheep in the order determined by two 3×3 Latin squares. Each period lasted 21 d, food intake and faeces output being recorded over the last 9 d. Mean daily dry matter (DM) intakes (g) were: C 1140, P 1060, R 1660 (SE of difference 89). Digestibilities of DM were: C 0·726, P 0·717, R 0·738 (SE 0·027).

The same procedure was used in a second trial, except that grass was processed daily and not frozen. Mean daily DM intakes (g) were: C 1400, P 1380, R 1730 (SE 67), and DM digestibilities: C 0·745, P 0·737, R 0·754 (SE 0·011). Mechanical treatment (R *v.* C) increased intake significantly, but comminution plus juice extraction (P *v.* C) had no significant effect.

These results suggest that the potentially detrimental effects of removing soluble nutrients from grass are counterbalanced by the beneficial effects of comminution. They also confirm that grass pulp is similar in acceptability and digestibility to unprocessed grass (Jones, MacLeod, Macdearmid & Houseman, 1974).

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The effects of a low protein intake by beef cows during pregnancy on the voluntary intake of roughage, the composition of the colostrum and the serum immune globulin concentration of their calves. By G. FISHWICK and D. CLIFFORD (introduced by R. G. HEMINGWAY), *Glasgow University Veterinary School, Bearsden, Glasgow*

The composition of bovine colostrum has been reviewed by Selman (1969) but there is no published information on the effects of diet during pregnancy. The present experiment examined the effects of a prolonged period of inadequate (Agricultural Research Council, 1965) protein intake during late pregnancy on the total protein content of colostral whey and the amount of immune globulin absorbed by the newborn calf.

Beef heifers (fourteen and sixteen/treatment; mean live weight, 410 kg) were given 2·7 kg/d molassed sugar-beet pulp (containing added vitamins and minerals) either alone (SBP, 109 g crude protein (CP)/kg dry matter (DM)) or supplemented with

30 g urea/kg (SBPU, 177 g CP/kg DM) for the last 14 weeks of pregnancy. In addition, oat straw (16 g CP, 429 g crude fibre (CF)/kg DM) was offered *ad lib.* during weeks 14 to 11 before calving. Hay (48 g CP, 322 g CF/kg DM) was given *ad lib.* for the remaining period of pregnancy. Without urea, voluntary straw and hay consumption was reduced (Table 1) and the intakes of digestible crude protein (DCP) were only about one-half the recommended (Agricultural Research Council, 1965) requirements. Metabolizable energy (ME) intakes were adequate (Agricultural Research Council, 1965) when both straw and hay were given.

Table 1. *Daily intakes of roughage dry matter* (DM), *metabolizable energy* (ME) *and digestible crude protein* (*DCP*) *by cows during late pregnancy, total protein concentration in the colostral whey, 48 h serum concentration of absorbed immune globulin, calf birth weight and live-weight gain to 9 weeks*

(Mean values with their standard errors where given)

	Straw			Hay					Live weight	
Pregnancy diet	DM (kg)	ME (MJ)	DCP (g)	DM (kg)	ME (MJ)	DCP (g)	Whey protein (g/l)	Immune globulin (ZST units)	Birth wt (kg)	Gain (kg/d)
SBP	3·06	50·6	127	5·68	71·5	200	133±12·5	26·1±3·79	28·1±1·66	0·79±0·050
SBPU	4·90	65·7	251	6·85	80·8	372	111±9·3	27·3±3·25	28·1±1·08	0·80±0·040

SBP, molassed sugar-beet pulp; SBPU, molassed sugar-beet pulp plus urea; ZST units, zinc sulphate turbidity units (McEwan, Fisher, Selman & Penhale, 1970).

After calving in individual pens, both groups of heifers were given hay *ad lib.* and 3·5 kg SBPU diet/d. Blood samples were obtained from the calves before sucking and 48 h later for the determination of serum immune globulin concentration (McEwan, Fisher, Selman & Penhale, 1970). Colostrum was obtained prior to suckling for total protein estimation. Results were available for eleven SBP calves (seven ♂, four ♀) and fifteen SBPU calves (six ♂, nine ♀) which were encouraged to suck to satiation within 3 h of birth. Neither the colostrum composition nor the concentration of absorbed immune globulin in the serum of the calves 48 h after birth differed significantly between treatments. The mean birth weights and live-weight gains to 9 weeks of the calves were satisfactory and were not affected by treatment.

One of us (G.F.) was in receipt of a research scholarship from the British Sugar Corporation Ltd.

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Experimental copper deficiency in the calf. By N. F. SUTTLE, *Moredun Research Institute, Edinburgh EH17 7JH*

The variable occurrence of diarrhoea, anaemia and skeletal abnormalities in outbreaks of hypocuprosis in grazing cattle (cf. Allcroft & Lewis, 1957; Underwood, 1971) may be attributable to differences in the severity of copper depletion or to the involvement of deficiencies or excesses of other elements. An attempt has been made to resolve this problem by studying the development of simple Cu deficiency in calves under experimental conditions.

Three groups of three Jersey calves were reared to 6 weeks of age on milk substitute (Nutrimilk 20; Scottish Agricultural Industries Ltd, Edinburgh) containing 0·8 mg Cu/kg dry matter. One group (A) received a Cu supplement, 2 mg Cu/kg. From 6 to 30 weeks all calves received a semi-purified diet consisting of (g/kg): oat hulls 300, dried skim milk 200, starch 170, cane-sugar 170, arachis oil 40, urea 30, $NaHCO_3$ 20, $CaHPO_4$ 10, Na_2SO_4 8, KCl 5, MgO 5, added water 40, and adequate amounts of vitamins A, D and E and trace elements other than Cu; this diet contained 1·3 mg Cu/kg, group A continued to receive supplementary Cu, but at a higher level of 7·5 mg/kg, group B continued to receive no supplement and group C received supplementary molybdenum (4 mg/kg). Daily food allowances were approximately 50 g/kg body-weight.

Cu depletion prior to weaning was without effect, but after weaning groups B and C developed signs of deficiency in the following order (cf. Table 1): first, hypocupraemia, secondly, growth retardation, impaired food conversion ratio (FCR) and rough hair coat, thirdly, intermittent diarrhoea and, fourthly, leg abnormalities. Diarrhoea occured the least frequently in group A. Anaemia was not observed and supplementary Mo did not exacerbate the deficiency.

Table 1. *Effects of a semi-purified, low-copper diet, with or without supplements of Cu (7·5 mg/kg) and molybdenum (4 mg/kg) on live-weight gain (LWG) food consumption (FC), food conversion ratio (FCR) and onset of clinical Cu deficiency in Jersey calves*

Group	Dietary supplement	LWG (kg)	FC (kg)	FCR (g FC/g LWG)*	Mean time of onset of sign (weeks)			
					Hypocupraemia	Rough coat	Diarrhoea	Leg abnormality
A	Cu	46·6	182	3·9	—	—	21	—
B	—	35·7	173	4·8	15	18	20	22†
C	Mo	34·7	181	5·2	15	18	21†	24†
SE of means (n=3)		4·8	15	0·6				

*Results for weeks 6–18.
†One calf/group unaffected.

Diminished cytochrome oxidase (*EC* 1.9.3.1) activity was found in the small intestine of groups A and B, thus confirming the findings of Fell, Dinsdale & Mills (1975), and it may have produced a malabsorption syndrome of which decreased FCR and diarrhoea were successive consequences. The calf is more susceptible to simple Cu deficiency than the lamb (cf. Suttle, Field & Barlow, 1970).

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The influence of dietary sulphate on the toxicity of lead to sheep. By J. N. MORRISON, J. QUARTERMAN, W. R. HUMPHRIES and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The work of Bird (1970) and Suttle (1974) on the availability of dietary copper suggests that the sulphur content of the diet of the ruminant may have an influence on the utilization of those metals that form insoluble sulphides. Since both lead sulphide and lead sulphate have a low solubility in aqueous systems, the influence of dietary S content upon Pb intoxication in the sheep has been investigated.

Four weanling rams (group A) were given a diet based on dried skim milk, urea and oat husks (Suttle & Field, 1968) and which contained 0·8 g S and 400 mg Pb/kg. Another four rams received a similar diet with a supplement of 3 g S as sodium sulphate/kg (group B).

The lambs given Pb and S supplements gained about 2 kg/week; in contrast, lambs given Pb alone ceased to gain weight within 4 weeks. Separate studies using the same basal diet but omitting Pb indicated that this difference was not solely due to the S content of the diet.

One ram of group A died after 5, one after 6 and one after 8 weeks. There was severe anaemia and substantially higher concentrations of Pb in blood, kidney, liver and testes than in group B animals (Table 1). At post-mortem these lambs were found to have pale, enlarged kidneys, multiple haemorrhages, oedema and Pb lines in the bones. Cortical bone indices of group A were, on average, about one-third lower than those of group B. The epididymes of group A lambs were coloured throughout with a black pigment and had Pb contents of 2·4 mg/kg compared with 1·2 mg/kg for group B.

Table 1. *Influence of dietary sulphur content on blood haemoglobin and tissue lead content of sheep given diets containing* 400 *mg Pb/kg* (*results from animals surviving* 8 *weeks of treatment*)

	Haemoglobin† (g/l)	Blood Pb† (mg/l)	Liver Pb (mg/kg fresh wt)	Kidney Pb (mg/kg fresh wt)	Testis Pb (mg/kg fresh wt)
Low-S diet (two survivors of four treated)*	55, 109	0·59, 0·40	19·7, 9·5	17·7, 34·3	1·5, 0·5
S-supplemented diet (four survivors of four treated)	136±6	0·18±0·03	1·9±0·1	7·6±3·5	0·3±0·04

*Individual values.
†After 7 weeks of treatment.

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This experiment strongly suggests that the S content of the diet of the sheep markedly influences the fate and toxicity of dietary Pb.

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Ammonium polyphosphate in the drinking-water as a source of phosphorus for growing sheep. By R. G. HEMINGWAY and G. FISHWICK, *Glasgow University Veterinary School, Bearsden, Glasgow*

Ammonium polyphosphate solutions are widely used as components of liquid fertilizers. It was found that this material did not adversely affect the intake of drinking-water by cattle and sheep.

Three groups each of six growing wether sheep (mean live weight, 25 kg) in metabolism cages were given a basal low-phosphorus diet (0·44 g P/d) consisting of molassed sugar-beet pulp (800 g/d, 0·57 g P/kg dry matter) and barley husk siftings (150 g/d, 0·62 g P/kg dry matter). One group of sheep received no P supplement. The second group was given an additional 1·75 g P/d as dicalcium phosphate (176 g P/kg) which was intermixed with the food. The third group was given 1·75 g additional P/d as 8·8 ml of an ammonium polyphosphate solution (99 g nitrogen and 147 g P/kg, 0·1 g fluorine/kg; sp. gr. 1·35). This was supplied once/d in 1 l of drinking-water. To equalize N intakes these sheep were given 7·4 g urea instead of 10 g/d. There were no palatability problems and additional drinking-water was subsequently given as required to satisfy their voluntary intake of about 4–5 l/d.

Following an introductory 7 d feeding period, when all the sheep were given 1·75 g additional P/d as dicalcium phosphate, the three diets were given for 14 d and P balances were measured during days 8–14. The sheep were weighed at the start and end of the 14 d period and blood samples were obtained on day 14.

Both dicalcium phosphate in the food and ammonium polyphosphate in the drinking-water significantly increased P retention and blood P concentration to similar

Table 1. *Mean live-weight gain, phosphorus balance results and blood P concentrations for sheep given P supplements*

Supplement . . .	Nil (A)	Dicalcium phosphate (B)	Ammonium polyphosphate (C)	SEM	Significance of differences
Live-wt gain (kg/d)	0·11	0·17	0·17	0·026	NS
P balance (g/d)					
Intake	0·41	2·14	2·18	—	—
Urine	0·02	0·11	0·08	0·034	NS
Faeces	0·66	1·00	1·11	0·047	B, C>A***
Retention	−0·27	1·03	0·99	0·066	B, C>A***
Blood P concentration (mmol/l)	1·05	2·30	2·53	0·15	B, C>A***

NS, not significant; ***$P<0·001$.

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extents. Both appeared to increase live-weight gain, but by amounts which were not significant over the short period of the experiment.

It is concluded that ammonium polyphosphate in the drinking-water is a fully satisfactory source of P. It is suggested that adding about 50 ml (68 g) /d of this material to the drinking-water of lactating cows would be a convenient means of supplying 10 g P/d, which is a commonly encountered dietary inadequacy. Additionally, ammonium polyphosphate, with 99 g N/kg, contains 620 g crude protein/kg (assuming $N \times 6{\cdot}25$) and on this basis 50 ml/d would provide 42 g crude protein.

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