*The Two Hundred and Fifty-sixth Scientific Meeting of the Nutrition Society was held in the University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD, on Friday, 30 March 1973, at 09.00 hours, when the following papers were read:*

**The effective critical temperature in groups of pigs.** By L. E. MOUNT, W. H. CLOSE and M. W. A. VERSTEGEN*, *ARC Institute of Animal Physiology, Babraham, Cambridge*

Under thermally neutral conditions the increase in heat production associated with feeding must be dissipated, but in the cold this additional heat satisfies part of the increased thermal demand of the environment. In an attempt to establish the lower limits of thermal neutrality, in relation to feeding levels in groups of pigs, effective critical temperatures have been estimated from determinations of the extra thermoregulatory heat production (ETH) required in the cold.

Metabolizable energy (ME) intake, heat loss and nitrogen balance were measured in eight experiments, in each of which a group of four castrated male pigs (20–40 kg body-weight) lived for 3 weeks continuously in a calorimeter equipped as a pig pen. The combinations of calorimeter temperature and feeding level (g food/kg body-weight) were 20°, 39; 20°, 45; 8°, 45 and 8°, 52; two experiments were carried out at each combination (Verstegen, Close, Start & Mount, 1973).

ETH at 8° was estimated as follows. The partial efficiency of energy retention (ER) in the 20° experiments was found to be 0·665 from the linear regression of ER on ME intake, so that $\dfrac{ER}{0\cdot665}$ was the fraction of ME associated with production ($ME_p$). $ME\text{-}ME_p = ME_8$, where $ME_8$ is the sum of the maintenance ME requirement ($ME_m$) at thermal neutrality (assumed at 20°) and ETH. ETH is then given by $ME_8\text{-}ME_m$.

ETH showed different degrees of reduction during the course of the different experiments at 8°, but in each instance the mean value was positive, indicating a mean environmental level below thermal neutrality. In the 8°, 45 experiments ETH was 111 kJ/kg$^{0\cdot75}$ per d, and in the 8°, 52 experiments ETH was 34 kJ/kg$^{0\cdot75}$ per d. Taking, from a combined series of experiments, an approximate value of ETH of 12 kJ/kg$^{0\cdot75}$ per d for each °C below the critical temperature, the critical temperature for the 8°, 45 groups was about 17° $(8+\frac{111}{12})$ and for the 8°, 52 groups about 11° $(8+\frac{34}{12})$.

In the 8°, 45 experiments the mean value of ETH as a percentage of the intake of gross energy was 6·5%, and in the 8°, 52 experiments it was reduced to 1·7%.

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## The energy costs of maintenance and production in the growing pig.

By W. H. CLOSE, M. W. A. VERSTEGEN* and L. E. MOUNT, *ARC Institute of Animal Physiology, Babraham, Cambridge*

Estimates of the energy costs of maintenance and production (protein and fat synthesis) were obtained on four groups of four young castrated male pigs, 20–40 kg body-weight, maintained at 20° and given either 39 or 45 g food/kg body-weight daily. Measurements of metabolizable energy (ME) intake, heat loss (HL) and nitrogen balance were made continuously over 3-week periods, and energy retention (ER) and the deposition of protein and fat were calculated (Verstegen, Close, Start & Mount, 1973).

The mean maintenance energy requirement (MEm) was calculated from the regression equation:

$$ER/kg^{0.75} = 0.665 \ (\pm 0.04) \ ME/kg^{0.75} - 278 \ (\pm 23) \ (n = 28). \tag{1}$$

If the amount of ME above the maintenance requirement was assumed to be converted into ER with a constant efficiency at each feeding level, MEm was the ME when ER = 0, giving MEm of 418 kJ/kg$^{0.75}$.

Mean values of the energy costs of production were calculated by two different regressions, of heat loss and of ME available for production (MEp), where MEp = ME − MEm, on protein and fat deposition:

$$HL = 14.3 \ (\pm 7.3)X_1 + 7.0 \ (\pm 4.7)X_2 + 511 \ (n = 28) \tag{2}$$

$$ME_p = 41.2 \ (\pm 5.0)X_1 + 57.0 \ (\pm 4.4)X_2 \ (n = 28) \tag{3}$$

where $X_1$ and $X_2$ were the daily depositions of protein and fat, respectively, in g.

By adding the energy values of protein and fat, 23.8 and 39.7 kJ/g (Brouwer, 1965), to the coefficients obtained in equation 2 the energy costs of protein and fat synthesis were 38.1 and 46.7 kJ/g respectively. Values of 41.2 and 57.0 kJ/g were obtained in equation 3. The energy costs of protein and fat synthesis were within the ranges, 37.7–66.9 kJ/g protein and 46.0–58.6 kJ/g fat, of other published values.

Equation 2 gives an estimate of the MEm, 511 kJ/kg$^{0.75}$ per d, based on heat loss, compared with the estimate of 418 on the energy retention basis (equation 1), and both may be compared with the range of 435–515 found by other workers.

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## The optimum duration of metabolic balance experiments with groups of pigs.

By M. W. A. VERSTEGEN*, L. E. MOUNT and W. H. CLOSE, *ARC Institute of Animal Physiology, Babraham, Cambridge*

In a series of measurements of gross energy (GE) and metabolizable energy (ME) intake, heat loss and nitrogen balance in groups of growing pigs (Verstegen,

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Close, Start & Mount, 1973), the duration of measurement in each experiment was 17 d. This was divided into three periods each of 3 d and four periods each of 2 d; complete energy and N balances were established for each period. Two replicates were carried out with each of four treatments combining environmental temperature and feeding level. The question that arises is whether experimental time could have been used differently to give more information, specifically by decreasing the length of each experiment and increasing the number of replicates. Dammers (1964) has shown that a total duration of 5–7 d gives reliable results for individual animals.

The coefficients of variation $(CV = \dfrac{SE \times 100}{mean})$ for a number of quantities during progressively increasing lengths of time in the course of each experiment are given in Table 1. The rate of fall in CV became small after a total of 7 d for heat loss, after 12 d for N balance and ME/GE, and after 14 d for gain in body-weight. Increasing the number of days on experiments beyond these times would not have increased appreciably the reliability of the results.

Table 1. *The coefficient of variation $(\dfrac{SE}{mean} \times 100)$ of the mean body-weight gain, N balance, ME intake and heat loss (HL) in the pig for total periods of 5 to 17 d.*

| No. of periods | Total days of collection | Body-wt gain | N balance | ME:GE | HL/kg per d | HL/kg$^{0.75}$ per d |
|---|---|---|---|---|---|---|
| 2 | 5 | 20·19 | 6·87 | 1·83 | 2·55 | 2·74 |
| 3 | 7 | 16·34 | 6·63 | 1·29 | 1·80 | 2·07 |
| 4 | 10 | 12·91 | 5·26 | 0·96 | 2·00 | 2·00 |
| 5 | 12 | 10·21 | 4·50 | 0·88 | 2·13 | 1·90 |
| 6 | 14 | 9·00 | 4·14 | 0·81 | 2·08 | 1·80 |
| 7 | 17 | 8·63 | 4·09 | 0·68 | 2·23 | 1·82 |
| $\dfrac{SE \text{ within groups}}{SE \text{ between groups within treatments}}$ | | 0·64 | 0·63 | 0·34 | 0·72 | 0·27 |

To make the best use of the experimental material, the variation between groups of animals on the same treatment must be known. It can be shown that the optimum number of periods is proportional to the ratio of the standard error within groups of animals to the standard error between groups within treatments (Henry, 1968). The ratios for the present experiments are given in Table 1, and indicate that it would have been more efficient to increase the number of replicates and reduce the number of periods.

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**Effect of anaesthetic on sucrose–fed rats.** By A. E. BENDER, K. B. DAMJI and K. S. ISMAIL, *Nutrition Department, Queen Elizabeth College, London W8 7AH*

Sucrose fed to experimental animals can have deleterious effects on the kidneys and even on life-span when compared with starch, and can increase the concentrations of a great variety of enzymes (Bender & Damji, 1972).

Animals under the apparent stress of sucrose (700 g/kg diet) were subjected to the additional stress of an anaesthetic, pentobarbitone (60 mg/kg body-weight intraperitoneally). Adult male rats of the Sprague–Dawley strain, weighing about 200 g, were used. 'Sleeping time' was taken as the index of the combined effects of the stresses.

Table 1 shows (a) that the 'sleeping time' of rats fed on sucrose with 200 g casein/kg diet was 70% of that of the starch-fed controls; (b) similarly, the 'sleeping times' of sucrose-fed rats were significantly shorter on diets containing either 100 g casein/kg or 50 casein/kg; (c) 'sleeping times' were lengthened on both diets when the protein content was reduced to 100 g and particularly 50 g/kg; (d) when the two groups of rats in each experiment were transferred from sucrose or starch to stock diet for 7 d the difference between them was abolished.

Table 1. *Sleeping times of rats injected with pentobarbitone (60 mg/kg body-weight) fed on diets containing starch or sucrose for 30 d*

| Dietary protein (g/kg) | Dietary carbohydrate | Sleeping time min | $\frac{\text{sucrose}}{\text{starch}}$ (%) | Significance of difference $P$* | Sleeping time after 7 d on stock diet (min) |
|---|---|---|---|---|---|
| 200 | Starch | 141 | | | 154 |
| | Sucrose | 98 | 70 | 0·001 | 138 |
| 100 | Starch | 153 | | | 125 |
| | Sucrose | 125 | 80 | 0·05 | 142 |
| 50 | Starch | 235 | | | 124 |
| | Sucrose | 177 | 75 | 0·014 | 122 |

*Mann-Whitney test.

Dietary sucrose does not increase the specific activity of the drug-metabolizing enzyme *p*-nitrobenzoate reductase and decreases the specific activity of biphenyl 4-hydroxylase and cytochrome P 450 (Dickerson, Basu & Parke, 1971). However, in all our experiments sucrose induced considerable enlargement of the liver, so that total capacity may have been considerably increased.

At the low protein level (50 g/kg) rats were less able to metabolize the drug and two died. The remainder recovered their normal capacity in this respect after 7 d on stock diet.

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**Lipid peroxide in the developing rat brain.** By R. WALKER, D. N. RUDRA and J. W. T. DICKERSON, *Department of Biochemistry, University of Surrey, Guildford*

The pigment 'lipofuscin' has been considered to be a normal constituent of nerve cells (Greenfield & Meyer, 1963). There is evidence that the amount of lipofuscin in human (Strehler, Mark, Mildvan & Gee, 1959) and dog (Munnell & Getty, 1968) myocardium and in mouse and rat brain (Reichel, Hollander, Clark & Strehler, 1968) increases with age. It is formed by the autoxidation of polyunsaturated fatty acids, and the process can be retarded by dietary supplementation with vitamin E (Weglicki, Reichel, & Nair, 1968). However, reports on the changes with age of the lipid peroxide content of the rat brain are conflicting, for whereas Pritchard & Singh (1961) reported an increase to 15 d of age with a subsequent decrease to a lower value at maturity, Yoshikawa & Hirai (1967) reported a steady rise between 1 and 18 months of age. This subject has been re-examined.

Wistar male rats of known ages between 7 and 189 d were decapitated; the brain was quickly removed, dissected into forebrain, cerebellum and brain stem, and the parts were weighed and quickly frozen. Lipid peroxide was determined in the individual parts of at least five brains at each age from animals of different litters by the thiobarbituric acid method (Bieri & Anderson, 1960); the results were expressed in terms of malondialdehyde (MDA).

In the forebrain and cerebellum the concentration of MDA rose sharply between 7 and 14 d and fell gradually to a near-mature value by 56 d. The absolute amounts in these parts increased rapidly between 7 and 14 d and had reached adult values by 21 d. In the brain stem the concentration of MDA fell between 7 and 14 d and again between 21 and 28 d. The absolute amounts, however, rose gradually between 7 and 21 d and fell to a near-mature value by 28 d.

These results were discussed in the light of previous reports.

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**The effect of a long–term reduction in rumen capacity on the intake of hay by a cow.** By J. A. BINES, P. A. JONES and D. J. NAPPER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

It has been claimed (Tulloh, 1966) that a hypertrophy of the alimentary tract may occur during lactation, permitting the milking cow to maintain a larger appetite

than the dry cow in order to meet the nutritive demands of lactation. Further examination of the results suggests that the increase in gut capacity may be only a secondary effect resulting from a reduction in the amount of fat within the abdominal cavity during early lactation. The weight (kg) of the total contents of the cavity were remarkably similar for both lactating and non-lactating cows:

|                  | Lactating | Non-lactating |                    |
|------------------|-----------|---------------|--------------------|
| Digesta          | 82·6      | 58·3          |                    |
| Fat              | 18·9      | 47·1          |                    |
| Alimentary tract | 26·3      | 22·5          | (from Tulloh, 1966)|
| Total            | 127·8     | 127·9         |                    |

It should be possible to test this hypothesis by artificially reducing the capacity of the rumen in an animal which is not lactating and whose fat depots therefore remain relatively constant in size.

A dry fistulated cow was given *ad lib.* access to medium-quality hay. After 6 weeks to establish intake, three water-filled bladders with a total volume of about 33 l were placed in the rumen and remained there for 17 weeks during which time hay remained available *ad lib.* After removal of the bladders, intake was measured for a further period. In previous experiments using this technique (Campling & Balch, 1961), bladders were not left in the rumen for long enough for adaptive changes of this kind to be observed.

The mean daily intakes (kg) of hay were:

|                             |        |        |      |
|-----------------------------|--------|--------|------|
| Before insertion of bladders |       |        | 9·9  |
| With bladders               | weeks: | 1–6    | 7·3  |
|                             |        | 7–12   | 7·3  |
|                             |        | 13–17  | 5·6  |
| After removal of bladders   | week:  | 5      | 8·8  |

After insertion of the bladders, intake fell rapidly and remained low. There was no indication that a hypertrophy of the alimentary tract occurred to enable the intake to return to the original level, indeed, after week 12, there was a further fall in intake. After removal of the bladders, intake rose slowly, and was still below the original level at the end of the 5th week. Intake of water, though more erratic than food intake, followed it closely.

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**The effect of urea on the intake of straw by fat cows and thin cows.** By J. A. BINES and D. J. NAPPER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

It has been shown (Bines, Suzuki & Balch, 1969) that thin cows will eat more of a nutritionally adequate diet than the same cows when fat. However, when straw

was given as the sole food, intakes were similar, and it was suggested that a possible reduction in the protein status of the thin animals may have concealed the expected difference. If this were so, it would be expected that the difference in intake could be restored by means of nitrogen supplementation of the diet.

Four non-lactating cows were given constant intraruminal infusions of 75 g urea in 9 l water daily when fat and when thin; this treatment was compared with the infusion of 9 l/d water. The cows were given *ad lib.* access to oat straw for 5 h/d. Measurements were made of intake and digestibility of the straw, the rate of breakdown of cellulose (cotton thread) in the rumen and the retention of N by the cows. The results were:

|  | Thin cows | | Fat cows | | |
|---|---|---|---|---|---|
|  | Urea | Water | Urea | Water | SEM |
| Intake of dry matter (kg/d) | 5·1 | 3·9 | 4·4 | 3·5 | 0·3 |
| Intake of digestible energy (MJ/d) | 43·1 | 25·9 | 39·3 | 25·9 | 3·3 |
| Dry-matter digestibility coefficient | 0·46 | 0·36 | 0·47 | 0·40 | 0·01 |
| Time (h) for 25% loss of weight of cotton in rumen | 23·8 | 93·5 | 31·3 | 90·1 | 14·3 |
| N retained (g/d) | −2·2 | −21·4 | −14·1 | −30·2 | 3·6 |

Intakes of dry matter and digestible energy were similar in fat and thin cows in the absence of N supplementation. When urea solution was infused into the rumen intakes rose substantially, that of thin cows tending to rise more than that of fat cows. Infusion of urea also increased the rate of breakdown of cellulose in the rumen and the digestibility of the straw, the increases being similar for fat and thin cows. The amount of N retained also increased as a result of urea infusion.

Since rate of breakdown in the rumen and the protein status of the animals were both improved by infusion of urea, it is difficult to estimate from the results of this experiment the relative contribution of these two factors to the apparent increase in intake by the thin, supplemented cows. Further work is required to clarify this point.

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**Ammonia toxicity in sheep.** By J. B. SOAR, P. J. BUTTERY and D. LEWIS, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

Tetanic spasms, coma and eventual death are among the signs of acute ammonia toxicity. However, at sublethal levels, ammonia has numerous effects on metabolism. Ammonia toxicity was induced in mature wethers by continuous infusion of ammonium acetate (pH 7·4) into a jugular vein. Samples of blood were withdrawn from the opposite jugular vein. At infusion rates of 0·8 mg $NH_3$-N/min per kg body-weight, blood ammonia concentrations were observed to rise and plateau at approximately 12 mg $NH_3$-N/l blood, followed by a rapid rise at which time signs of acute toxicity became evident. The length of the plateau was inversely related to the infusion rate such that slow rates (0·7 mg $NH_3$-N/min per kg body-weight) caused

an initial rise in blood ammonia which then remained constant throughout the infusion (2 h). Infusion rates of 1·0 mg $NH_3$-N/min per kg caused a rapid rise in blood ammonia, accompanied by the signs of acute toxicity. These results can be interpreted as evidence of a buffering system of finite capacity existing to prevent excessive increases in blood ammonia leading to acute toxicity.

Ammonia toxicity was associated with a percentage rise in plasma glutamine of $50 \pm 15$ (mean $\pm$ SEM, five determinations), the other amino acids remaining constant or being depressed. Blood urea showed no marked change but blood glucose was found to rise from a basal level of $0·48 \pm 0·03$ g/l (mean $\pm$ SEM, six determinations) to $1·09 \pm 0·06$ g/l at the onset of acute toxicity. A similar situation is observed in the blood glucose concentrations of the non-ruminant (Prior, Clifford & Visek, 1970). No change in plasma magnesium was found, an observation that is not consistent with the suggestion of Chow & Pond (1972), that ammonia toxicity is a result of the depletion of available magnesium due to the formation of magnesium ammonium phosphate. Plasma potassium concentrations were found to rise slightly during the ammonia infusion. However, a marked percentage fall of $12·5 \pm 1·92$ (five determinations) was observed when the infusion was stopped. This observation is consistent with the suggestion that ammonia interferes with the potassium pump, this being the underlying cause of ammonia toxicity (Lund, Brosnan & Eggleston, 1970).

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**The use of *n*-paraffin-grown yeast in diets for chicks.** By A. I. LABIB* and D. HEWITT, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The growth of chicks given all-vegetable diets made up from maize meal, ground oats, wheat middlings, grass meal, *n*-paraffin-grown yeast (kindly supplied by BP Proteins Ltd, London) and sesame meal was compared with that of chicks given a standard diet based on cereals, soya-bean meal and fish meal. The diets were given to 1-week-old broiler chicks for 3 weeks. A suboptimal level, 180 g/kg, of dietary protein was selected to ensure that differences in protein quality were reflected by differences in growth rate.

In the first experiment the use of yeast as the sole supplementary protein was studied. Chicks given the standard diet which contained 160 g soya-bean meal and 70 g fish meal/kg gained 21·8 g/d; chicks given a diet containing 190 g yeast/kg gained only 13·4 g/d. Supplementing the yeast-containing diet with 2 g methionine/kg increased weight gain by 1 g/d. Neither additional methionine nor supplements of lysine, arginine and glycine increased growth further. To investigate whether the

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growth depression caused by high levels of yeast is associated with the RNA in the yeast, a preliminary experiment was carried out in which 19 g yeast RNA (BDH Chemicals Ltd, Poole, Dorset) per kg was added to the standard diet based on fish meal and soya-bean meal modified to contain the same amount of true protein as the yeast-containing diet. The growth of the chicks was depressed slightly after 1 week but significantly after 3 weeks, whereas growth on the yeast-containing diet was significantly depressed after only 1 week.

In a subsequent experiment all-vegetable diets containing different proportions of yeast and sesame meal supplemented with lysine proved more satisfactory and showed little indication of a growth depression due to the presence of yeast up to a level of 165 g/kg.

**Nutritive value and toxicity of *Sauropus androgynous*.** By A. E. BENDER and
  K. S. ISMAIL, *Nutrition Department, Queen Elizabeth College, London W8 7AH*

The leaf of *Sauropus androgynous*, reported to contain 100 g protein/kg (Willimont, 1949), is commonly eaten in Malaysia, where it is called Chekor manis or asin-asin. Since it grows wild in South-east Asia, it is a potentially valuable source of protein.

A survey carried out in and around Kuala Lumpur on 458 families showed that the average consumption was 180 g per head eaten once a week.

Analysis yielded 70 g protein/kg fresh weight, 350 g/kg dry weight. Amino acid analysis gave a chemical score of 68, limited by the sulphur amino acids.

An attempt to measure net protein utilization failed because the rats completely refused to eat the diet. Heat-treated and acetone-extracted leaf were also refused, but when the leaf was extracted with hot water the rats accepted the diet, and results then were: net protein utilization 0·6, protein efficiency ratio 2:1.

The aqueous extract of leaf was supplied as drinking-water to rats fed on the stock diet; although their food consumption was approximately the same as that of the controls given tap-water, they gained weight only slowly and exhibited bleeding around the nose and paws. This observation suggested that the refusal to eat the food was because of its toxicity rather than unpalatability.

The aqueous extract was examined by thin-layer chromatography (TLC) in an attempt to identify the substance or substances responsible. Since leaf was involved, the chromatograms were tested for alkaloid by treatment with iodoplatinate reagent (Waldi, Schnackerz & Munter, 1961). A spot developed with $R_F$ value 0·68 in chloroform–acetone–diethylamine (5:4:1, by vol.) compared with a value of 0·67 for papaverine (Waldi *et al.* 1961). Repeat runs in cyclohexane–$CHCl_3$–diethylamine (5:4:1, by vol.) and methanol gave $R_F$ values of 0·40 and 0·73, compared with values for papaverine of 0·42 and 0·70.

The aqueous solution was concentrated by evaporation under reduced pressure, and the recrystallized material showed colour reactions similar to those for papaverine (Clarke, 1969; British Pharmacopoeia, 1968).

The identity of the substance was confirmed by comparison of the nuclear magnetic resonance spectrum with that of a sample of authentic papaverine.

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An approximate quantitative determination was made by separating the papaverine spot on TLC plates from a known amount of leaf and measuring the extinction at 252 nm in a spectrophotometer. The value was 5·8 g/kg fresh leaf. Thus, individuals consuming 180 g leaf would consume about 1 g papaverine compared with the therapeutic dose of up to 200 mg/d (Merck Index, 1960).

During the survey, subjects warned the interviewer that excessive consumption of Chekor manis caused dizziness and drowsiness.

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**The energy cost of nitrogen metabolism in chicks.** By P. J. BUTTERY, K. N. BOORMAN and EILEEN BARRATT, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

In an attempt to assess the energy cost of nitrogen metabolism in the young cockerel, experiments have been conducted to determine the energy required to synthesize uric acid and for the maintenance and deposition of skeletal muscle in a 900 g hybrid cockerel consuming approximately 20 g protein and 1·26 MJ metabolizable energy (ME) daily. Using a perfused chicken liver preparation (Locke, Buttery & Boorman, 1972) the synthesis of 1 mol uric acid from glycine was found to be associated with an uptake of 1·49 mol oxygen. This uptake is equivalent to 0·71 MJ/mol uric acid synthesized if glucose is assumed to be the energy source. The birds under investigation would excrete approximately 1 g N/d as uric acid (Solberg, Buttery & Boorman, 1971) and therefore the energy cost of the uric acid synthesized would be at least 13·4 kJ/d. This is probably an underestimation since no account has been taken of any possible energy loss to the bird as a consequence of the formate units and glycine incorporated into the uric acid.

Under the conditions chosen for this study the body-weight gain of the chicks would be expected to be approximately 40 g/d. Of this gain 47% would be skeletal muscle containing 210 mg/g protein (dry-weight basis). The increase in muscle protein is therefore 3·9 g/d. On the assumption that each mol of amino acid requires 3 or possibly 4 mol ATP, or its equivalent, to be incorporated into protein, and that this energy is derived from glucose, the production of 3·9 g of protein would require 8·0 kJ (3 mol ATP) or 10·9 kJ (4 mol ATP). The total energy 'loss' to the animal would, therefore, be 91·7 kJ or 94·2 kJ assuming the ME of protein to be 21·4 kJ/ mol. However, these calculations neglect any consideration of protein turnover. The half-life of breast-muscle protein in the chick, measured by the method of Waterlow & Stephen (1968), was found to be 9·12 d, i.e. 7·7% of the protein is re-

newed each day. For total skeletal muscle, this rate of renewal is equivalent to the deposition of 6·8 g protein/d. The energy cost of this renewal would be 14·2 or 18·8 kJ, depending on the number of mol ATP used in the calculation (see above).

Thus skeletal muscle deposition and maintenance require 106–113 kJ/d and uric acid synthesis at least 13·4 kJ/d.

The assumptions and values for growth rate used in these calculations are based on accepted values and information gathered in this laboratory.

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**The influence of oral contraceptives on the effect of low–protein–high–sucrose or low–protein–high–starch diets on male rats.** By D. B. JEFFERYS and I. R. WHITE (introduced by BETTY L. COLES), *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

The difference between starch and sucrose as components of a high-carbohydrate-low-protein diet was investigated. Sucrose is the main component of the diet in the West Indies, whereas starch is the principal one in Africa. Differences in the resultant clinical picture may be reflections of the carbohydrate difference. The combined effect of oestradiol and progesterone has been found to affect sucrose handling (Jefferys & White, 1973).

Five groups of male Wistar rats (290) g were used, five in each group. A control group received a diet containing (g/kg): protein 150, carbohydrate 660, fat 130, salts 10 (18·4 MJ/kg diet). The other groups received (g/kg): carbohydrate (sucrose or starch 800), fat 130, protein 50 and salts 20 (18·0 MJ/kg diet). One sucrose and one starch group also received 1·0 mg norethisterone and 0·05 mg mestranol (Norinyl-1) per 25 g food. The food was offered *ad lib*.

After 27 d, the rats were starved for 24 h, and blood was removed from the heart under heavy diethyl ether anaesthesia. The serum protein concentrations and the Whitehead index (Whitehead & Dean, 1964) were determined. The liver lipid was extracted and weighed.

The control group gained 52 g during the experiment, the others lost weight. The starch and sucrose groups showed similar weight losses, but the starch group consumed 40% more energy. The oral contraceptive (pill) significantly reduced the energy intake and increased the weight loss with both carbohydrates.

The total serum protein concentrations were decreased for all four groups, compared with the controls. When the pill was given, the serum proteins for the starch group were increased but those for the sucrose group were not. Both carbohydrate

regimens with the pill raised the albumin and $\alpha_2$-globulin concentrations, but the $\beta$-globulin fractions were reduced still further. A decreased albumin concentration was accompanied by increased $\gamma$-globulin concentrations with the carbohydrate regimens (Coles & Macdonald, 1966). When carbohydrate intakes were high the concentration of liver lipids was raised, rats receiving the pill producing even greater increases, the most marked increase being in those given sucrose and the pill. The Whitehead index, a possible measure of nutritional status, was raised for all groups except those receiving starch and the pill.

Sucrose appeared to be less harmful than starch in a low-protein diet. Though less sucrose than starch was eaten, the serum albumin and liver lipid concentrations were higher in the sucrose groups. The Whitehead index showed no significant difference. The pill decreased energy intake and increased the weight loss, but the serum albumin and $\alpha_2$-globulin concentrations were higher.

We thank Dr B. L. Coles and Professor I. Macdonald under whose supervision these experiments were carried out.

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### The influence of dietary carbohydrates and proteins on faecal nitrogen excretion in rats. By V. C. MASON* and R. M. PALMER (introduced by R. H. SMITH), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The influence of various dietary carbohydrates and proteins on faecal nitrogen excretion was examined in a series of studies involving both young and adult rats. In the carbohydrate studies egg albumen was used as the only source of N and rice starch was used in the control diet. It was observed that whereas maize and tapioca starches had little effect on the amount of faecal N excreted, relative to rice starch, both raw potato starch and yam starch, and to a lesser degree cellulose, in the diet resulted in a considerable increase in faecal N associated with a parallel increase in the bacterial diaminopimelic acid (DAPA). The ratio of DAPA increase to faecal N increase indicated that the latter was largely due to bacterial growth.

In the studies with various dietary proteins of differing apparent digestibilities the type of protein did not markedly influence DAPA excretion. Thus, for example, the faeces derived from blood meal (apparent digestibility 0·69) contained slightly less DAPA (25·9 mg/kg dry matter intake) than the faeces from a diet containing fish meal (digestibility 0·90, 28·0 mg DAPA/kg dry-matter intake). However, the ratio of DAPA to faecal N was significantly lower when the relatively indigestible blood meal was consumed (0·07 g DAPA/16 g faecal N compared with 0·24 g/16 g faecal N with the fish-meal diet). These results were interpreted to show that microbial growth in the hind gut is rarely limited by the availability of N.

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It was concluded that when highly digestible proteins are consumed the amount of N excreted in the faeces is primarily determined by the level of bacterial activity in the hind gut, which in turn is primarily influenced by the type and pretreatment of the carbohydrates in the diet. Thus, because they do not take account of this bacterial activity, methods which rely on amino acid analyses of the faeces for determining the availability of dietary amino acids (e.g. Kuiken & Lyman, 1948) should be regarded with reserve.

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**Processing maize and urea efficiency: in vitro evaluation and growth trial in kids.** By L. P. BORGIDA, M. DURAND and J. DELORT-LAVAL, *Laboratoire de Recherches sur la Conservation et l'Efficacité des Aliments, Institut National de la Recherche Agronomique, 78350–Jouy-en-Josas, France*

Some heat treatments applied either to immature cereals (Durand, 1970) or to a mixture of urea and grains (starea) (Helmer, Bartley, Deyoe, Meyers & Pfost, 1970) improve urea-nitrogen utilization by rumen microflora, because of a higher susceptibility of starch to enzymes. Three maize processings, popping, flaking and extrusion, which were shown in vitro to intensify $\alpha$-amylase action (C. Mercier, personal communication), were compared when included in a concentrate for growing kids containing (g/kg): ground maize 400, either untreated or treated ground maize 400, straw 150, urea 20, minerals and vitamin mixture 30.

Of each concentrate, 12 g were incubated for 5 h with 200 ml whole rumen content from sheep adapted to a similar diet and an equal volume of artificial saliva. Compared with untreated diet, extrusion and flaking and, to a lesser extent, popping produced, within the first 30 min, a higher soluble-carbohydrates peak and then a more pronounced pH decrease with increased volatile fatty acid formation; this involved a significant increase in the disappearance of urea N and $NH_3$-N from the medium (Table 1).

Table 1. *Changes in volatile fatty acid (VFA) production, pH and ammonia-nitrogen + urea-N consumption after 5 h incubation of concentrates*

(Mean values with their standard errors; $n=4$)

|  | Untreated diet | Popped diet | Flaked diet | Extruded diet |
|---|---|---|---|---|
| VFA production (mmol/l) | $69\pm1$ | $71\pm1$ | $86\pm7$ | $97\pm5$ |
| pH | $6\cdot15\pm0\cdot04$ | $6\cdot00\pm0\cdot05$ | $5\cdot80\pm0\cdot07$ | $57\cdot\pm0\cdot02$ |
| $NH_3$-N + urea-N consumption (mg N/l) | $152\pm9$ | $207\pm8$ | $242\pm12$ | $233\pm2$ |

Two pairs of early-weaned (5th week) male and female kids were offered each concentrate *ad lib.* plus 200 g hay/d. For all diets, concentrate intake was high and growth fairly good. The effect of treatment was quite different on males and females.

For males, values were in good agreement with in vitro values; growth rates were increased by 20% with flaked and extruded diets and by 10% with the popped diet. Dry matter as well as N feed conversion followed the same trend (Table 2).

Table 2.  *Growth rate and food conversion ratio (g food dry matter : g weight gain) in kids, 7–16 weeks old, given different diets*

|  | Untreated diet | | Popped diet | | Flaked diet | | Extruded diet | |
|---|---|---|---|---|---|---|---|---|
| Sex | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Growth rate (g/d) | 168 | 141 | 187 | 170 | 203 | 152 | 200 | 124 |
| Food conversion ratio | 4·29 | 4·83 | 3·86 | 4.23 | 3·61 | 4·68 | 3·90 | 5·43 |

For females, extrusion had a depressive effect on growth, whereas popping resulted in 20% better growth, and flaking was intermediate.

Further studies are in progress to explain this sex effect and to determine the optimum level for growth of both urea and treated cereal.

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**Effects of different dietary nitrogen sources on metabolism in the stomach of the calf.** By R. H. SMITH and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Three calves received equal daily amounts of concentrates and roughage, the former morning and evening and the latter evening only. The diets were flaked maize and hay (A) or mixtures of similar energy content containing flaked maize and straw supplemented with decorticated groundnut meal (B), fish meal (C), maize gluten (D) or urea (E). Diet A contained 16 g and the other diets 26 g nitrogen/ kg dry matter. All were given for at least 18 d before sampling. Before a morning feed, mean concentrations (g/l) of total non-ammonia N (NA-N), $NH_3$-N and RNA-N in strained rumen fluid were 0·70–1·15, 0·07–0·20 and 0·065–0·082 respectively. At 1·5 h after a feed these concentrations generally showed little change or had decreased, but for diet E, $NH_3$-N had increased to 0·90 g/l. At 5·5 h after feeding, concentrations still showed little change for diet A, but RNA-N concentrations had increased by about 0·042 for diet D and 0·064 for diets B, C and E. It was estimated therefore (McAllan & Smith, 1972) that the concentrations of total microbial N in these samples had increased by 0·38–0·58 g/l. For diet E a quantitatively similar decrease in $NH_3$-N concentration occurred.

Two calves examined at pasture showed no diurnal changes in rumen RNA-N concentrations, which were slightly higher than the maximum values for the stall-fed calves.

Apart from lower maximum $NH_3$-N concentrations, changes in the composition of duodenal contents generally reflected those in rumen fluid. Ratios of NA-N:

RNA-N were, however, greater in duodenal than in rumen contents, presumably mainly owing to secretion of endogenous N into the abomasum. From these ratios and assuming little gain or loss of RNA-N between rumen and duodenum (Smith & McAllan, 1971), it was calculated, for samples of duodenal contents taken from calves at pasture or 6 h after giving diets A or E, that concentrations of endogenous N were 0·27–0·51 g/l (14–26% of NA-N). It was also calculated (McAllan & Smith, 1972) from RNA-N concentrations in similar samples of duodenal contents that microbial N supplied 68, 67, 79, 69, 47 and 71% of the NA-N for calves at pasture and for those receiving diets A, B, C, D and E respectively.

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**Metabolism of casein in the rumen and some effects of formaldehyde treatment.** By A. P. WILLIAMS, A. B. MCALLAN and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

In vitro incubation of samples of casein with calf rumen contents showed that there were marked differences between products prepared in different ways. In particular, rennet-precipitated casein led to much less ammonia production than lactic acid-precipitated casein. This was true also in in vivo experiments.

Following the technique of Ferguson, Hemsley & Reis (1967), lactic acid–precipitated casein was treated with formaldehyde solution (10 g formaldehyde/kg protein). Casein treated in this way showed greatly reduced ammonia production on in vitro incubation with calf rumen contents, whereas its available methionine content, determined by microbiological assay (Boyne, Price, Rosen & Stott, 1967), was reduced by only 16%. Two calves were given a basal diet of about equal amounts of straw and flaked maize (12 g nitrogen/kg dry matter) or diets of the same energy content but with some flaked maize replaced by untreated or formaldehyde-treated casein to give different N contents (19, 26 or 34 g N/kg dry matter). The animals were allowed at least 2 weeks to adapt to each new diet.

Two hours after feeding, concentrations of $NH_3$-N (g/l, mean values $\pm$ SE) in rumen fluid were 0·010 ± 0·002, 0·074 ± 0·019 and 0·145 ± 0·006 respectively for the three diets containing increasing amounts of untreated casein. Corresponding values for treated casein were only 0·007 ± 0·002, 0·005 ± 0·001 and 0·013 ± 0·009 respectively. Plasma urea–N concentrations increased progressively with increasing amounts of dietary casein from 25–35 mg/l for the unsupplemented diet to 125–155 mg/l with the highest casein supplements. There was little difference between treated and untreated casein, e.g. 141 mg/l and 139 mg/l respectively with the highest casein supplements.

The mean amount (g/kg dry matter ± SE) of α-dextran (estimated as glucose liberated by mild acid hydrolysis) in bacteria separated from the rumens of the calves

4 h after they had received the basal diet was 145 ±20. With increasing intake of un-treated casein corresponding bacterial samples showed decreasing amounts of dextran to 54 ± 19 g/kg at the highest casein level. This effect was like that found when similar diets were supplemented with urea (Smith & McAllan, 1972), but was not shown when formaldehyde-treated casein formed the supplement; with the highest intake of treated casein bacterial dextran content was 148 ± 37 g/kg. This finding shows that the form, as well as the amount, of dietary N may influence the synthesis of microbial carbohydrate in the rumen.

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**$N$–steroyl–DL–methionine – a new form of protected methionine for ruminant feeds.** By P. N. LANGAR, P. J. BUTTERY and D. LEWIS, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

Methionine has been reported to be the first limiting amino acid in ruminant diets (Nimrick, Hatfield, Kaminski & Owens, 1970; Wakeling, Lewis & Annison, 1970). The possibility has been investigated of using $N$-steroyl-DL-methionine (S-met) to supplement ruminant diets, and the extent of its protection against degradation in the rumen has been measured.

When S-met (equivalent to 5 g DL-methionine) was introduced into the rumen of a sheep through a fistula, 35·3% was recovered (as methionine and S-met) at the proximal duodenum during the first 24 h and 29·2% in the subsequent 24 h period. In a similar experiment with 5 g DL-methionine, 17% was recovered during the first 24 h; 10 h after methionine dosing, free methionine concentrations in the rumen had fallen to the basal level ($0·05 \times 10^{-3}$M). Methionine and its analogues were assayed by the method of McCarthy & Sullivan (1941).

Further experiments were carried out with varying intraruminal doses of DL-methionine or the equivalent of DL-hydroxy-4-methylthiobutyric acid (MHA) or S-met. The methionine or derivatives were given at feeding time for 12 d. On the 11th and 12th days a 48 h duodenal sample (Thompson & Lamming, 1972) collection was made to estimate the percentage of material escaping fermentation (Table 1). A quantity of S-met equivalent to 7·5 g methionine caused the sheep to refuse food on day 5, and a similar effect was observed with 10 g or 20 g doses of DL-methionine, food intake ceasing on days 10 and 5 respectively.

These results indicate that S-met is substantially protected from rumen degradation and, together with the observation that S-met is able to correct for methionine deficiency in rats (R. Fahnenstich, private communication), suggest that S-met is a suitable form of protected methionine for ruminant feeds.

Table 1. *Recovery of methionine and methionine derivatives at proximal duodenum of sheep in two separate experiments with each material (percentage of dose recovered/24 h)*

| Daily dose as methionine (g) | DL-methionine | | MHA | | S-met | |
|---|---|---|---|---|---|---|
| 2·5 | 12·6 | 2·6 | 22·4 | 19·3 | 22·8 | 24·2 |
| 5·0 | 17·0 | 1·1 | 25·5 | 13·2 | 34·8 | 26·2 |
| 7·5 | 19·5 | 3·6* | 11·6 | 7·6 | † | † |

Results are from two consecutive 24 h periods at the end of each 12 d feeding period.
MHA, DL-hydroxy-4-methylthiobutyric acid; S-met, *N*-steroyl-DL-methionine.
*Food intake reduced. †Food refused.

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**The methionine requirement of growing lambs.** By J. R. MERCER* and E. L. MILLER, *Department of Applied Biology, University of Cambridge, Cambridge CB2 3DX*

The methionine requirement of four growing lambs was determined by continuously infusing into the abomasum a mixture of amino acids containing either 0, 0·5, 1·0 or 1·5 g methionine/24 h. The responses to the infusions were measured by the urinary excretion of ^{35}S after an abomasal injection of [35S]methionine and by changes in plasma urea concentration.

The composition of the diet (g/kg dry matter (DM)) offered throughout the experiment was: rolled barley 812, chopped barley straw 100, molassine meal 50, urea 5, dicalcium phosphate 5, sodium sulphate 5, trace element and vitamin premixes 20 and chromic oxide 3. The diet was given hourly, the total daily intakes of DM and nitrogen being 712 and 14·22 g respectively. Each lamb received the four amino acid infusion mixtures according to a Latin square design. Each mixture contained essential amino acids, except for methionine, in the same proportions as in Peruvian fish meal. Non-essential amino acid N was supplied by glycine, glutamic acid and proline (Dean & Scott, 1962). Total N infused was 3·84 g/d. The amino acid solution (pH 4·5) was infused at 13·5 ml/h for 9 d. The first 5 d were an adaptation period (Nimrick, Hatfield, Kaminski & Owens, 1970). On the 6th day, 30 μCi L-[35S]methionine were injected into the abomasum and excretion of ^{35}S in urine was determined over the last 4 d (Downes, Reis, Sharry & Tunks, 1970). Samples of blood from a jugular vein were taken each morning of the last 5 d of infusion, and a number of samples of abomasal digesta were taken 2 d after the termination of the infusion.

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The main results are shown in the table:

| | Methionine infused (g/24 h) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0·5 | 1·0 | 1·5 | SEM | (df) |
| Urine $^{35}$S (ratio, excreted in 4 d:injected) | 0·079$^a$ | 0·081$^a$ | 0·097$^c$ | 0·117$^b$ | | |
| Urine $^{35}$S (confidence limits, $P=0·05$) | 0·066– | 0·068– | 0·083– | 0·103– | | (5) |
| | 0·092 | 0·095 | 0·112 | 0·133 | | |
| Urine urea excretion (g N/d) | 7·00$^a$ | 6·08$^a$ | 5·83$^a$ | 5·70$^a$ | 0·335 | (6) |
| Plasma urea concentration (mg/l) | 278$^a$ | 247$^b$ | 230$^c$ | 259$^b$ | 7·2 | (70) |

$^{abc}$ Significant differences ($P<0·10$) indicated by unlike superscripts.

The similarity of $^{35}$S excretion when 0 or 0·5 g methionine was infused is assumed to indicate methionine is limiting and is optimally utilized. The increased $^{35}$S excretion when 1·0 and 1·5 g methionine were infused is assumed to indicate methionine is in excess of requirement for maximum utilization. The methionine requirement was calculated as the quantity of methionine corresponding to the intersection of the lines joining 0 with 0·5 and 1·0 with 1·5 g methionine treatments, together with the quantity of methionine passing through the abomasum from the rumen each day. For lambs growing at a mean rate of 154 g/d, the methionine requirement was calculated as 2·35 g/d. A similar calculation using plasma urea concentration as the indicator of response gave a requirement of 2·63 g/d.

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**Contribution of microbial and undegraded dietary protein to the potential protein utilization in young ruminants.** By E. R. ØRSKOV and A. W. BOYNE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

To estimate the value of protein or non-protein nitrogen supplements for young ruminants it is essential to know the contributions to their N metabolism made by microbial protein and by the undegraded protein of the diet.

Many workers have found that the production of microbial protein is related to that of carbohydrates fermented in the rumen, so it was suggested (Ørskov, 1970) that the N utilization by the animal should be considered relative to its energy intake. In the experiment to be described N utilization has been expressed as g N retained divided by MJ of metabolizable energy (ME) provided by the diet above estimated maintenance requirement.

The lambs used for the experiment were weaned on to a basal diet of barley with urea added to give a crude protein content in the dry matter of 130 g/kg (Ørskov, Fraser & McDonald, 1972). This diet was offered *ad lib.* In addition 50 g/d fish-protein concentrate (Astra Nutrition, Sweden) was administered in liquid form (FPC) in such a way that it bypassed the rumen and therefore did not contribute to the metabolism of rumen bacteria. This amount of FPC was chosen

because preliminary evidence showed that further addition failed to increase N retention. At about 20 kg live weight half of the lambs had the FPC withheld. These are designated the basal group, and the experiment continued with the basal and FPC groups until the lambs were about 65 kg live weight. Measurements of N balance were made at frequent intervals on all animals. In each animal of the basal group there was considerable fluctuation in N utilization, but there was no evidence of a trend with live weight. The mean utilization was calculated for each of the five animals to give 0·67, 0·70, 0·75, 0·78 and 0·79 g N retained/MJ of ME above maintenance, with a general mean of 0·74. By contrast, there was a very marked fall in N utilization with live weight for the FPC groups. The initial values at about 15 kg live weight ranged from 1·3–1·7 g N retained/MJ of ME above maintenance and had fallen to that of the basal group at weights which ranged from 30 to 48 kg for individual animals, with a mean of 40 kg for all six animals.

These results imply that for the young lamb there is no need to provide a protein supplement after it reaches a live weight of between 30 and 48 kg. However, in this experiment the lambs on the basal group grew somewhat slowly, and this along with their prolonged deprivation of N may have altered their potential for N retention. Consequently, in confirmatory experiments the protein supplement will be withdrawn at live weights which differ between one group of lambs and another. A number of breeds and different basal diets will be studied and it is hoped that either live weight itself within breeds, or rate of food conversion, which is also easily measured, may provide a practical guide to the stage of development at which protein supplementation may be discontinued.

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**The effect of energy source on the passage of nitrogen and amino acids to the duodenum in sheep fed on urea or fish meal.** By J. D. OLDHAM, H. SWAN and D. LEWIS, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

Ground barley straw, ground barley or molasses+wheat straw (60:40) were given in combination with either urea (Expt 1) or fish meal (Expt 2) to three mature cross-bred wethers, each fitted with a permanent rumen cannula and a re-entrant cannula into the proximal duodenum.

Samples of duodenal digesta were taken during continuous 24 h collections. Three collections were made from each sheep for each treatment. No correction was made to values obtained from measured digesta flow since Thompson & Lamming (1972), under similar conditions, showed that measured flow of dry matter and total digesta through the duodenum did not vary between three consecutive 24 h collections. Samples of rumen fluid were taken during duodenal collections.

Total collections of faeces and urine were made for 6 d during the period when flow measurements were made.

Nitrogen intakes did not differ between treatments (Table 1). The flow of N to the duodenum was lowest for molasses+urea, although only the values for straw or barley+urea were significantly greater than this ($P<0.05$). However, daily urinary N output was greater ($P<0.01$) for the molasses+urea treatment than for all other treatments. In addition this treatment yielded the lowest mean value for N balance and the greatest rumen-ammonia concentration after feeding. This suggests that the interaction between molasses and urea in the rumen may not be wholly beneficial to the ruminant. Barley treatments consistently gave the highest N balance ($P<0.01$).

Table 1. *Daily nitrogen intake, passage of N and total essential amino acids (EAA) to the duodenum, N balance and rumen-ammonia concentration 2 h after feeding, in sheep on the different experimental treatments*

| | Expt 1 | | | Expt 2 | | | |
|---|---|---|---|---|---|---|---|
| Treatment | Urea + straw | Urea + barley | Urea + molasses | Fish meal + straw | Fish meal + barley | Fish meal + molasses | SE range |
| N intake (g/d) | 23·99 | 24·85 | 23·38 | 21·36 | 22·27 | 21·33 | 1·90–2·84 |
| Duodenal N (g/d) | 22·60 | 20·24 | 13·16 | 19·27 | 18·38 | 17·30 | 2·97–3·77 |
| Total EAA (mmol/16 g N intake) | 201·3 | 204·2 | 116·0 | 195·9 | 204·3 | 196·5 | |
| Total EAA (mmol/16 g duodenal N) | 213·7 | 250·7 | 206·1 | 217·1 | 247·5 | 242·3 | |
| N balance (g/d) | +4·68 | +10·83 | +0·47 | +6·03 | +11·79 | +5·39 | 2·88–4·50 |
| Rumen-ammonia concentration (μmol/ml) | 19·0 | 13·0 | 24·0 | 11·5 | 8·6 | 9·3 | 1·6 –2·7 |

Passage of amino acids to the duodenum generally reflected that of N with molasses+urea, providing the smallest daily quantities (mmol) of every amino acid except methionine. The fish-meal treatments yielded consistently lower values than urea treatments for daily passage of methionine.

The composition of the essential amino acids (EAA) at the duodenum did not differ between treatments when each EAA was expressed as a ratio to valine, except for the fish-meal treatments which tended to produce low proportions of methionine.

These results suggest that the carbohydrate sources investigated can be ranked in the following order: barley>straw>molasses, in terms of the effectiveness with which they influence the utilization of N in the ruminant.

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## The influence of the level of feeding on the digestion of a diet consisting of barley, hay and flaked maize. By J. C. Hodgson and P. C. Thomas, *Hannah Research Institute, Ayr KA6 5HL*

In sheep receiving a diet of ground barley, ground hay and flaked maize (56:24:20) in twenty-four hourly meals each day there were wide variations in the composition of the mixture of rumen short-chain fatty acids between sheep or in the same sheep on different occasions and the molar percentage of propionic acid was correlated with the clearance rate of the rumen fluid phase (Hodgson & Thomas, 1972). The experiment described here was designed to determine the influence of level of feeding on the flow of fluid from the rumen and on the mixture of short-chain fatty acids in the rumen fluid.

Five rumen-cannulated sheep were given a diet of ground barley, ground hay and flaked maize (56:24:20) in four experimental periods at levels of feeding of 33, 42, 55 and 67 g dry matter (DM)/kg $W^{0.73}$ per d. Food was given in four equal meals each day at 06·00, 10·00, 16·00 and 22·00 hours and water was provided as an intraruminal infusion (2 l/d). Access to drinking-water was limited to a short period at 10.00 hours and the animals drank negligible amounts.

The concentration of total short-chain fatty acids increased ($P < 0.01$) and the rumen pH decreased ($P < 0.01$) with increases in the level of feeding, and the pH was correlated ($P < 0.001$) with the concentration of total short-chain fatty acids. At each level of feeding there were wide variations between animals in the mixture of short-chain fatty acids in the rumen fluid. At 33, 42, 55 and 67 g DM/kg $W^{0.73}$ per d, respectively, the ranges between animals in the molar percentages of acetic acid, propionic acid and butyric acid were: 53–63, 14–30 and 12–21; 41–62, 19–30 and 11–33; 46–56, 25–35 and 9–19; and 43–54, 20–32 and 10–27.

Rumen volume and outflow rate were determined with polyethylene glycol as marker. Although there was no correlation between the rumen volume, outflow rate or clearance rate and the level of feeding, there was a significant ($P < 0.01$) relationship between the molar percentage of propionic acid (y) in the mixture of rumen acids and the clearance rate of the rumen fluid phase (x, rumen vol./d). The regression $y = 36.78 - 7.23x$ was similar to that of $y = 37.27 - 8.05x$ reported by Hodgson & Thomas (1972) and confirms the importance of the clearance rate of the rumen fluid phase as a factor in the control of the fermentation in the rumen.

### REFERENCE

Hodgson, J. C. & Thomas, P. C. (1972). *Proc. Nutr. Soc.* 31, 57A.