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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Thirtieth Scientific Meeting of the Nutrition Society was held in the Surgery Lecture Theatre, Royal Infirmary, Glasgow, on Friday, 26 February, 1971, at 10.30 hours, when the following papers were read:

The induction of hypocalcaemia in ewes during pregnancy and parturition.

By N. S. RITCHIE (introduced by R. G. HEMINGWAY), *Department of Animal Husbandry, Glasgow University Veterinary School, Bearsden, Glasgow*

Hemingway & Ritchie (1965) discussed the significant role played by hypocalcaemia in the development of hypomagnesaemic tetany. To investigate this relationship, an experiment was carried out to attempt to induce hypocalcaemia and hypomagnesaemia simultaneously in lambed ewes.

Nineteen Cheviot ewes were fed indoors from 16 December to 7 May on a low-calcium diet of hay and concentrates supplying 1·1 g Ca and 0·9 g magnesium. A control group of eight ewes received the same diet plus a daily supplement of 3 g Ca as calcium carbonate. The ewes lambed over the period 17 March to 10 April with a lambing percentage of 155. On 7 May they were transferred to good pasture as the sole feed. Blood samples were taken from each ewe on twenty-two occasions and analysed for plasma Ca and Mg concentration. The mean results of these

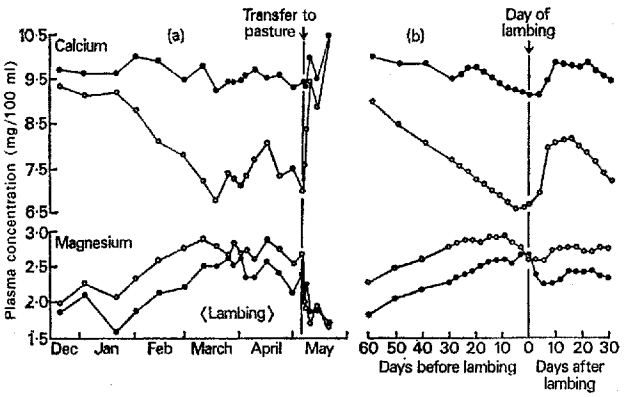

Fig. 1. Mean plasma calcium and magnesium concentrations of two groups of ewes on varying dietary Ca intakes. ○–○, nineteen unsupplemented ewes; ●–●, eight supplemented ewes; (a), on a calendar date basis; (b), rearranged in relation to lambing date.

analyses for the two groups are shown in Fig. 1(a). Hypocalcaemia was induced in the unsupplemented group. Before transference to pasture, the mean plasma Ca concentration was 7·02 mg/100 ml, but this was rapidly restored to a normal value of 9·5 mg/100 ml within 4 d of pasture grazing. Over the same 4 d period the plasma Mg concentration of this group fell from 2·6 mg/100 ml to 1·7 mg/100 ml. At no time,

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however, was there any severe degree of both hypocalcaemia and hypomagnesaemia simultaneously present. No hypomagnesaemic tetany occurred.

There were fluctuations in the mean plasma values around lambing. The individual results were therefore rearranged according to lambing date to give values, by interpolation where necessary, at 3 d intervals before and after lambing. Fig. 1(b) shows the mean results. There was an apparent rise in the plasma Ca concentration of both groups immediately after parturition, unassociated with the dietary supply. There was also an apparent inverse relationship with the plasma Mg concentration.

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Hemingway, R. G. & Ritchie, N. S. (1965). *Proc. Nutr. Soc.* **24**, 54.

Zinc in human health and disease. By E. CANNING and G. S. FELL (introduced by D. P. CUTHBERTSON), *Biochemistry Department, Royal Infirmary, Glasgow*

Levels of zinc in the plasma, red cells, tissue and urine of patients thought likely to have abnormalities of zinc metabolism have been obtained. A severe generalized illness is usually present before consistently low values (<80 μg zinc/100 ml) are found. The levels for series of patients with liver disease, malabsorption, various malignant tumours and other conditions are presented and compared to normal control subjects. This is contrasted with the largely normal values obtained for leg ulcer cases who nevertheless benefited from oral zinc therapy. From this we suggest that the action of oral zinc is pharmacodynamic and not necessarily related to a true zinc-deficiency state. We are measuring red cell and tissue zinc in patients to determine if a degree of zinc depletion does exist.

The changes in zinc metabolism following accidental and surgical trauma are considered and the potential losses of zinc in urine are discussed, with our attempts to relate this to calorie intake and nitrogen and potassium excretion.

An automatic method for sampling digestia in vivo. By R. A. EVANS, R. F. E. AXFORD and N. W. OFFER, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caernarvonshire*

The use of re-entrant cannulas for sampling flow of digesta through the alimentary canal has been reviewed (Ash, 1969). As a basis for judging the accuracy of the manual method of collection, indigestible markers such as chromic oxide and polyethylene glycol have been employed. The recovery of markers from duodenal re-entrant cannulas shows that manual collection depresses the flow of digesta, and workers in this field have had to employ the strategem of correcting observed flow for incomplete recovery. The extensive literature on the recovery of markers

indicates dissatisfaction with the situation, and Corse & Sutton (1970) have pointed out the need for caution in interpreting results from experiments in which markers have been used.

A machine is described which samples continuously and automatically the flow of digesta through the duodenum of a sheep, returning the bulk to the animal (Axford, Evans & Offer, 1971). It has been employed for periods as long as 4 months without interruption. The recovery of markers administered by mouth is quantitative, showing that the automatic method of collection does not depress flow. The method permits long-term studies of daily variations in flow-rate and passage of nutrients when the animal is on a constant ration (Table 1). The establishment of a steady state after an abrupt change in ration can also be monitored, which permits a more economic use of experimental resources.

Table 1. *Variations in flow rate and composition of the duodenal digesta in a Welsh wether fed a constant daily ration of 700 g milled hay containing 13·4 g nitrogen*

Day	Flow (l/d)	Dry matter (g/d)	Nitrogen (g/d)	Cr_2O_3 recovery (%)
1	8·46	389	17·8	98·5
2	6·64	378	14·9	99·0
3	7·42	408	16·3	101·0
4	6·41	359	12·5	102·5
5	6·67	405	14·3	111·0
6	6·01	388	13·2	111·0
7	5·93	360	12·7	100·0
8	5·96	392	12·2	99·0
9	6·28	383	12·9	96·5
10	6·21	407	13·0	101·0
11	6·15	385	12·6	96·0
12	6·52	414	12·7	126·5
Mean ± SE	6·55 ± 0·21	389 ± 5	13·8 ± 0·5	103·5 ± 2·5

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The effect of formaldehyde treatment of protein supplements upon their in vitro fermentation and utilization by sheep. By J. G. HUGHES and G. L. WILLIAMS, *Department of Agriculture, University College of North Wales, Bangor, Caernarvonshire*

An in vitro technique was adopted for evaluating the effectiveness of various formaldehyde treatments in preventing ruminal degradation of protein. Treatment of casein with 1% solution of formaldehyde gave optimum results in that the apparent solubility of casein on incubation with rumen liquor was reduced by HCHO treatment from 90·0% to 4·9%.

In a feeding trial using thirty-six 6-month-old Welsh Mountain wether lambs fed at three levels of energy intake, lambs receiving 50 g HCHO-treated casein as a supplement had significantly higher growth rates ($P<0.01$) at the lower levels of energy intake than those receiving 50 g untreated casein. Total wool production over the 300 d feeding period was also higher ($P<0.05$) in those lambs receiving treated casein. Differences in fleece weight in favour of the treated group were +67·6%, +75·0% and +20·7% at the three levels of energy intake. The response to HCHO-treated casein diminished with increasing energy intake. Wool production measured periodically on a defined area of skin was higher at all times in the treated group.

In order to select a suitable protein source for a further feeding trial, a number of conventional protein supplements were examined in treated and untreated form. Apparent digestibility in vitro and ammonia production on incubation with rumen liquor were measured. Of the protein meals examined, extracted groundnut meal showed the best potential. Formaldehyde treatment reduced its solubility in rumen liquor from 70% to 20% and in vitro ammonia production was only 10% of that from untreated meal.

The protection of dietary protein by formaldehyde treatment and its effect on the composition of duodenal digesta in 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, Caernarvonshire*

The effect upon growth rate and wool production of incorporating formaldehyde-treated casein in the diet of lambs has been described (Hughes & Williams, 1971). We have investigated its effect upon the rate of passage through the intestine of digesta, dry matter, total nitrogen, amino acids, ammonia and nucleic acids.

A sheep fitted with re-entrant cannulas in the proximal duodenum and restrained in a metabolic crate was connected to an automatic device for collecting continuous representative samples of duodenal digesta (Axford, Evans & Offer, 1971; Evans, Axford & Offer, 1971). Faeces were also collected for analysis. The sheep was fed

Table 1. *Effect upon flow pattern of digesta of formaldehyde treatment of casein incorporated in the diet of sheep*

Measurement	Casein	Formaldehyde-treated casein
Total N in ration (g/d)	24·74 ± 0·46	24·81 ± 0·45
Flow of digesta (l/d)	8·80 ± 0·45	8·39 ± 0·45
Dry matter of digesta (g/d)	453 ± 5	464 ±39
Total N in digesta (g/d)	19·55 ± 0·27	25·85 ± 1·73
Amino acid N in digesta (g/d)	10·40 ± 0·14	14·83 ± 0·33
Ammonia N in digesta (g/d)	2·33 ± 0·10	1·69 ± 0·27
Nucleic acid N in digesta (g/d)	8·25 ± 0·59	8·20 ± 2·04
Total N accounted for in digesta (g/d)	20·98 ± 0·62	24·72 ± 2·09
Total N in faeces (g/d)	5·71 ± 0·42	6·08 ± 0·42

a ration of meadow hay (500 g/d), barley meal (300 g/d) and casein (80 g/d) for a 10 d equilibration period and then for 12 d during which samples were taken. For the succeeding 12 d a similar ration of equal N content was fed in which the casein was replaced by casein which had been pre-treated with 1% formaldehyde solution. Formaldehyde treatment of the casein in the diet led to an increase in the total amino N, a decrease in the ammonia N, and no change in the nucleic acid N reaching the duodenum. The total faecal N was unchanged (Table 1). Alterations in the individual amino acids resulting from the change in diet will be discussed.

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The capacity for the removal of glucose from the small intestine by mature sheep. By E. R. ØRSKOV, R. W. MAYES and A. PENN, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

We have observed that when over 200 g glucose/d were infused via the abomasum in a lamb weighing 25 kg the concentration in the terminal ileum rose, indicating inefficient absorption. An experiment has now been conducted with two mature female sheep of 55 kg and 45 kg live weight. They received each day 900 g of a basal diet of cubed dried grass in two equal feeds at 08.00 and 20.00 hours. Each day, 2 l of a 0·9% NaCl solution was given as a continuous infusion via an abomasal cannula. Glucose was added to this solution, the amount given increased by 20 g/d. On alternate days samples of ileal fluid were obtained from an ileal cannula at 2 h intervals and faecal samples were also obtained. The samples were pooled daily and kept frozen until analysed for glucose by the glucose oxidase method. Chromium-EDTA, prepared by the method of Binnerts, van't Klooster & Frens (1968), was used as an indigestible marker.

Most of the increment infused in excess of 300 g/d (Fig. 1) passed the terminal ileum and when more than 400 g/d were infused glucose was excreted in the faeces, which became increasingly fluid and acid (pH$<$5), indicating a high concentration of acid fermentation products.

Indirect evidence for a low rate of absorption of glucose in ruminants was also provided by Reid (1952) when he compared rate of increase in blood glucose following abomasal administration in sheep with that of non-ruminants and it may well limit the extent to which postruminal digestion of α-linked glucose polymers may be encouraged with methods of bypassing the rumen (Ørskov, Benzie & Kay, 1970). The young lamb, however, seems capable of digesting large quantities of lactose given with milk and it is possible that an adaptation might occur if such feeding was continued into maturity.

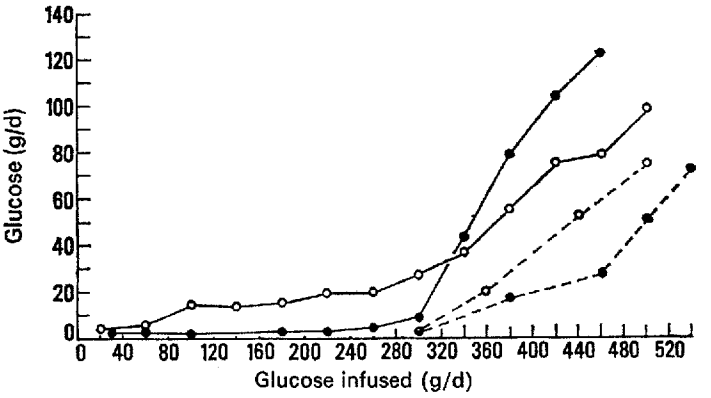

Fig. 1. Effect of glucose infusion via the abomasum on glucose passing the terminal ileum (—) and excreted in faeces (– – –) of sheep 1 (●) and sheep 2 (○).

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The inhibition by chloroform of methane production by rumen contents.

By C. G. HARFOOT and J. W. CZERKAWSKI, *Hannah Dairy Research Institute, Ayr*

Bauchop (1967) studied the effects of carbon tetrachloride, chloroform and methylene chloride on methane production and hydrogen uptake by rumen contents in vitro. This communication describes the effects of inhibiting methanogenesis on the total rumen fermentation in vitro and the extent of inhibition of methane in vivo.

The in vitro experiments were done using an artificial rumen as described by Czerkawski & Breckenridge (1969). The inoculum used in each vessel was 40% strained rumen contents from sheep. The substrates infused were 83 mg glucose/100 ml rumen contents per h and 42 mg sodium formate/100 ml rumen contents per h. Addition of 3·75 mg $CHCl_3$/100 ml rumen contents at the start and at 3 h after the start of incubation resulted in the complete inhibition of methanogenesis. With glucose the addition of $CHCl_3$ had little effect on the amount of substrate utilized, or on the production of lactic acid, or of total steam-volatile acids. It had little effect on the total gas produced or on the production of polysaccharides. However, with formate, although addition of $CHCl_3$ completely inhibited methane production, it also inhibited the utilization of formate, and the accumulation of hydrogen gas which took place was less than could be accounted for by the inhibition of methanogenesis and by the decreased utilization of formate. With glucose there was an increase in the production of propionic acid and an accumulation of

hydrogen gas, but these differences could account for only a small proportion of the hydrogen not used for methanogenesis.

The in vivo experiments were done using sheep with rumen cannulas given 500 g sugar-beet pulp at 09.00 hours. Immediately after the sheep were fed, 100 ml H_2O were injected into the rumen of one sheep. At the same time 100 ml H_2O containing 220 mg $CHCl_3$ were injected into the rumen of another sheep. Samples were removed from the rumen and their methanogenic activity was measured using glucose as substrate in the small-scale artificial rumen described by Czerkawski & Breckenridge (1970). No methane was produced in samples taken 30 min and 1 h after addition of $CHCl_3$; the methanogenic activity recovered partially in 24 h and recovery appeared to be complete in about 4 d. There was still some instability after recovery as shown by a decrease in methane production with samples of rumen contents taken soon after feeding. This was accompanied by an increased accumulation of hydrogen.

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The effect of group–feeding on the food requirements of Blackface ewes.

By JANET Z. FOOT and A. J. F. RUSSEL, *Hill Farming Research Organisation, 29 Lauder Road, Edinburgh EH9 2JQ*

Blackface sheep have distinct territorial patterns of behaviour when under hill-grazing conditions, consequently individuals of this breed are likely to take longer to become accustomed to group-feeding than are sheep of other breeds. The present experiment was designed to see if 6- to 7-year-old Blackface ewes brought off hill-grazing utilized the food on offer as efficiently when they were fed as a group as they did when fed individually. In addition, it was hoped to observe if being kept in a group, while still fed individually, increased the nutritional requirement of the individual.

There were six groups of twelve ewes on three treatments: (1) individually penned and individually fed, (2) group-penned and individually fed, (3) group-penned and group fed.

The individually fed animals in groups 1 and 2 received a range of food levels which it was intended would span the range of intakes by individual animals fed as a group (3). The main intake for the animals in each group was the same for the three management groups at each of two levels of feeding: 22 g/kg initial live weight (H) and 11 g/kg (L). Food intake of the individual animals in groups 3H and 3L were estimated every other week over a 7-week period from the total faecal output of these sheep plus estimates of apparent digestibility from certain of the individually fed animals which were also harnessed for faecal collection. The

sheep were weighed twice weekly before feeding. The daily weight change over the 7-week period was calculated and related to food intake within each group.

The food used in this experiment was a barley-based pellet containing 30% straw. This diet could be consumed rapidly; it was cleared within 0·5 h by the ewes in group 3H, and in half that time by those in group 3L. The calculated intakes ranged from 5·5 to 17·5 g/kg in group 3L (mean 11·5) and from 12·5 to 31·0 g/kg in group 3H (mean 22·1). These ranges were greater than those allocated within groups to the individually fed sheep, which were 7–15 g/kg and 18–26 g/kg respectively for the L and H treatments.

When food intake at zero weight change was estimated for the three groups it was found to be the same for the individually fed animals (19 g/kg), whether they were individually penned or group-penned. However, in the group-fed sheep food intake at zero weight change did appear to be higher (23 g/kg). This suggested that competition at feeding time resulted in a nutritional penalty to group-fed sheep.

The effect of infusing propane-1:2-diol into the rumen on the metabolism of sheep. By J. L. CLAPPERTON, KATHLEEN GRAHAM and J. W. CZERKAWSKI, *Hannah Dairy Research Institute, Ayr*

Czerkawski (1970) showed that, when propane-1:2-diol is added to a mixture of rumen liquor and phosphate buffer in an 'artificial rumen', there was partial inhibition of methane production and fermentation of the propane-diol. Experiments have been made in which 100 g propane-1:2-diol dissolved in water were infused into the rumen of wether sheep through a cannula while the animals were eating their daily ration of 500 g hay and 500 g long dried grass. This food was offered to the animals in two equal meals at 10.00 and 22.00 hours. Two experiments were made when the sheep were confined in a respiration chamber, and measurements of oxygen consumption and of carbon dioxide and methane production were made. Two experiments were also carried out with the sheep in a metabolism cage. In these experiments, polyethylene glycol (PEG) was added to the rumen contents as a soluble marker and samples of rumen liquor were taken at various times after feeding for the determination of pH, total and individual volatile fatty acids, lactic acid, PEG and propane-diol.

The results showed that the infusion of 100 g propane-1:2-diol daily increased the oxygen consumption of the sheep by 14% and the carbon dioxide production by 9%, and reduced the methane production by 9%. These changes were all statistically significant ($P<0·01$). When propane-diol was infused into the rumen, its concentration in the rumen liquor 30 min after feeding had risen to 16 g/l; it fell to less than 1 g/l within the following 3 h, the rate of fall of propane-diol concentration being much faster than the corresponding fall in PEG concentration. On average, there was a small reduction in the pH of the rumen liquor and no change in the total volatile fatty acid concentration. The proportion of acetic acid fell from 72% to

64% whereas that of the propionic acid increased from 20% to 27%; there was no change in the proportion of butyric acid. At the same time, the concentration of lactic acid in the rumen liquor was approximately 1 g/l 30 min after feeding; it then fell slowly until within 7 h the normal low concentration of lactic acid was attained.

The results indicate that part of the propane-1:2-diol was partly metabolized within the rumen and that part was absorbed unchanged.

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Factors limiting the utilization of glucose for milk fat synthesis in the ruminant. By J. M. CHESWORTH* and G. H. SMITH (introduced by J. A. F. ROOK), *Department of Agricultural Sciences, The University, Leeds LS2 9JT*

As compared with acetate, glucose is not appreciably used as a source of carbon for fatty acid synthesis in the ruminant mammary gland (see Hardwick, Linzell & Mepham, 1963). This has been attributed (Hardwick, 1966) to the relatively low activity of ATP citrate lyase in the ruminant mammary gland, and the consequent restriction upon the passage of acetyl CoA units formed from glucose out from mitochondria to the site of fatty acid synthesis.

We have reinvestigated the role of ATP citrate lyase as a factor restricting glucose metabolism. We have found levels of ATP citrate lyase in ruminant (cow) mammary gland which, although lower than those in the non-ruminant, are somewhat higher than those reported by Hardwick (1966). However, we have noted that the enzyme in crude preparations from the bovine mammary gland is inhibited by acetate and acetyl CoA, behaviour not observed in tissue from the non-ruminant (rat). Factors other than the amount of enzyme may therefore modulate its activity in vivo.

Other observations suggest restrictions on the pathway at an earlier stage. We have compared the ability of pyruvate and acetate to contribute to short-chain fatty acid synthesis in isolated mitochondria from the bovine mammary gland, by the pathway described by Smith & McCarthy (1969). Although it is uncertain whether this pathway operates in vivo, its occurrence in vitro provides a measure of the ability of the substrate to contribute to intramitochondrial acetyl coA. Similarly, the operation of the 'elongation' pathway (Wakil, McLain & Warshaw, 1960) may be so used in the non-ruminant. In mitochondria from the ruminant (cow), acetate was incorporated ten times as readily as was pyruvate, and in the non-ruminant (guinea-pig), although the total extent of incorporation was lower, pyruvate was incorporated five times as readily as was acetate.

Citrate from the milk of goats produced following the separate infusion of tracer quantities of [U-^{14}C]glucose and [1-^{14}C]acetate was isolated and cleaved into

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its acetyl- and oxaloacetate portions using a preparation of citrate lyase. Following acetate infusion, the specific activity of the oxaloacetate part was only slightly less than that of the acetyl part, indicating a limited entry of unlabelled materials into the tricarboxylic acid cycle. On the other hand, after the infusion of [U-^{14}C]glucose the specific activity of the acetyl unit was one-eighth that of the oxaloacetate unit. Taken together, these results indicate that glucose makes only a limited contribution to acetyl CoA, though it may enter the tricarboxylic acid cycle as oxaloacetate.

These two types of observation both suggest that in the ruminant mammary gland the oxidation of glucose to acetyl CoA, and therefore its contribution to ATP synthesis, may be limited. Pyruvate enters the tricarboxylic acid cycle preferentially through conversion into oxaloacetate (possibly through the action of acetyl CoA upon pyruvate dehydrogenase and pyruvate carboxylase). This, rather than net oxidation of glucose, may account for most of that fraction of the carbon dioxide produced from glucose in the gland which remains after the contribution of the pentose cycle has been subtracted.

One of us (J.M.C.) was in receipt of a Ministry of Agriculture, Fisheries and Food postgraduate scholarship.

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Triglyceride fatty acids of lambs reared on a lipid-free diet By W. R. H. DUNCAN and D. A. GARTON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and G. MATRONE, *Biochemistry Department, North Carolina State University, Raleigh, North Carolina 27607, USA*

The relatively high proportions of stearic acid and *trans*-octadecenoic acid which characterize the internal depot triglycerides of sheep are associated with the absorption and apparently selective incorporation of these acids which are produced in the rumen by microbial hydrogenation of dietary C_{18} unsaturated acids (see Duncan & Garton, 1967; Garton & Duncan, 1969).

To determine the proportion of stearic acid in triglycerides which results from endogenous synthesis — and thus to get an indication of the increment normally occasioned by stearic acid of exogenous origin — analyses were made of triglycerides of four lambs which received no dietary lipid (other than in colostrum) until they were killed at 6 months of age. After being suckled for a few days the lambs were bottle-fed on reconstituted non-fat milk solids (Carnation) and then weaned on to a semi-synthetic diet which comprised, in parts by weight: glucose, 30·6; starch, 30·6; soya-bean protein, 13·0; cellulose, 3·0; $NaHCO_3$, 6·0; $KHCO_3$, 4·0; $CaHPO_4$, 1·8; $CaCO_3$, 1·0; vitamin mix, 5·0; mineral mix, 3·0 and, as binding agent, glycerol,

2·0. At slaughter, samples of perinephric and subcutaneous (rump) adipose tissue were taken for extraction and analysis of their constituent triglycerides according to Duncan & Garton (1967). The results with respect to the three major fatty acids are given in Table 1, together with the composition of triglycerides from corresponding tissues of lambs which had been fed on grass cubes.

Table 1. *Major component fatty acids of adipose tissue trigycerides of lambs fed on a lipid-free diet or on grass cubes.*

(Values, to the nearest whole number, as mol/100 mol total fatty acids)

	Perinephric triglycerides			Subcutaneous triglycerides		
Lambs fed on lipid-free diet	16:0	18:0	18:1	16:0	18:0	18:1
No. 1	20	23	44	24	11	46
No. 2	23	22	41	26	7	46
No. 3	22	24	44	22	13	54
No. 4	24	27	39	28	11	45
Mean value	23	24	42	25	11	48
Lambs fed on grass cubes (Means for three animals)*	20	34	31	22	12	46‡

*From Garton & Duncan (1969). †Includes 12 mol/100 mol *trans* 18:1. ‡Includes 7 mol/100 mol *trans* 18:1.

Whereas the subcutaneous triglycerides contained similar proportions of stearic acid whether or not the diet contained lipid, the perinephric triglycerides were notably different. In the absence of dietary lipid, stearic acid accounted for about 10% less of the total fatty acids of the perinephric triglycerides than was present when the diet consisted of grass cubes which contain C_{18} unsaturated acids (Garton, 1960) available for hydrogenation in the rumen. Nevertheless, even when given a lipid-free diet, the lamb produces perinephric triglycerides having a higher content of stearic acid than is found in the perinephric triglycerides of non-ruminant herbivores and this may reflect differences in stearoyl desaturase activity.

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The distribution of free amino acids between plasma and blood cells of chicks fasted or fed a protein-free diet. By A. G. STEPHENS and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caernarvonshire*

The definition of the requirements of an animal for amino acids and the quantitative assessment of the quality of dietary protein are current problems in nutrition.

Their investigation has involved, *inter alia*, the determination of the pool size of free amino acids in tissues, such as liver and muscle, and in particular of the concentration of free amino acids in blood plasma (Dean & Scott, 1966; Zimmerman & Scott, 1967*a*,*b*; McLaughlan & Illman, 1967). The blood cells constitute 30–40% of the total blood volume and are suspended in the plasma, but their significance in the transport and storage of amino acids has not received attention. We have therefore studied the free amino acid levels in plasma and blood cells of groups of 4-week-old chicks on a variety of diets. We here report the effect of a 24 h fast following on *ad lib.* consumption of a diet containing 20% casein and of *ad lib.* consumption of a protein-free diet (Table 1). Blood was taken by heart puncture,

Table 1. *Free amino acids in plasma and blood cells of 4-week-old chicks (mg/100 ml)*

	20% casein diet *ad lib.*		24 h fast following 20% casein diet		Protein-free diet *ad lib.*	
Amino acid	Plasma	Cells	Plasma	Cells	Plasma	Cells
Asp	4·6	14·1	3·2	5·7	2·9	8·2
Thr	26·1	16·3	14·7	7·5	7·7	9·4
Ser	9·7	20·9	8·3	14·2	10·4	15·2
Glu	9·4	60·1	7·6	48·8	6·3	43·6
Gly	4·6	37·2	4·9	27·7	4·8	22·5
Ala	5·9	32·8	4·8	23·1	5·0	15·8
Cys	2·6	0·3	4·3	0·9	1·4	0·5
Val	4·5	8·0	4·2	5·7	2·5	4·7
Met	1·4	1·8	1·2	1·9	0·8	3·2
Ileu	2·3	2·4	3·1	1·8	1·8	2·8
Leu	3·1	6·7	3·8	4·7	2·5	5·0
Tyr	5·9	6·8	2·9	3·5	3·2	6·7
Phe	2·2	3·4	2·0	1·6	1·7	2·7
His	3·8	20·9	3·9	20·1	3·5	20·2
Trn	1·3	0·0	1·1	0·0	1·3	0·4
Orn	1·0	0·6	1·4	0·4	0·9	0·9
Lys	20·5	52·9	22·3	38·6	19·0	23·3
Arg	2·2	53·0	3·2	30·0	4·8	28·6

and the hematocrit was measured. The free amino acids were determined in plasma (Thomas, Evans, Robins & Siriwardene, 1967) and lysed whole blood after deproteinization with sulphosalicylic acid and hydrolysis of amides to the free acids. The free amino acids in blood cells were calculated by difference. The blood cells clearly carry the major part of the free amino acid burden and this is subject to depletion when the chick is deprived of dietary protein. The pattern of depletion is not the same for plasma as for blood cells.

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Determination of the composition of mammary cells in the sheep. By A. KONAR, P. C. THOMAS and K. G. TOWERS, *Department of Agricultural Sciences, The University, Leeds LS2 9JT*

Four lactating ewes were fitted with indwelling polyethylthene cannulas in their jugular veins. Each ewe was separated from its lambs and the mammary gland was milked out completely after an intravenous injection of 0·5 i.u. oxytocin (Syntocin). Two hundred and fifty ml of isotonic sodium thiocyanate solution were administered intravenously and allowed to diffuse into body tissues to act as a marker substance for extracellular fluid; 0·5 g of polyethylene glycol (PEG) in 5 ml of the ewe's own milk was injected through a fine polyethylthene cannula into each half of the mammary gland to act as a marker substance for milk. Five hours after the thiocyanate treatment, blood and milk samples were taken for analysis, the sheep was killed and the mammary gland immediately excised. Each half of the gland was homogenized and analysed for chemical constituents, including thiocyanate and PEG. Using these results, and assuming that the composition of extracellular fluid was similar to that of blood plasma and that milk held within the gland was similar to that obtained on milking, the composition of cell contents was determined.

Mean values (with standard errors) for the contents of lactose (g/100 g cell water), potassium, sodium and chloride (mg/100 g cell water) in the cells of eight gland units were: $4{\cdot}82 \pm 0{\cdot}32$; $654{\cdot}6 \pm 32{\cdot}1$; $109{\cdot}1 \pm 5{\cdot}4$; $41{\cdot}1 \pm 5{\cdot}8$. With the exception of one gland unit, the osmotic activity, calculated on the basis of lactose and free ions, suggested that the intracellular fluid was either hypertonic or contained water-soluble constituents which were not in solution. Pooled results from the four experiments showed a close direct relationship ($r = 0{\cdot}96$) between the intracellular contents of lactose (L, g/100 g cell water) and potassium (K, mg/100 g cell water) such that $L = 0{\cdot}0123K - 3{\cdot}46$.

To account for the interrelationships between the lactose and potassium contents in cow's milk, Rook & Wood (1958) postulated that lactose was synthesized within the alveolar cell and expelled into the lumen together with a potassium-rich secretion. More recently, Brew (1969) has envisaged lactose to be synthesized in the Golgi region of the cell and transported to the lumen in lactose 'particles' formed by pinched-off portions of Golgi membrane. The present results are consistent with certain features of both these hypotheses since significant concentrations of lactose were present within the mammary cells and lactose and potassium contents in the cell were closely related. The basis of this relationship is uncertain but may reflect an association of lactose and potassium within the Golgi vesicles.

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The value of oats in diets for laying hens. By J. DAVIDSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Of the cereals available for feeding, oats is the one with the lowest energy concentration, and probably the best balance of amino acids (Davidson, Mathieson & Williams, 1962). Because of this low energy content it is not used to any great extent in broiler production but it should still be suitable for egg production. To prepare a diet having a given protein to energy ratio a smaller amount of protein concentrate has to be added to oats than to other higher-energy cereals. One might expect hens to eat more of such a ration to allow for the lower energy content (Bolton, 1958, 1959), but this apparently depends on the strain of bird used (Morris, 1968). Compensation for a low-energy ration is not necessarily essential. Provided the amino acid intake is adequate, alteration in energy intake may only affect fat deposition.

An experiment was carried out with groups of sixty-three Thornber 606 hybrids in their 1st year of lay, fed diets (Table 1) which were mainly oats supplemented with either 6 or 8 % white fish meal or a mixture of oats, wheat and barley with 10% fish meal. These three diets contained 12·6, 13·8 and 14·7% crude protein with predicted metabolizable energy (ME) concentrations of 2·28, 2·29 and 2·46 Mcal/kg respectively. A commercial diet having 17·6% crude protein and unknown ME value was also given.

Over the 32-week period there was no significant difference in food intake or egg production although the oats diet with 6% fish meal did give rise to significantly larger eggs. The results indicated that diets based mainly on ground oats, containing about 13% crude protein, could support as good egg production as diets based on a mixture of higher-energy cereals containing more protein, even when the birds

Table 1. *Experimental laying diets*

	High oats, low fish meal	High oats, medium fish meal	Mixed cereal, fish meal
Barley, ground	5	5	23·5
Oats, Sussex ground	73	71	30
Wheat, ground	5	5	24
White fish meal	6	8	10
Grass meal	4·5	4·5	5
Ground limestone	4·5	4·5	6
Steamed bone flour	0·8	0·8	–
Salt (containing 2·5% $MnSO_4 \cdot 4\ H_2O$)	0·5	0·5	0·5
Adisco + riboflavin*	0·7	0·7	1
	100·0	100·0	100·0

* Proprietary mixture containing 1000 i.u. vitamin A, 250 i.u. cholecalciferol and 500 μg riboflavin per g (Isaac-Spencer & Co. Ltd, Aberdeen).

did not increase food consumption to allow for decreased dietary energy concentration. This could be borne in mind when formulating diets for layers, if the price of oats is advantageous.

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Invertase activity in the rumen contents of sheep given molassed sugar-beet pulp. By J. W. CZERKAWSKI and J. LUMSDEN, *Hannah Dairy Research Institute, Ayr*

The observation that both sucrose and its constituent monosaccharides were fermented equally efficiently in the artificial rumen (Czerkawski & Breckenridge, 1969) suggested that there was considerable invertase activity in the rumen contents. Such activity has been demonstrated in rumen protozoa and bacteria (Christie & Porteous, 1957; Howard, 1959; Thomas, 1960), and the object of this work was to investigate the distribution of invertase activity in various fractions of rumen contents of sheep given sugar-beet pulp and its variation with respect to feeding. Optimum pH values for invertase activity occured between pH 4·5 to 7·5. Both the cell-free extracts and unfractionated rumen contents obeyed the Michaelis–Menten equation; with the rumen contents of sheep given sugar-beet pulp at pH 5·8 the Michaelis constant was 10·5 mM and the maximum production of reducing sugar was 3·1 μmol/ml.h.10 μl rumen contents.

The distribution of invertase activity in various fractions depended on the pH of the mixture in which it was determined and the time of sampling before and after feeding. The protozoal fractions contained 60–80% and the bacterial fractions contained 10–25% of the total invertase activity, but the cell-free rumen fluid contained only 3–10% of the total. At pH 5·8 the activity of the protozoal fractions (expressed as units/mg protein nitrogen) was four to six times greater than the activity of the bacterial fractions. The activity of the protozoal fractions was greater in samples taken from the rumen of sheep given sugar-beet pulp than in samples from sheep given other rations, such as hay or dried grass. There was considerable variation in the enzyme activity with respect to the time of feeding. With sugar-beet pulp rations the ratios of activities determined at pH 5·8 and 7·0 showed maxima 1–3 h after feeding.

Dilution of the rumen contents resulted in a proportional decrease in the invertase activity and a disproportionately large decrease in fermentative activity. Calculations showed that in the rumen of the sheep given molassed sugar-beet pulp the rate of sucrose hydrolysis would be marginally greater than the rate of utilization of the resulting reducing sugars. In the artificial rumen the rate of hydrolysis of sucrose was several times greater than the rate of utilization of the resulting reducing sugars. Thus, in the artificial rumen the invertase activity is not likely to be rate-limiting.

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Total body water of rats as measured with different amounts of injected tritiated water. By A. J. GORDON, J. H. TOPPS and T. W. BEGG, *School of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD*

The use of tritiated water (TOH) to measure the total body water of animals frequently gives an overestimate when compared with the value obtained by desiccation. Kay (1963) has suggested that the amount of radioactivity injected per unit body-weight may influence the magnitude of this discrepancy. He found that with pigs weighing 27, 55 or 90 kg, each of which was given the same dose of TOH, the estimate of total body water was far more precise with the smallest than with either of the two heavier groups of animals.

Forty male rats, aged 7 weeks, which had been used in two experiments to measure protein quality and which weighed between 70 and 190 g, were injected with amounts of TOH ranging from 0·2 to 1·5 μCi/g $W^{0.75}$. After an equilibrating time of 3 h the rats were anaesthetized and blood was withdrawn by heart puncture before they were killed. Water was removed from the blood by vacuum sublimation, its radioactivity measured and the TOH space calculated. Total body water of the slaughtered rats was obtained by drying to constant weight in a microwave oven.

The results showing the differences between TOH space and total body water are given in Table 1.

Table 1. *Difference between tritiated water space (TOHS) and total body water (TBW) of rats injected with different amounts of radioactivity*

Expt	No. of rats	Radioactivity injected (μCi/g$W^{0.75}$)	$\frac{\text{TOHS-TBW}}{\text{TBW}} \times 100$
1	6	0·2	6·8
	6	0·5	11·1
	6	0·8	10·3
	6	1·0	8·6
	4	1·5	10·5
2	3	0·2	6·2
	2	0·7	10·3
	4	1·0	10·5
	3	1·5	12·7
Total	40		9·7±0·65

Contrary to the suggestion of Kay (1963) the amount of radioactivity used had no significant effect on the magnitude of the difference between TOH space and total

body water. Although TOH space was highly correlated with and gave a reliable prediction of total body water, the use of body-weight was found to be a more accurate indicator of both total body water and the percentage of body fat.

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Isobutylidene diurea: a slow-release non-protein nitrogen source for ruminants. By J. J. PARKINS, N. S. RITCHIE and R. G. HEMINGWAY, *Glasgow University Veterinary School, Bearsden, Glasgow*

Isobutylidene diurea (IBDU) $(CH_3)_2$ CH. CH. $(NHCONH_2)_2$ is a non-hygroscopic, sparingly water-soluble material containing 32% nitrogen. Its principal agricultural use is as a slow-acting nitrogen fertilizer for rice. Its physical properties, reactions in the soil and the effects on plant growth have been described by Hamamoto (1966).

In a preliminary experiment isonitrogenous amounts of IBDU and urea admixed with maize meal were given separately by rumen fistula to a cow which had been deprived of food overnight. Urea (0·22 g/kg live weight) produced the normally expected increases in blood and rumen ammonia concentrations (Table 1). In contrast, IBDU effected little elevation in ammonia concentrations.

Table 1. *Concentrations of ammonia in the blood (μg/100 ml) and rumen liquor (mg/100 ml) resulting from the administration (per rumen fistula) of IBDU and urea admixed with maize meal*

Time after administration (h)	IBDU		UREA	
	Blood	Rumen	Blood	Rumen
0	nd	0·0	nd	2·5
0·5	nd	15·0	82·5	38·0
1	nd	17·5	100·0	80·0
1·5	nd	15·0	107·0	105·0
2	nd	12·5	80·0	85·0
3	nd	10·0	56·0	45·0
7	nd	8·7	nd	20·0

nd, not detected.

In vitro studies with rumen liquor indicated that only 10% of the total nitrogen available from IBDU was released over a 3 h period at pH 6 (37°). Hydrolysis of IBDU is enhanced as the pH is decreased from neutrality to pH 2. The hydrolysis product (2 methyl propanal) was detected (gas–liquid chromatography) in vitro but not in vivo in rumen liquor.

The apparent digestibility of IBDU was determined by differences in adult sheep fed a barley diet ± IBDU supplying 3% crude protein (Table 2). There was an immediate increase in urinary nitrogen output. The apparent digestibility of the

nitrogen in IBDU was 64·6% 2 weeks after feeding commenced. IBDU was fully digested after about 1 month of continuous feeding. The apparent digestibility of the crude fibre showed a parallel increase with time.

Table 2. *Apparent digestibility (%) of IBDU and crude fibre by adult sheep fed a barley diet*

	IBDU	Crude fibre
No IBDU	0	28·1
After 1–2 weeks feeding	64·6	29·3
After 2–3 weeks feeding	94·0	35·2
After 5–6 weeks feeding	102·0	33·0

A single dose of 100 g IBDU (≡70 g urea) administered as a drench to a 25 kg sheep produced no signs of toxicity in circumstances where about 15 g urea would normally be fatal.

It is possible that some of the dietary IBDU remains unhydrolysed until it reaches the abomasum.

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Isobutylidene diurea, soya-bean meal and urea as dietary nitrogen supplements for growing lambs. By J. J. PARKINS, N. S. RITCHIE and R. G. HEMINGWAY, *Glasgow University Veterinary School, Bearsden, Glasgow*

A previous communication (Parkins, Ritchie & Hemingway, 1971) has described the manner of the slow release of nitrogen from isobutylidene diurea (IBDU) when fed to ruminants.

Four groups, each of seven Greyface lambs (mean live weight, 16·9 kg) were offered *ad lib.* one of four pelleted diets. The control diet (11·5% crude protein) was composed of 70% barley, 10% maize, 10% wheatings, 5% locust beans and 3% soya-bean meal together with adequate minerals and vitamins. The other three diets each contained 14·5% crude protein, nitrogen supplementation being provided by appropriate amounts of either IBDU, soya or urea incorporated in place of some of the barley. The lambs were weighed each week over the trial period of 70 d.

The IBDU- and soya-supplemented lambs grew steadily over the whole period and made significantly better total mean live-weight gains with superior food conversion ratios than either the control or urea-supplemented lambs (Table 1). The lambs given urea grew rather better than the control lambs during the first 35 d but at a significantly lower rate (and with a much inferior food conversion ratio) than all three other groups of lambs between 36 and 70 d. Over the whole 70 d period there was no response to urea but significant responses to both IBDU and soya.

Table 1. *Mean live-weight gains (kg), food conversion ratios (kg diet/kg live-weight gain) and plasma urea concentrations (mg/100 ml) of groups of seven Greyface lambs*

	Days	Control	Urea	Soya	IBDU	SE of mean
Live-weight gain	0–35	7·32	9·14	10·38**	10·38**	0·69
	36–70	10·76	8·82*	11·22	10·57	0·54
Total	0–70	18·08	17·96	21·60*	24·40*	1·01
Food conversion ratio	0–35	4·20	3·88	3·26	3·17	–
	36–70	4·45	5·42	4·69	5·06	–
Total	0–70	4·35	4·63	4·01	4·09	–
Plasma urea concentration	35	19·0	33·4**	28·6*	30·0*	3·05
	46	15·9	32·9***	33·9***	34·1***	2·05
	61	21·6	36·1**	49·7***	42·4***	3·04
	70	35·1	51·3**	56·9***	48·6*	3·98

Significance of difference from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Plasma urea concentrations were determined at intervals throughout the 70 d period. Those lambs fed the control diet had significantly lower plasma urea concentrations than those for all three supplemented groups.

It is concluded that IBDU is a useful source of non-protein nitrogen which may be superior to urea.

We would like to thank Mitsubishi Chemical Industries Ltd for the provision of IBDU for the experiments of this and the preceding communication.

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Protein metabolism and phenylacetic acid excretion in the urine of sheep.

By A. K. MARTIN, *Hannah Dairy Research Institute, Ayr*

Experiments in this laboratory have shown that sheep may excrete between 0·02 and 7·50 g phenylacetic acid in 24 h.

When two sheep were given hay the average phenylacetate excretion in the urine was 0·18 g/24 h and when two others were given dried grass 0·38 g were excreted per 24 h. To measure the contribution of body tissue metabolism to phenylacetic acid excretion these sheep were fasted for 10 d. Excretion of this acid by all the sheep increased during the initial days of starvation to a maximum on the 3rd or 4th days of 0·96 g for sheep which had received hay and 1·28 g for those which had been given dried grass. The stimulation of phenylacetic acid production by fasting suggests that when sheep are fed only a small proportion of the phenylacetylglycine in their urine is derived from body tissue metabolism.

Phenylalanine may be metabolized by the rumen microflora to phenylacetic acid (Scott, Ward & Dawson, 1964). To study this metabolism in the present experiments

casein was infused into the rumen or abomasum of sheep maintained on a diet of hay or of 20% hay–80% maize. Increments in phenylacetate excretion over the amounts excreted in control periods were found when casein (7, 14, 21, or 28 g nitrogen/24 h for 7 d periods) was infused into the rumen. A significant linear relationship was observed between increments of phenylacetic acid and the amount of casein infused ($0{\cdot}001 < P < 0{\cdot}01$) indicating an average of 246 ± 22 mg phenylacetic acid/g casein N infused. Only small increments (20 ± 15 mg phenylacetic acid/g casein N) were obtained following casein infusion into the abomasum.

Casein is more completely degraded in the rumen than most proteins so experiments were made with proteins that are subject to varying rates of fermentation. Two energy-rich foods, sugar-beet pulp or rolled barley, provided 70% of the N intake (21 g N/24 h) of four sheep given hay–concentrate diets. On average, 15 mg phenylacetic acid/g N intake were excreted in the urine when sugar-beet pulp was given and 30 mg/g N when barley was given. When protein concentrates, field beans or extracted linseed cake, replaced the energy-rich foods 102 and 105 mg phenylacetic acid/g N intake were excreted respectively. Re-utilization of the products of protein degradation may be more extensive in energy-rich rations; it has been shown (Allison, 1965) that phenylacetate may be utilized by rumen micro-organisms to synthesize phenylalanine. The amounts of phenylacetic acid excreted by sheep may then be largely dependent on the extent of protein degradation and protein synthesis occurring in the rumen.

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Redistribution of calcium in sheep induced by stress. By G. MOSELEY and R. F. E. AXFORD (introduced by R. A. EVANS), *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caernarvonshire*

A variety of minor surgical interferences caused consistent reductions in the plasma calcium concentrations of Welsh Mountain ewes and wethers in seventeen experiments. A similar effect was observed in a series of animals treated with a solution containing 0·3 mg adrenaline hydrochloride intravenously. The reductions developed rapidly, accounting for up to 10% of the initial concentration within 40–60 min.

In the same animals, plasma non-esterified fatty acids (NEFA) rose to a maximum after 5–15 min and remained elevated for 1 h at a lower level.

The fall in plasma calcium varied with the rise in NEFA for the surgically stressed sheep ($r = 0{\cdot}933$) and for the adrenaline-treated sheep ($r = 0{\cdot}953$).

Subcutaneous fat biopsy samples were taken from some of the sheep. These showed increases of calcium content which correlated with the fall in plasma calcium ($r = 0{\cdot}838$).

Omental fat samples were taken from two unstressed wethers immediately after they had been shot and found to contain 9·1 ±0·3 μg calcium/g wet weight. In one of the two, after administration of 0·3 mg adrenaline hydrochloride intravenously before blood circulation ceased, the calcium content of the omental fat rose to 56·4 ± 4·4 μg/g.

In vitro investigations showed that sheep omental fat, incubated in serum, accumulated calcium in response to adrenaline stimulation.

It is suggested that stressful conditions can cause movement of significant amounts of calcium into the fat depots of sheep, which may have implications in the development of hypocalcaemic disorders.

The nutritional evaluation of single-cell proteins. 1. By A. A. WOODHAM and P. S. DEANS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Although protein concentrates produced from yeasts grown on hydrocarbon oil have been available now for some time, much of the published work has resulted from testing sponsored by the producers (Shacklady, 1967; Evans, 1968; Ko & Yu, 1968; de Groot, Til & Feron, 1970*a*, *b*). Experiments using pigs and poultry have led to the conclusion that the hydrocarbon-grown yeasts constitute satisfactory supplements for cereal-based diets and that the only important nutritional deficiency is likely to be methionine.

Amino acid analyses done at this Institute on both yeasts and bacterial protein products have confirmed the methionine deficiency, and also suggested that some other of the essential amino acids might be on the borderline of adequacy when these materials are called upon to form the sole additional protein source in cereal-based diets for chicks.

Evaluations of six samples using the total protein efficiency (TPE) chick growth method (Woodham, 1968) revealed significant differences which were still detectable even after methionine addition (Table 1). Supplementation of a yeast-protein sample with leucine and glycine in addition to methionine gave no better growth than methionine alone, whereas supplementary lysine had a deleterious effect either

Table 1. *Total protein efficiency (TPE) of various samples of unicellular protein with and without additional amino acids. Some values for field beans, fish meal and groundnut meal are included for comparison*

	Sample no. and type								
	2 Yeast	3 Yeast	4 Yeast	5 Bacterial	6 Yeast	7 Bacterial	Beans	Fish meal	Groundnut
Unsupplemented	2·34	2·37	2·18	1·85	2·00	2·06	1·90	3·20	2·21
+0·5% Met	2·42	2·50	2·27	2·00	2·17	2·30	3·06	–	–
+0·5% Met and 0·5% Lys	2·03	2·06	–	1·95	–		–	–	–
+0·5% Lys	2·22	–	–	–	–	–	–	–	–
+0·5% Met and 0·25% Leu and 0·25% Gly	2·41	–	–	–	–	–	–	–	–

alone or with methionine. For a bacterial protein sample in which a low nutritive value was combined with low available lysine (Palmer & Smith, 1971) suggesting that processing damage had occurred, the addition of lysine had no growth-depressing effect.

The magnitude of the response to methionine is disappointing. Similar supplementation of methionine-deficient plant protein sources such as field beans has produced dramatic improvements in chick feeding trials. For example the TPE of a sample of Throws MS winter beans has been increased from 1·90 to 3·06 by the addition of 0·5% methionine. The explanation for the comparatively small improvement by similar treatment of hydrocarbon-grown yeast requires elucidation.

The amino acid supplementation experiments have also emphasized the unsatisfactory nature of the figures currently available for the chick's requirements for essential amino acids. In particular, these and other experiments have suggested that published requirements for glycine and leucine are too generous.

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The nutritional evaluation of single-cell proteins. 2. By R. PALMER and R. H. SMITH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Six single-cell protein preparations (four yeasts and two bacterial) were fed to rats as sole sources of protein using the procedure of Miller & Bender (1955). The net protein utilization (NPU), true digestibility (TD) and biological value (BV) of the samples were determined. The NPU values given in the table are the means of four or five estimations. Amino acid analyses were carried out at this Institute on all the samples; available methionine was determined by microbiological assay with *Streptococcus zymogenes* (Ford, 1962). Thus, except for their low total sulphur amino acid content, it was predicted that all six preparations should meet the essential amino acid requirements of the rat. In agreement with this prediction the NPUs of all six preparations were increased on methionine supplementation. At higher levels of supplementation than those given in the table some preparations gave further improvements in NPU: with 1·8% added methionine yeast no. 2 gave NPU=82, and bacterial preparation no. 5 gave NPU=71. The addition of other amino acids gave no further improvement at optimum methionine levels (e.g. yeast no. 4 with 1 g methionine+0·5 g histidine+0·5 g lysine gave NPU=75).

	Sample no. and type					
	2 (Yeast)	3 (Yeast)	4 (Yeast)	5 (Yeast)	6 (Bacterial)	7 (Bacterial)
Nitrogen (%)	10·50	10·40	9·68	8·72	14·22	13·65
Nucleic acid N (% total N)	9·0	12·3	14·2	15·0	18·4	7·5
Total methionine (g/16 g N)	1·8	1·6	1·9	1·7	1·8	1·8
Available methionine (g/16 g N)	1·6	1·5	1·6	1·6	1·5	1·7
Total S amino acids (g/16 g N)	2·8	2·5	2·6	2·4	2·8	2·8
Total lysine (g/16 g N)	7·6	7·8	7·9	7·5	5·9	6·0
Available lysine (g/16 g N)	6·0	5·9	5·0	5·3	4·1	4·1
TD	94	93	91	79	90	91
NPU	56	58	50	44	38	46
NPU + 0·3 g L-Met/100 g protein	58	62	69	50	49	NI
NPU + 0·6 g L-Met/100 g protein	68	69	73	54	54	57
NPU + 0·9 g L Met/100 g protein	74	78	75	60	63	68
BV	60	62	54	56	42	51
BV + 0·9 g L-Met	79	84	82	76	70	75

NI, no information.

RNA was estimated in all the samples by a modification of the procedure of Schmidt & Thannhauser (1945) and DNA by the method of Burton (1956). Previous work (Mason and Palmer, unpublished) has suggested that bacterial DNA and RNA are almost completely absorbed from the alimentary canal of the rat. Since our NPU values are based on total nitrogen consumed it seems that these values may be depressed by this digestible non-protein fraction.

The unsupplemented proteins tested gave NPUs ranging from 38 to 58, the yeast preparations nos. 2 and 3 giving the highest values. The addition of methionine substantially increased the values for all the samples. By correcting the nitrogen intake of each group of rats the NPU values of the proteins were increased still further. Thus the yeast preparation no. 4 when fed with supplements of 0·3, 0·6 and 0·9 g L-methionine gave respectively NPUs of 80, 85 and 87.

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The uptake of linoleic acid by the newborn lamb. By R. C. NOBLE and J. H. MOORE, *Hannah Dairy Research Institute, Ayr*

Although the concentrations of the C18 polyunsaturated fatty acids in the various tissues of the newborn lamb are very low, it has been shown that there are pronoun-

ced increases in the concentrations of these acids during the first 3–4 d after birth in spite of the extremely low concentration of polyunsaturated fatty acids in the colostrum and milk (Leat, 1966; Noble, Steel & Moore, 1970*a*,*b*, 1971). The present report provides some information obtained from a polyunsaturated fatty acid balance experiment with lambs during the suckling period. Groups of lambs were slaughtered at intervals of 0, 10, 20 and 30 d after birth, having received a diet of cow's milk. The intake of linoleic acid by each lamb was obtained from the anaylsis of each daily milk sample and analysis of homogenates of the whole carcasses of the lambs gave results for the total amount of linoleic acid contained in all the tissues of each lamb. At birth the total amount of linoleic acid in the body of the lamb was only about 300 mg. After 10 d the total amount of linoleic acid in the carcass had increased to about 10 000 mg and after 20 d it had reached a level of 25 000 mg. The amount of linoleic acid found at 30 d was similar to that found in the carcasses at 20 d. The total dietary intake of linoleic acid by the lambs during the first 10 and 20 d was found to be virtually the same as the total amount of linoleic acid found in the homogenates of the whole carcasses at these times. However, by the 30th day after birth the intake of linoleic acid had exceeded that found in the carcass. Although the amount of the C20 polyunsaturated fatty acids in the body of the lamb at birth was greater than that of linoleic acid, after birth the amount of the C20 polyunsaturated fatty acids increased only slightly. It would appear that during the first 20 d after birth, the lamb is able to retain in its tissues all of the linoleic acid presented in the diet.

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