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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Ninety-sixth meeting of The Nutrition Society was held at the Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh, on Friday, 23 February 1968, *at* 11.30 *h, when the following papers were read :*

Some comparisons of performance of lambs with and without rumen ciliate protozoa. By MARGARET I. CHALMERS, J. DAVIDSON, J. MARGARET EADIE and J. C. GILL, *Rowett Research Institute, Bucksburn, Aberdeen*

Until the experiments of Abou Akkada & El-Shazly (1964, 1965) and those of Christiansen, Kawashima & Burroughs (1965) no significant effects on the performance of ruminants due to the absence of rumen ciliate protozoa had been reported. In view of their findings we have compared lambs with and without rumen ciliates over the first 14 months of growth.

Sixteen Cheviot lambs were isolated from other ruminants from 2 days of age. The lambs were randomly divided into two comparable groups at 3 weeks of age and individually penned in two separated areas. They were milk-fed for 13 weeks, offered dried grass *ad lib.* from 4 weeks of age and at 13 weeks the milk was replaced by a concentrate ration. At 29 weeks a set ration of two parts dried grass or hay to one part concentrates was introduced.

At 5 weeks of age the lambs in one group were inoculated with a rumen ciliate population. Regular microscopic examination of rumen fluid samples confirmed that the established populations were comparable throughout the experiment.

Complete data on individual feed intakes were recorded and statistical analysis showed no difference in food consumption between groups. Similarly, height and length of lambs did not differ significantly but body girth measured at 47 weeks was greater ($P<0.01$) in the ciliate-free lambs.

Table 1. *Some comparisons of ciliate-free and faunated lambs (mean values)*

Measurement (no. of animals)		Ciliate-free (C−)	Faunated (C+)	Difference (C−)–(C+) with SE	Significance
Live-weight gain (kg/week), 6–59 weeks	(7, 8)	0·82	0·80	+ 0·02 ±0·023	NS
Nitrogen digestibility (%)	(2, 2)	77·2	76·7	+ 0·5 ±1·6	NS
Nitrogen retention (g/day)	(2, 2)	4·8	5·1	− 0·3 ±0·84	NS
Ammonia-N (mg/100 ml rumen liquor), *ad lib.* ration	(7, 8)	9·6	19·7	−10·1 ±1·8	$P<0.001$
Ammonia-N (mg/100 ml rumen liquor), set ration	(6, 6)	14·4	24·6	−10·2 ±2·2	$P<0.01$

NS, not significant.

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The table indicates that no significant differences in other features of general performance were found. The higher ammonia levels in the faunated animals are in agreement with the results from other workers but on a high daily intake of nitrogen this would not be expected to influence performance.

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Component fatty acids of plasma lipids of lambs with and without rumen ciliate protozoa. By A. K. LOUGH (introduced by G. A. GARTON), *Rowett Research Institute, Bucksburn, Aberdeen*

Seven ciliate-free and eight faunated lambs from the experiment described in the preceding abstract (Chalmers, Davidson, Eadie & Gill, 1968) were used. When the animals were 6 months old and being fed daily on a diet of 300 g concentrates (mostly ground maize) and dried grass to appetite, samples of venous blood were obtained. Plasma lipids were extracted with chloroform–methanol and their component fatty acids, as methyl esters, were analysed as described by Duncan & Garton (1963).

No significant differences were observed between ciliate-free and faunated lambs in respect of mean values for plasma total lipids (320 and 338 mg/100 ml respectively; SED ± 28) and for plasma total fatty acids (129 and 146 mg/100 ml respectively; SED ± 11).

As shown in Table 1, the total fatty acids of the plasma of the faunated lambs had a significantly higher proportion of C_{18} mono-unsaturated acid and a corresponding significantly lower proportion of polyunsaturated acids (18:2 and 18:3) than did the fatty acids of the plasma of ciliate-free lambs. These observations corroborate those of Klopfenstein, Purser & Tyznik (1966) who, in a similar study, compared lambs, defaunated by treatment with copper sulphate, with re-faunated lambs.

Table 1. *Component fatty acids of plasma total lipids*

Fatty acid	% by weight of plasma total fatty acids: Ciliate-free	Faunated	SED	Significance
16:0	13·6	13·9	±0·5	NS
16:1	3·7	3·7	±0·4	NS
18:0	26·8	26·2	±1·5	NS
18:1	24·4	29·7	±1·3	$P<0·01$
18:2	15·7	11·8	±1·0	$P<0·01$
18:3	5·2	4·1	±0·19	$P<0·001$
Others	10·6	10·6	±0·7	NS

SED, standard error of difference of mean; NS, not significant.

As discussed by Garton & Duncan (1964), the fatty acid composition of sheep plasma lipids reflects the extent to which dietary fatty acids have undergone microbial hydrogenation in the rumen. It is therefore concluded that C_{18} unsaturated fatty acids provided by the concentrates and dried grass were more effectively hydrogenated when ciliate protozoa were present in the rumen than when they were absent. However, because of the complex interrelationships between rumen micro-organisms, it does not necessarily follow that ciliate protozoa, of themselves, were directly responsible for the additional hydrogenation which took place in the faunated lambs.

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Effect of copper intake on copper metabolism in rats. By N. F. Suttle, B. S. W. Smith and A. C. Field, *Moredun Institute, Edinburgh*, 9

The effects of dietary copper and Cu status of the rat on Cu metabolism were assessed by radioisotope balance technique. Pairs of adult hooded Lister rats, on a sucrose–dried skim-milk diet containing 10% cellulose and 1 μg/g inherent Cu, were given 0, 20 or 200 μg/g added Cu, as $CuSO_4.5H_2O$, for 2 days or 4 months prior to the oral or intraperitoneal administration of 68 μc ^{64}Cu, as $CuCl_2$ of specific activity 4·25 μc/μg. Faeces and urine were collected separately for 48 h, and radioactivity in the excreta was measured in a well-type scintillation counter. The experiment was repeated on twenty-four different rats, collecting the excreta for 72 h. There were no differences between experiments and the results for 48 h have been pooled in the data presented in Table 1.

Table 1. *Effect of dietary Cu concentrations and length of pretreatment on the metabolism of ^{64}Cu in the 48 h after oral or intraperitoneal administration of the isotope*

Cu supplement (μg/g)	Pre-treatment	% Oral dose: No. of rats	Excreted: In urine	Excreted: In faeces	Retained	% Intraperitoneal dose: No. of rats	Excreted: In urine	Excreted: In faeces	Retained
0	2 days	4	1·9a	52·3a	45·8a	4	5·0a	6·1a	88·9a
	4 months	4	1·8a	41·7a	56·5a	4	4·4a	4·4a	91·2a
20	2 days	4	0·8b	73·3b	25·9b	4	3·4a	38·8b	57·9b
	4 months	4	0·9b	70·9b	28·3b	4	5·6a	43·6b	50·8b
200	2 days	4	0·3b	87·3c	12·4c	4	1·3b	63·4c	35·5c
	4 months	4	0·2b	95·3c	4·5c	4	1·4b	48·4b	50·0b
	Residual SD		±0·6	±7·0	±6·3		±1·4	±9·4	±10·3

Means not followed by the same index letter within columns are significantly different ($P<0·05$).

https://doi.org/10.1079/PNS19680050 Published online by Cambridge University Press

Prior to commencing the experiment, all rats received the diet containing 20 μg Cu/g. Withdrawing the Cu supplement increased the urinary excretion and retention and decreased the faecal excretion of ^{64}Cu after oral dosage, the last two effects being more marked after 4 months depletion. After IP dosage, faecal excretion fell regardless of pretreatment; urinary excretion was unaffected and retention, therefore, increased. With 200 μg added Cu/g, faecal excretion was increased and the urinary excretion and retention of ^{64}Cu were decreased after oral dosage, the first two effects being more marked after 4 months supplementation. After IP dosage, urinary excretion was decreased regardless of pretreatment but an increase in faecal excretion was only observed after 2 days supplementation.

Using the combined 2 day and 4 month data for 72 h, values of 52·2, 30·6 and 20·0% for true absorption were obtained on diets containing 0, 20 and 200 μg added Cu/g, respectively. It appears that absorptive efficiency decreases as Cu intake increases; the amount of Cu absorbed still increases but Cu absorbed in excess of requirements is largely excreted via the faeces. The effects of the Cu status of the rat on these processes are relatively small.

The effects of pyridoxine deficiency on the liver lipids of the chick. By J. H. MOORE*, M. E. COATES and D. L. WILLIAMS†, *National Institute for Research in Dairying, Shinfield, Reading*

The higher concentrations of linoleate and the lower concentrations of arachidonate in the tissue total fatty acids of pyridoxine-deficient rats or chicks has led to the suggestion that pyridoxine may function as a cofactor in the biosynthesis of arachidonate from linoleate (Witten & Holman, 1952; Dam, Kristensen, Nielsen & Søndergaard, 1958). However, other investigators (e.g. Kirschman & Coniglio, 1961; Peluffo, Brenner & Mercuri, 1963) could find no evidence for the direct involvement of pyridoxine in the synthesis of arachidonate.

In Expt 1, three groups (A, B and C) of twenty chicks each were given, from 1 day of age, purified diets that contained respectively 2500, 250 or 50 μg pyridoxine per 100 g diet. These diets contained 0·3% linoleate. After 3 weeks, the mean weights of the chicks in groups A, B and C were 269, 264 and 179 g respectively. In the liver total fatty acids, the percentages of linoleate were similar in all groups (mean, 10·4%) but the percentages of arachidonate in groups A, B and C were 10·0, 10·2 and 5·3 respectively. Triglycerides constituted 23% and phospholipids 64% of the total liver lipids in groups A and B but in group C, triglycerides accounted for 41% and phospholipids only 48% of the total liver lipids. The level of pyridoxine in the diet did not affect the fatty acid compositions of the liver triglycerides, phospholipids or cholesteryl esters. In Expt 2, two groups (D and E) of twelve chicks each were given, from 1 day of age, purified diets that contained respectively 400 or 60 μg pyridoxine per 100 g diet. These diets contained 3·0% linoleate.

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After 3 weeks, the mean weights of the chicks in groups D and E were 321 and 170 g respectively. In the total liver fatty acids in groups D and E the mean percentages of arachidonate were 23 and 14 and the mean percentages of linoleate were 16 and 26 respectively. Triglycerides accounted for 21 and 43%, and phospholipids 67 and 47% of the total lipids in groups D and E respectively. Again, the level of pyridoxine in the diet did not affect the fatty acid compositions of the liver triglycerides, phospholipids or cholesteryl esters.

In both experiments, the lower concentrations of arachidonate in the liver total fatty acids of the deficient chicks were due to the higher proportions of trigylcerides and lower proportions of phospholipids in the liver lipids. The concentration of arachidonate in the liver phospholipids was ten to fifty times greater that that in the liver trigylcerides. In Expt 2, the higher concentration of linoleate in the liver total fatty acids in group E was due to the fact that the concentration of linoleate in the liver triglycerides was twice that in the liver phospholipids. These experiments show that the proportions of the different lipid classes in chick liver are markedly changed by pyridoxine deficiency, but no evidence was obtained for a direct involvement of pyridoxine in the biosynthesis of arachidonate.

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Abnormal changes in the volume of the rumen contents of sheep given molassed sugar-beet pulp. By J. W. Czerkawski and D. R. Paterson, *Hannah Dairy Research Institute, Ayr*

Sheep with rumen fistulas were kept on maintenance rations of sugar-beet pulp given as single daily meals at 9·00 h, mixed with equal weights of water, or with solutions of polyethylene glycol (PEG, 10 g/day) during alternative 2-week periods. Faeces were collected daily, and were bulked weekly and analysed for dry matter, nitrogen and PEG. Samples of rumen contents were analysed during 4–5 days of each second marker week (Hydén, 1955). The rumen samples were taken before and immediately after feeding and eight to ten times throughout the remainder of the days. The sheep ate their rations in less than 30 min and drank 200–400 ml of water soon afterwards.

The recovery of PEG in faeces was 92–96% and its excretion pattern lagged almost exactly 1 week behind the intake. The proportion of nitrogen in the faecal non-PEG dry matter was almost constant. In one sheep which was 12 weeks on the rations, excretion of dry matter varied from week to week in a definite cyclic way, with an amplitude that was much greater than might have been expected from the intermittent administration of the marker. The amplitude tended to decrease during the experiment. The non-PEG dry matter also increased and decreased regularly,

with the maxima occurring 2 weeks after the rumen sampling weeks. The experiment with another sheep was shorter (9 weeks) and although only one minimum and one maximum value of faecal dry matter could be observed, the lag after administration of marker and the magnitude of the changes were similar to those obtained with the first sheep. It could not be ascertained from these results whether the periodicity in the amounts of dry matter excreted in the faeces was due to some psychological effect of sampling or whether it was caused by the marker.

The concentrations of PEG in the rumen fluid enabled the volumes of the rumen contents and the relative rates of outflow to be calculated (Hydén, 1961; Warner, 1966). The apparent volumes of the contents increased in one sheep from 4·5 l. after 4 weeks on the ration to 9·4 l. after 8 weeks and to 17·0 l. after 12 weeks. The rumen volume of the second sheep was 4·7 l. after 6 weeks. The calculated relative rates of outflow of fluid from the rumen were low (0·5/day after 4 weeks) and decreased to 0·26/day after 12 weeks.

Since the volume of the rumen contents of sheep on constant maintenance rations is unlikely to increase greatly (Hydén, 1961), and certainly not fourfold, and since the fraction of rumen volume flowing out should not change when the animals are in a steady state, and should be, in sheep, not less than 1–2 l./day (Hydén, 1961), it must be provisionally concluded that the simple theory of steady state and constancy of rumen volume (Warner, 1966) might not apply to some sheep on a sugar-beet pulp diet.

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Sodium requirement of the fowl at the onset of lay. By R. S. ANDERSON, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Although an increase in salt intake is known to enhance water intake and excretion in the fowl (Kare & Biely, 1948), there is little information on the effect of low dietary levels of salt on water turnover. Lumijarvi, Frank & Hill (1967) reported, however, that in colostomized birds water intake and urine output were markedly increased when salt intake was restricted. The effects of low levels of dietary sodium on normal laying birds are described in the present report.

Three diets containing 0·03, 0·07 and 0·10% sodium (mainly as sodium chloride) were fed *ad lib.* to three groups of four 19-week-old Shaver 288 pullets. Lighting (12 h on, 12 h off) and temperature (60–70°F) were controlled.

All diets were adequate for normal weight gain during the prelay period, but the birds receiving the 0·03% sodium diet showed a marked decrease in food intake and body-weight after the onset of lay. When this diet was replaced by one containing 0·07% sodium, food intake and body-weight increased rapidly. The birds which received the 0·07% sodium diet throughout the experiment showed a slight transient decrease in body-weight which commenced about 3 weeks after the onset of lay.

The water : food ratio and droppings moisture of the birds receiving the low-sodium diet were greater than those of the other two groups. Although the mean water intake of the low-sodium group was also greater than that of the other two groups, there were large individual variations and the difference was not significant.

There was a delaying effect of low sodium intake on the onset of lay, but egg production and average weight were similar in all three groups during the first 4 weeks after the onset of lay. The egg production of the low-sodium group then decreased rapidly to 32%, that of the medium-sodium group showed a slight transient decrease to 78%, while that of the high-sodium group remained above 89%.

The mean plasma sodium concentration, 148 ± 2 m-equiv./l. (mean $\pm$ SD), of the birds receiving 0·03% sodium was significantly lower than those of the birds receiving 0·07%, and 0·10% sodium (156 ± 2 m-equiv./l. and 155 ± 2 m-equiv./l. respectively).

These findings indicate that, as in other animals, sodium deficiency in the fowl is characterized by anorexia and weight loss and that at the onset of lay there is an increased sodium requirement for which dietary levels below 0·10% are inadequate. There is some evidence of increased water intake at low dietary sodium levels, but it is likely that the effects of salt deprivation on water balance of the colostomized fowl (Lumijarvi *et al.* 1967) are more readily induced than in the intact fowl (Hart & Essex, 1942).

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Restricted and ad lib. feeding of dairy cows on diets differing in crude fibre and metabolizable energy contents. By S. AMIR*, J. N. AITKEN and M. KAY, *Rowett Research Institute, Bucksburn, Aberdeen*

For the first 6 weeks of lactation, twenty cows were fed *ad lib.* on a pelleted diet (A) containing 17% straw and 12% crude fibre. For weeks 7–24 they were divided into four groups, two of which were fed *ad lib.* and two according to the Agricultural Research Council (1965) standards. The two groups on each feeding level were given pelleted diets containing either (B) 7% or (C) 29% straw. These two diets contained 8 and 16% crude fibre, respectively, and were estimated to supply 2·62 and 2·46 Mcal metabolizable energy per kg dry matter. Food intake, milk production and live-weight changes were recorded. Samples of rumen liquor were taken from two to four cows in each group of five, on three occasions.

The main results are shown in Table 1. The cows fed *ad lib.* had intakes about 50% higher than those restricted, but responded by gaining weight rather than by giving more milk (although their milk contained more solids-not-fat than that of the restricted cows). The two diets promoted equal intakes of metabolizable energy

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Table 1. *Food intake, milk production and live-weight change in cows fed* ad lib. *or restricted, on diets containing* (*B*) 2·62 *or* (*C*) 2·46 *Mcal/kg dry matter* (*mean values for 7th–24th weeks of lactation*)

	Diet				SE and significance of differences
	B *ad lib.*	B restricted	C *ad lib.*	C restricted	
Dry-matter intake (kg/cow day)	14·7	8·8	17·4	12·6	±0·82***
Metabolizable-energy intake (Mcal/cow day)	36·4	23·0	36·3	27·8	±1·7***
Milk yield (kg/cow day)†	18·1	15·5	14·9	16·5	±1·3
FCM yield (kg/cow day)†‡	12·8	11·7	13·5	14·0	±0·82
Butterfat in milk (%)†	2·06	2·43	3·13	3·17	±0·26**
Solids-not-fat in milk (%)	9·51	8·81	9·01	8·78	±0·10***
Live-weight change (kg/cow day)	0·39	−0·12	0·37	−0·09	±0·14***

†Adjusted by covariance for initial value. ‡4% fat-corrected milk. **$P<0·01$. ***$P<0·001$.

when fed *ad lib.* The rumen volatile fatty acids from cows on diet B had the molar proportions, acetic acid, 47%, propionic, 41% and butyric, 9%. The corresponding values for diet C were 58, 21 and 18%. The difference between the diets in this respect probably accounts for the difference in milk fat percentage.

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The protein requirements of early-weaned calves. By M. KAY and N. A. MACLEOD, *Rowett Research Institute, Bucksburn, Aberdeen*

Twenty-seven Friesian bull calves were given from 14 days of age one of three isocaloric diets containing either 20% (H), 17% (M) or 14% (L) crude protein in the dry matter. They were weaned at about 30 days when they weighed 50 kg and from then until they reached 95 kg live-weight gains and feed intake were recorded. At approximately 65 kg live weight (period 1) and again at 90 kg (period 2) digestibility and nitrogen balance measurements were made with each calf.

The main results are given in Table 1. There were slight differences in dry-matter intake between calves on the three treatments but there were no significant differences in their growth rate. When the rates of gain were adjusted to a feed intake of 2 kg/day (1·7 kg dry matter) calves given diet L grew more slowly. The high-protein diet appeared to be no better than the medium-protein diet, even during the first few weeks after weaning. The digestibility of dry matter was lower for treatment L. Nitrogen retention (g/day) was low for treatment M in period 1, probably because dry-matter intake was low. The proportion of absorbed nitrogen

which was retained varied from 61% (treatment H) to 69% (L) in period 1 and from 43% (H) to 68% (L) in period 2.

Table 1. *Feed intake, live-weight gain, digestibility and nitrogen retention in calves given diets containing 20% (H), 17% (M) or 14% (L) crude protein*

	Treatment			SE and significance of differences
	H	M	L	
Dry-matter intake (kg/day)	1·74	1·60	1·82	±0·16
Live-weight gain (kg/day)	0·81	0·73	0·72	±0·06
Live-weight gain at feed intake of 1·7 kg/day (kg/day)	0·79	0·77	0·69	±0·022***
Dry-matter digestibility (%)				
Period 1	78	77	73	±1·6*
Period 2	77	77	74	±1·4
Apparent nitrogen digestibility (%)				
Period 1	74	68	65	±1·7***
Period 2	76	75	69	±1·7**
Nitrogen retention (g/day)				
Period 1	27	18	18	±2·9*
Period 2	27	30	27	±3·1
Nitrogen retention (% of intake)				
Period 1	45	44	45	±2·7
Period 2	33	45	47	±2·8***

*$P<0.05$. **$P<0.01$. ***$P<0.001$.

Dietary factors influencing starch disappearance in various parts of the alimentary tract and caecal fermentation in early-weaned lambs.

By E. R. Orskov and C. Fraser, *Rowett Research Institute, Bucksburn, Aberdeen*

Eight lambs of Cheviot or Cheviot cross breeding were fitted with abomasal cannulas at 2–3 weeks of age; 2 weeks later two of them were fitted with cannulas in the terminal ileum and two with cannulas in the caecum. They were weaned at approximately 5 weeks of age and were introduced to a series of dietary treatments over feeding periods of 11 days, the order of allocation of treatments being randomized. On the last day of each period samples of abomasal, ileal and caecal contents and of faeces were obtained at 2 h intervals and composite samples were analysed. Dry-matter disappearance was estimated by using polyethylene glycol as an indigestible marker and starch was estimated as α-linked glucose by the method of MacRae & Armstrong (1966). Glucose was determined by the glucose-oxidase method.

The basal diet (RB) contained 80% rolled barley and a protein–mineral supplement based on soya-bean meal. There was approximately 17% crude protein in the dry matter of the diet. Diet RBG consisted of 60% of the basal and 40% of chopped dried grass. These two diets were given at two levels, an estimated *ad lib.* level and 70% of that. Two other diets, one (GB) containing ground barley in place of the rolled barley in the basal and one (RBM) in which maltose was substituted for 30% of the rolled barley, were each given at the high level only.

https://doi.org/10.1079/PNS19680050 Published online by Cambridge University Press

The percentage of the ingested starch which escaped rumen fermentation increased with level of feeding (from 4 to 7·5% on RB and from 7·5 to 13% on RBG) and decreased slightly with ground barley (GB) and with the maltose substitution (RBM). The high values with diet RBG as compared with RB suggest that the dried grass increased the rate of passage of smaller particles from the rumen. There was considerable variation among individual values for each diet, particularly at the higher feeding level, the highest result for diet RB being 18% and for diet RBG, over 20%.

The proportion of the dietary starch reaching the terminal ileum increased, at an increasing rate, as that in the abomasum increased above about 5%. The highest individual values at the ileum were 6% for diet RB and over 9% for diet RBG, which suggests that the small intestine has only a limited capacity for starch digestion. This agrees with findings of Walker (1959) that lambs have a very low amylase activity in the intestine.

The molar percentage of acetic acid in the caecum varied from 57 to 78, the lowest values being found when a high proportion of the dietary starch escaped rumen fermentation.

The faeces contained less than 1% of the ingested starch, irrespective of diet. It appeared that starch passing the terminal ileum was effectively removed during the fermentation in the caecum and large intestine.

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The influence of level of dietary phosphorus on the effect of manganese on shell formation. By MARGARET LONGSTAFF and R. HILL, *Royal Veterinary College, University of London*

The relationship between the levels of dietary phosphorus and manganese on shell formation in pullets at the start of egg laying has been studied. Four diets, two of high-Mn content (106–7 μg/g), one with 0·7 and the other 1·5% P, and two of low-Mn content (6–7 μg/g) one with 0·7 and the other 1·5% P were given to pullets from 16 weeks of age: there were ten birds per dietary group.

From the number of shell-less eggs of the first ten laid by each bird, and the thickness and strength of the first six shells produced, a significant interaction between dietary Mn and P was observed. The effects of low Mn were greater with high than moderate P, but with high Mn the level of P had no significant effect.

Of three low-Mn diets used in a previous experiment (Longstaff & Hill 1967), that giving the poorest shells, similar to one used by Lyons (1939), had a high P content and this may explain the more marked effects with this than other diets of similar Mn content.

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Skeletal manganese in pullets given diets of different Mn content. By R. HILL and J. W. MATHERS, *Royal Veterinary College, University of London*

Pullets were sacrificed, some before sexual maturity (group I), and others at the point-of-lay (groups H and L) or after 6–7 months of egg production (groups HH, LL and HL) having been given diets of high (106–7 μg/g) or low (6–7 μg/g) manganese content. The mean Mn content of the skeleton before sexual maturity was 153 μg (I). At the point-of-lay, birds given the high-Mn diet (H) had a slightly larger content (180 μg) and those given the low-Mn diet (L) a slightly smaller Mn content (126 μg), but there were no significant differences among these three values. After the 6–7 month egg-laying period, birds given the low-Mn diet, whether throughout the experiment (LL) or only from the point-of-lay (HL) had similar skeletal Mn contents (179 and 172 μg respectively), both slightly larger than the low-Mn group killed earlier (L). Birds given the high-Mn diet to the end of the experiment (HH) contained very much more Mn (564 μg) than any other group.

In general, these similarities and differences were reflected in the concentration of Mn in dry fat-free bone or ash, but some non-significant differences in Mn content given as weight of Mn were increased and became significant when given as concentration, there being differences in weight of dry fat-free bone and percentage of ash among treatment groups.

No evidence was obtained supporting the view that the skeleton represents a substantial store of Mn (Underwood, 1962).

REFERENCE

Underwood, E. J. (1962). *Trace Elements*, 2nd ed., p.190. London: Academic Press Inc.

Metabolism of riboflavine in goats with a caecal fistula. By D. W. WEST and E. C. OWEN, *Hannah Dairy Research Institute, Ayr*

Anaerobic fermentations in vitro of riboflavine, in the presence of contents of the rumen or ruminant caecum, produce metabolites of riboflavine. These metabolites, which appear in urine and milk when riboflavine is fed can be shown, by taking fistula or stomach-pump samples, to originate in the rumen (West, Owen & Taylor, 1967) but we did not know whether fermentations elsewhere in the gut could give rise to riboflavine metabolites. Two different surgical approaches were used: that of Redman, Teague, Henderickx & King (1964) who placed in-dwelling cannulas in the caecum of the pig and that of Faitelberg & Semenjuk (1956) who united the wall of the caecum of sheep with the flank of the animal at the site of fistula so permitting us to employ the same type of perspex fitment for the fistula as was already in use at this Institute for fistulas elsewhere in the gut. Subsequent operations on goats were therefore done with the latter technique. The operation caused the animals little, if any, inconvenience. Experiments with goats, so operated, showed that, when riboflavine was fed, metabolites and riboflavine appeared in

milk, urine and caecal samples, but that, when riboflavine was placed in the caecum, riboflavine appeared by itself in urine and milk. These experiments on caecal function are being continued.

To Dr D. Robertshaw, who carried out the first operations by each of the techniques, the authors are indebted for both his help and advice.

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Redman, D. R., Teague, H. S., Henderickx, H. K. & King, N. B. (1964). *J. Anim. Sci.* **23**, 1032.
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The One Hundred and Ninety-seventh meeting of The Nutrition Society was held at the Sir John Atkins Laboratories, Queen Elizabeth College, Campden Hill Road, London, W8, on Thursday, 14 March 1968, at 16.30 h, when the following papers were read:

Energy metabolism of young rats fed different levels of protein and/or calories. 1. The partition of the metabolizable energy intake of pair-gained and pair-fed litter-mates. By K. J. McCRACKEN*, *Dunn Nutritional Laboratory, Infant Nutrition Research Division, University of Cambridge and Medical Research Council*

Diets containing suboptimal levels of protein have given slower growth and also decreased efficiency of energy retention than complete diets fed at the same calorie intake (e.g. Forbes, Swift, Black & Kahlenberg, 1935; Hamilton, 1939). Blaxter (1962) has suggested that at least a part of the decreased efficiency may be explained by the greater proportion of fat in the body gain of rats fed low-protein diets and the lower efficiency of fat formation compared to protein formation. However, this could not account for the large difference in requirement for energy maintenance apparent in the results of Miller & Payne (1962).

We studied the energy balance of young male hooded Lister rats maintained at 28° in continuous light and fed either a low-protein diet (5% protein from casein+methionine, 10% maize oil, 30% sucrose, 50% dextrin) or a control diet containing 20% protein with extra casein at the expense of dextrin, both diets being supplemented with mineral and vitamin mixtures.

Three treatments were compared using litter-mates starting at 55–60 g, over the

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