PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Fifty-fourth Scientific Meeting (One Hundred and First of the Scottish Group) was held in Lecture Hall 4A, Mathematics Building, University of Glasgow, University Gardens, Glasgow G11 5JR, on Friday, 23 February 1973, at 11.30 hours, when the following papers were read:

The effect of the addition of trichloroacetamide and chloroform to the rumen of sheep on the fermentation. By J. L. CLAPPERTON, *Hannah Research Institute, Ayr KA6 5HL*

Trei, Parish, Singh & Scott (1971) and Trei, Singh & Scott (1972) have shown that when either trichloroacetamide or a hemiacetal compound of starch and chloral hydrate was incorporated into the food of lambs methane production was suppressed, the ratio of acetic acid: propionic acid in the rumen liquor was reduced and more energy was retained by the animals. Their results, however, suggested that the magnitude of these effects diminished with time. Clapperton & Czerkawski (1972) in experiments lasting for only 10 d showed that small amounts of chloroform given into the rumen of sheep had similar initial effects.

The present experiments were designed to investigate the effects of trichloroacetamide and chloroform when they were given into the rumen of sheep for 98 d continuously. Nine 2-year-old Greyface wether sheep each fitted with a permanent rumen cannula were offered 600 g/d of a mixture of two parts of chopped hay and three parts of molassed sugar-beet pulp. The diet was given in two equal meals daily. After an initial period when no additive was given, groups of three sheep were given: no additive, 170 mg/d trichloroacetamide or 140 mg/d chloroform. Rumen volatile fatty acids were measured at intervals of 14 d and the gas production of the rumen liquor was determined in vitro (Czerkawski & Breckenridge, 1970).

The results showed that after 14 d both trichloroacetamide and chloroform caused a reduction in the ratio of acetic acid: propionic acid in the rumen liquor, the values obtained being: no additive 2·66, trichloroacetamide 1·94, chloroform 1·61. There were changes in the ratio throughout the experiment with all the treatments, but the differences between the treatments diminished, the corresponding values for the period from 56 to 98 d being 3·87, 2·91 and 2·59 respectively. Initially, both compounds completely suppressed methane production; with chloroform the effect persisted throughout the experiment but, with trichloroacetamide, methane production returned to normal after 60 d.

The results suggest that the rumen micro-organisms are able to adapt to the presence of trichloroacetamide in the rumen but that they are less able to adapt to the presence of chloroform.

REFERENCES

Clapperton, J. L. & Czerkawski, J. W. (1972). *Proc. Nutr. Soc.* **31**, 55A.
Czerkawski, J. W. & Breckenridge, G. (1970). *Lab. Pract.* **19**, 717.
Trei, J. C., Parish, R. C., Singh, Y. K. & Scott, G. C. (1971). *J. Dairy Sci.* **54**, 536.
Trei, J. C., Singh, Y. K. & Scott, G. C. (1972). *J. Anim. Sci.* **34**, 510.

Determination of hypoxanthine, xanthine and uric acid in ruminants' urine. By M. A. RAZZAQUE and J. H. TOPPS, *School of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD*

Ruminants excrete considerable quantities of the purine derivatives hypoxanthine, xanthine, uric acid and allantoin, most of which come from microbial nucleic acids synthesized in the reticulo-rumen. Methods used to determine these compounds other than allantoin are either subject to interference from other urinary constituents or involve radioisotopes. Recent work has shown that the pattern of excretion of purine derivatives differs between certain species, and the analytical procedure shown in Fig. 1 was devised to examine such differences. The procedure is based on a two-stage ion-exchange chromatographic separation of purines using the resins AG 50W × 4 (200–400 mesh) in the hydrogen form and AG 1W × 10 (50–100

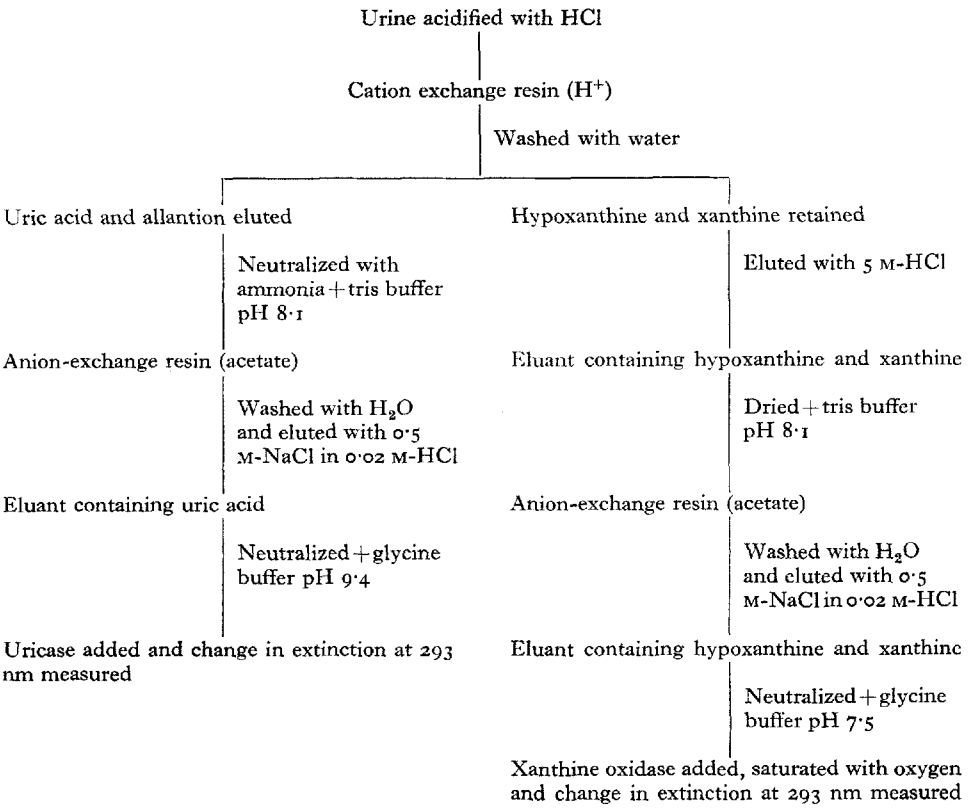

Fig. 1. Scheme of analysis.

mesh) converted into the acetate form (Biorad Laboratories, California, USA), followed by enzymatic conversion of hypoxanthine and xanthine to uric acid and of uric acid to allantoin.

Recoveries of uric acid and of hypoxanthine+xanthine were 95–98 and 88–99% respectively.

Metabolism of nucleic acids by sheep and red deer. By M. A. RAZZAQUE and J. H. TOPPS, *School of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD* and E. D. GOODALL and R. N. B. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In earlier studies (Maloiy, Kay, Goodall & Topps, 1970) it was observed that sheep excreted more urinary allantoin and uric acid than red deer. We have now re-examined this difference to see if it could be related to the production of nucleic acids in the rumen.

Two sheep and one red deer, all fitted with rumen cannulas, were housed in metabolism cages and given a pelleted diet consisting largely of barley straw, maize starch and groundnut meal and containing 26·3 g nitrogen/kg. This diet was delivered from a continuous feeding device at either 0·9 or 1·8 of their maintenance level of nutrition.

The concentrations of nucleic acids in the rumen contents of the two sheep and the deer were estimated by the method of McAllan & Smith (1969) and were found to be similar, averaging 132, 115 and 172 mg nucleic acid N/l respectively. Later, when the animals and another red deer were given a diet of pelleted dried grass, values of 40, 40 and 35, 70 mg nucleic acid N/l were recorded. The sheep excreted approximately 50% less nucleic acid in their faeces than the deer but the amount was related to the amount of food consumed and to the excretion of faecal N (47·9, 40·9 and 49·3 mg nucleic acid N/g faecal N respectively).

Table 1 shows that, despite these similarities in nucleic acid production, excretion of purines in the urine differed greatly between sheep and deer.

Table 1. *Dietary nitrogen intake (g/24 h), urinary N (g/24 h) and purine N (mg/24 h) excretion by sheep and red deer*

Animal	Dietary N	Urinary N	Hypoxanthine +xanthine	Uric acid	Allantoin	Total purine
			0·9 × maintenance requirement			
Sheep 245	15·5	13·2	15·8	25·9	212	254
Sheep 247	16·6	17·2	35·2	8·6	300	344
Deer A	23·3	23·9	105·5	3·6	181	290
			1·8 × maintenance requirement			
Sheep 245	31·1	23·8	50·3	43·7	674	768
Sheep 247	33·2	28·9	43·9	15·5	604	663
Deer A	46·7	41·2	207·2	5·6	257	470

These results indicate that the sheep and deer differed in their catabolism of purines rather than in the microbial synthesis of nucleic acids.

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REFERENCES

Maloiy, G. M. O., Kay, R. N. B., Goodall, E. D. & Topps, J. H. (1970). *Br. J. Nutr.* **24**, 843.
McAllan, A. B. & Smith, R. H. (1969). *Br. J. Nutr.* **23**, 671.

The lipid response to female gonadal hormones, of male rats given a high-sucrose diet. By D. B. JEFFERYS and I. R. WHITE, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

The lipid response to dietary sucrose seems to depend on the sex of the consumer. Men and post-menopausal women on high-sucrose diets show a rise in fasting serum triglyceride concentrations, which is not shown by pre-menopausal women (Coltart & Macdonald, 1971). The combined oral contraceptive, however, is associated with a rise in fasting triglyceride concentrations (Stokes & Wynn, 1971).

Eighteen mature male Wistar rats (270 g) were fed *ad lib.* on a diet of (g/kg) sucrose 75, calcium caseinate 20 and salt mixture (Briggs & Williams, 1963) 5. Four groups were injected daily; one received 2 μg oestradiol subcutaneously in 0·2 ml oil, the second 4 mg progesterone, the third a combined dose of the hormones, and a control group oil alone. Two rats were killed on day 0; after that one from each group was killed on days 7, 14, 21 and 28. After a 24 h fast and 2 h before death each animal received 6·65 μCi [^{14}C]sucrose by stomach-tube. Serum lipid was extracted by the Carlson (1959) method, and liver lipid by the Folch, Lees & Sloane Stanley (1957) method. The lipid fractions were separated by thin-layer chromatography and the radioactivity in each fraction was determined. The specific activity of the perirenal fat was also measured.

The control group showed a marked increase in the amount of liver triglyceride (mg/g) 27·3 day 0, 39·7 day 28; with oestradiol, the triglyceride content (12·7 mg/g) was only 30% of the final control value ($P<0·005$), whereas progesterone (25·5 mg/g) produced no alteration from the day 0 value. The combined regimen produced a response different from that of either oestradiol or progesterone alone, raising the liver triglyceride (47·5 mg/g) above the control value ($P<0·005$). The specific activity of the perirenal fat revealed that oestradiol increases incorporation of sucrose into adipose tissue compared with the mean control value (counts/min per mg lipid) control 8·2, oestradiol 18·1 ($P<0·005$). The total serum lipid concentrations were increased for all four groups (g/l) control 2·54, oestradiol 2·41, progesterone 2·34, combined 3·23. The combined hormone regimen raised the serum concentration above the control value ($P<0·01$).

There appears to be synergism between oestradiol and progesterone affecting the handling of dietary sucrose. Oestradiol alone increases the incorporation of sucrose into adipose tissue, and this may be responsible for the reduction in the serum and liver lipid concentrations. Progesterone has no definite actions alone, but the combined preparation raises the liver and serum concentrations of triglyceride.

REFERENCES

Briggs, G. M. & Williams, M. A. (1963). *Fedn Proc. Fedn Am. Socs. exp. Biol.* **22**, 261.
Carlson, L. A. (1959). *Acta Soc. Med.* **64**, 288.
Coltart, T. M. & Macdonald, I. (1971). *Br. J. Nutr.* **25**, 323.
Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Stokes, T. & Wynn, V. (1971). *Lancet* ii, 677.

Effect of ensilage on the lipids of pasture grasses. By A. K. LOUGH and L. J. ANDERSON (introduced by G. A. GARTON), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Total lipids were extracted (Nichols, 1964) from samples of mixed pasture grasses (rye-grass, timothy and meadow fescue) and from two samples of a similar mixture of grasses which had been ensiled (without additives) on different farms for 12 months. The lipids were fractionated (Duncan & Garton, 1962) into unesterified fatty acids, neutral lipids and complex lipids, including phospholipids, and from each fraction the total fatty acids were isolated as their methyl esters. After pigments associated with the methyl esters had been removed by adsorption on to silicic acid, the esters were analysed by gas–liquid chromatography using both a polar (adipate) and a non-polar (Apiezon) liquid phase (Duncan & Garton, 1963), to give the results shown in Table 1.

Table 1. *Fatty acid composition of unesterified fatty acids (UFA), neutral lipids (NL) and complex lipids (CL) of fresh grasses and of ensiled grasses*

(Values as % by weight, to nearest whole number, of fatty acids in each lipid class; values in parentheses give % contributed by each class to fatty acids of total lipids)

	Fresh grasses			Silage 1			Silage 2		
Fatty acid	UFA (4)	NL (22)	CL (74)	UFA (43)	NL (50)	CL (7)	UFA (48)	NL (42)	CL (10)
16:0	33	8	10	20	22	28	27	19	22
18:0	8	7	2	1	3	7	5	4	4
18:1*	13	7	3	15	15	35	22	10	10
18:2*	6	10	10	31	26	14	16	21	10
18:3	17	58	72	17	24	7	3	35	0
Others†	23	10	3	16	10	9	27	11	54

*For silages 1 and 2 values include acids having *trans* bonds.
†For silages 1 and 2 these include acids not present in fresh grasses; among these are unidentified components which have carbon numbers (Woodford & van Gent, 1960) of 19·4 and 20·5 on the polar liquid phase.

As found previously by Garton (1960), linolenic acid (18:3) was the major component of the esterified fatty acids of mixed pasture grasses. In the ensiled grasses, on the other hand, this acid was present in much lower proportion in the esterified fatty acids and indeed was absent altogether from the complex lipids of silage 2. Concomitantly, the complex lipid fraction of both silage samples was found to represent a much smaller proportion of the total lipids than it does in fresh grasses; this was associated with the presence of a considerably increased contribution of unesterified fatty acids to the total lipids.

These observations indicate that lipolysis takes place when grass is ensiled and, the finding that *trans* unsaturation was present in 18:1 and 18:2, indicates that isomerization similar to that which occurs by microbial action in the rumen (Kepler, Hirons, McNeill & Tove, 1966) can also occur in silage; reduction of double bonds also takes place, though apparently it does not include the hydrogenation of octadecenoic acids.

REFERENCES

Duncan, W. R. H. & Garton, G. A. (1962). *J. Lipid Res.* **3**, 53.
Duncan, W. R. H. & Garton, G. A. (1963). *Biochem. J.* **89**, 414.
Garton, G. A. (1960). *Nature, Lond.* **187**, 511.
Kepler, C. R., Hirons, K. P., McNeill, J. J. & Tove, S. B. (1966). *J. biol. Chem.* **241**, 1350.
Nichols, B. W. (1964). In *New Biochemical Separations* p. 321 [A. T. James and L. J. Morris, editors]. London: D. Van Nostrand Co. Ltd.
Woodford, F. P. & van Gent, C. M. (1960). *J. Lipid Res.* **1**, 188.

Nature of the association between unesterified long-chain fatty acids and particulate matter in duodenal digesta of sheep. By A. SMITH and A. K. LOUGH (introduced by G. A. GARTON), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The major lipid constituents of post-ruminal digesta of adult sheep are unesterified long-chain fatty acids (see Garton, 1967). These acids, which include stearic, palmitic and octadecenoic acids, are almost entirely associated with particulate matter in digesta (Lennox, Lough & Garton, 1968). Unesterified fatty acids are solubilized in bile salt micelles as a prerequisite for their absorption from the small intestine (see Lough, 1970). It was therefore of interest to investigate the nature of the association between long-chain fatty acids and the particulate fraction of sheep duodenal digesta.

Duodenal contents, free from bile and pancreatic juice, were collected from a grass-fed sheep provided with a re-entrant cannula immediately distal to the pylorus. The particulate matter which was obtained by centrifuging at 40 000 *g* and which was washed with distilled water in the centrifuge was entirely free of gastric HCl. It was then freeze-dried, packed into a glass column and extracted successively with hexane, light petroleum (b.p. 40–60°), chloroform–methanol (1:1, by vol.), and chloroform–methanol–acetic acid (1:1:0·1, by vol.). The solvent was changed when extraction at each stage was complete.

All the unesterified fatty acids associated with the particulate matter were extracted into hexane. Furthermore, most of the small amount of neutral lipids which are also associated with the digesta were removed by hexane. Phospholipids and other complex lipids were mainly extracted into chloroform–methanol (1:1, by vol.); only traces of these polar lipids were eluted by the acidic solvent mixture.

The observation that the unesterified fatty acids are readily and completely extracted from association with particulate matter into solution in a non-polar solvent such as hexane indicates that these acids are fully protonated and thus cannot have been present in any ionic form. Moreover, since these long-chain acids are so readily taken into solution in hexane it is apparent that, contrary to the suggestions of Leat & Harrison (1969) and Harrison & Leat (1972), they are not bound to the proteins of the particulate matter, nor are they in the form of an insoluble complex, but are merely adsorbed on the surfaces of the particles.

REFERENCES

Garton, G. A. (1967). *Wld Rev. Nutr. Diet.* **7**, 225.
Harrison, F. A. & Leat, W. M. F. (1972). *J. Physiol., Lond.* **225**, 565.

Leat, W. M. F. & Harrison, F. A. (1969). *Q. Jl exp. Physiol.* **54**, 187.
Lennox, A. M., Lough, A. K. & Garton, G. A. (1968). *Br. J. Nutr.* **22**, 237.
Lough, A. K. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 519 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press Ltd.

An indirect estimation of rate of formation of long-chain fatty acids in the rumen of sheep. By J. W. CZERKAWSKI, *Hannah Research Institute, Ayr KA6 5HL*

It was shown that, by gradually increasing the proportions of linseed oil fatty acids in the rations of sheep, it is possible to incorporate up to 80 g/d without marked changes in the digestibility of the basal ration (Czerkawski, 1966). During subsequent experiments, first with two sheep, then with four and three sheep, in some sheep there was considerable accumulation of palmitic and a small accumulation of myristic acids in the rumen, particularly when high-fat rations were being consumed. It was shown that these high concentrations of palmitate were not due to β-oxidation of C_{18} acids, nor were they an artefact due to sampling of unevenly distributed material in the rumen contents. Further experiments showed that, when the rumen contents with high concentrations of palmitic acids were incubated with [^{14}C]acetate, there was considerable labelling of palmitic and myristic acid.

Starting with the equation of Warner (1966), and assuming that (a) the concentration of fatty acids in the rumen just before one feed is the same as the concentration just before the next feed and (b) although the 18-carbon atom fatty acids are hydrogenated, they are neither synthesized nor degraded in the rumen, the following expression may be derived for the specific rate of formation of palmitic acid in the rumen:

$$K = \frac{2{\cdot}3}{t\text{-}T} \log \frac{C_{16}}{C_{18}} \cdot \frac{(\bar{C}_{18}+C_{18})}{(\bar{C}_{16}+C_{16})} \ h^{-1},$$

where C_{16} is the concentration of palmitic acid, C_{18} is the concentration of total 18-carbon acids, $\bar{C}_{16}$ and $\bar{C}_{18}$ are the potential increments in concentration due to food intake (intake/volume) for palmitic and 18-carbon acids respectively, t is the time between feeds and T is the time taken for the animal to eat its food. The rate of formation of palmitic acid is obtained by multiplying K by C_{16} and the rumen volume.

Calculations showed that when the concentrations of palmitic acid were exceptionally high in the rumen, the rumen contents of sheep could be synthesizing between 10 and 30 g palmitic acid/d. This would require between 19 and 57 g acetic acid, which is a considerable proportion of the acetic acid formed in the rumen of sheep (120 g/d). Such synthesis would also require between 12 and 36 l hydrogen that would have to be rechannelled from other reductive processes such as methanogenesis and might partly account (Czerkawski, 1972) for the observed inhibition of methane production under these conditions.

REFERENCES

Czerkawski, J. W. (1966). *Br. J. Nutr.* **20**, 833.
Czerkawski, J. W. (1972). *Proc. Nutr. Soc.* **31**, 141.
Warner, A. C. I. (1966). *J. gen. Microbiol.* **45**, 213.

The establishment of rumen ciliate protozoa in sheep fed on whole barley to appetite. By J. MARGARET EADIE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Rumen ciliate protozoa cannot be established in cattle given pelleted barley to appetite (Eadie, Hobson & Mann, 1967). When restricted amounts of the same diet are given in three equal feeds daily very large rumen-ciliate populations will develop; this is believed to be due to changes in the extent and duration of falls in rumen pH (Whitelaw, Eadie, Mann & Reid, 1972). Ørskov & Fraser (1972) note that, when sheep are given to appetite whole, as opposed to pelleted barley, there is a marked increase in rumen pH. Experiments were carried out to determine whether rumen ciliates could survive when whole barley was given.

Two 3-year-old, cannulated Suffolks and two slightly younger Cheviots were used. A similar rumen-ciliate population was present in all four sheep. From a limited roughage–concentrate ration they were gradually changed to consuming either whole or pelleted barley to appetite, by way of an initial period of feeding three times/d. One animal of each breed was given whole barley and the controls the pelleted diet; both diets consisted of, per kg, 900 g barley and 100 g of a pelleted protein–mineral–vitamin supplement. Ciliates were not seen in the controls after a daily intake of 1650 g had been reached. Both *Entodinium* spp. and *Isotricha* spp. survived in the animals on whole barley up to a daily intake of 3150 g in set feeds and throughout the 6-week period of feeding to appetite. This included 1 week in which the intake of the Suffolk was 27 kg.

In a second experiment eight fattening lambs were given one mixed rumen-ciliate inoculum at 3·5 months old, when they had been eating to appetite for 9 weeks; four animals received whole barley and the controls the pelleted diet. Ciliates were not present before the inoculum. Rumen samples were examined 3, 7 and 14 d after inoculation, during which period food consumption was slightly lower in the controls. No ciliates were seen in samples from the animals fed on pelleted barley. Populations of *Entodinium* spp. and *Isotricha* spp., similar in number to those found with roughage diets, developed in all four lambs on whole barley. *Polyplastron* and *Dasytricha* were also present in two animals. Contrary to previous records, active ciliates were seen in samples with over 40% propionic acid in the volatile acid mixture.

The fact that rumen ciliates were able to survive, and even reach reasonable numbers, in animals fed on whole barley to appetite is a clear indication that rumen conditions with that diet were more stable than those when the same amount, or even less, barley was being consumed in the pelleted form.

REFERENCES

Eadie, J. M., Hobson, P. N. & Mann, S. O. (1967). *Anim. Prod.* **9**, 247.
Ørskov, E. R. & Fraser, C. (1972). *Proc. Nutr. Soc.* **31**, 101A.
Whitelaw, F. G., Eadie, J. M., Mann, S. O. & Reid, R. S. (1972). *Br. J. Nutr.* **27**, 425.

Energy metabolism of growing rats. 1. Effect of energy and protein intake on energy retention. By K. J. McCracken, *Agricultural and Food Chemistry Research Division, Ministry of Agriculture, Northern Ireland, and Agricultural Chemistry Department, Queen's University, Belfast*

McCracken (1968) showed that when differences in the pattern of intake are controlled by force-feeding, the heat production of rats fed on a low-protein diet is less than that of litter-mates fed on a normal-protein diet in isoenergetic amounts intended to maintain energy equilibrium. Stock (1972) 'pair-gained' two groups of rats trained to eat their daily ration in a 2 h period, and found that the heat production of the rats fed on the low-protein diet was higher than that of the controls. However, the differences in energy intake and therefore in energy retention between the two groups led to problems in the interpretation of his results. In order to extend the previous results of McCracken (1968) to a wider range of intakes and to obtain a better statistical measure of differences between treatments, a 3×3 factorial experiment has been conducted with fifty-four rats. The factors were dietary protein content (50, 100, 200 g/kg) and energy intake (1, 2, 2·5 MJ/kg starting weight per d). Male Lister hooded rats were weaned at 21 d of age on to the diet containing 200 g protein/kg. From 28 d of age (45 g body-weight) they were accustomed to force-feeding by gastric intubation during a 3 d period and then randomized to the experimental treatments. In addition, a number of animals were killed as starting controls. The experiment was conducted for a 7 d period, the rats being fed three times daily. Energy retention was assessed by the comparative slaughter technique. The results are summarized in Table 1.

Table 1. *Effect of energy and protein intake on energy retention and heat production of rats*

Energy intake	Low			Medium			High			SE of a mean
Protein level (g/kg)	50	100	200	50	100	200	50	100	200	
Metabolizable energy intake (kJ/d)	58·8	58·5	58·5	102·8	103·4	107·6	128·3	131·5	133·3	
Energy gain (kJ/d)	9·2	6·9	3·3	35·2	35·2	37·5	52·5	58·1	56·3	1·78
Heat production (kJ/d)	49·6	51·6	55·2	67·6	68·2	70·1	75·8	73·4	77·0	1·74
Heat production (MJ/d per kg)	0·97	0·96	0·98	1·22	1·16	1·14	1·31	1·18	1·19	0·03

The results were analysed statistically using the analysis of variance method. Carcass fresh weight, dry matter and protein increased significantly ($P<0·001$) with increase in energy intake or dietary protein. Carcass fat increased significantly

($P<0.001$) with increase in energy intake and decreased significantly ($P<0.001$) with increase in dietary protein. Daily heat production and energy retention increased significantly ($P<0.001$) with increase in energy intake but were unaffected by increase in dietary protein. Heat production per kg body-weight was not significantly affected by dietary protein at the two lower levels of energy intake, but on the high intake the heat production of the rats fed on the 50 g protein/kg diet was significantly higher ($P<0.01$) than that of the other two groups.

REFERENCES

McCracken, K. J. (1968). *Proc. Nutr. Soc.* **27**, 41A.
Stock M. J. (1972). *Proc. Nutr. Soc.* **31**, 15A.

Energy metabolism of growing rats. 2. Estimation of efficiency of deposition of protein and fat. By K. J. MCCRACKEN, *Agricultural and Food Chemistry Research Division, Ministry of Agriculture, Northern Ireland, and Agricultural Chemistry Department, Queen's University, Belfast*, and S. T. C. WEATHERUP, *Biometrics Division, Ministry of Agriculture, Northern Ireland, and Biometrics Department, Queen's University, Belfast*

There is considerable disagreement in the literature as to the efficiency of deposition of protein in non-ruminants. From theoretical considerations Blaxter (1962) calculated a value of 25·9 kJ/g protein deposited and a similar calculated value was reported by Schiemann (1963). Kielanowski (1965) concluded that a value of 28–32 kJ/g protein was probably applicable in all growing animals and reported a value of 48 kJ/g fat deposition in young pigs. However, Kielanowski & Kotarbinska (1970) reported values of 66 kJ/g and 53 kJ/g and Thorbek (1970) obtained values of 50 kJ/g and 51 kJ/g respectively for protein and fat deposition in the growing pig. In view of the lack of information in the rat and the similarity between the pig and the 'meal-fed' rat in the pattern of tissue deposition, an attempt has been made to estimate coefficients for fat and protein deposition using the results presented in the previous paper (McCracken, 1973).

Analysis of variance of energy retention *v.* energy intake at each level of dietary protein revealed that the three lines did not deviate significantly from parallelism and furthermore they were coincident. The equation for the pooled regression (kJ/d) is:

$$E\ (\text{stored}) = 0.675\ E\ (\text{intake}) - 33.5 \quad (r=0.996).$$

Consequently a multiple regression of the form $E\ (\text{intake}) = AW^{0.75} + BP + CF$ was applied to the pooled results. By calculating the line through the origin an equation of the form

$$E\ (\text{kJ/d}) = \underset{\pm 16}{468}\ W^{0.75} + \underset{\pm 4.2}{31.7}\ P + \underset{\pm 1.9}{52.1}\ F \qquad (W, \text{kg; P and F, g})$$

is obtained and all the coefficients are highly significant. The regression was also calculated within the range of the values. In this instance the equation becomes

$$E\ (\text{kJ/d}) = \underset{\pm 432}{670}\ W^{0.75} + \underset{\pm 11.2}{26.9}\ P + \underset{\pm 3.3}{50.9}\ F - \underset{\pm 45.8}{21.4} \quad (r=0.982)$$

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but only the coefficient for fat deposition is highly significant. However, the size of the coefficients in the two equations is similar and the results are in good agreement with the estimates made by Kielanowski (1965) in young pigs and lambs and by Walker & Norton (1971) in milk-fed lambs.

REFERENCES

Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants.* London: Hutchinson & Co. Ltd.
Kielanowski, J. (1965). *Publs Eur. Ass. Anim. Prod.* no. 11, p. 13.
Kielanowski, J. & Kotarbinska, M. (1970). *Publs Eur. Ass. Anim. Prod.* no. 13, p. 145.
McCracken, K. J. (1973). *Proc. Nutr. Soc.* **32**, 65A.
Schiemann, R. (1963). *Deutsche Akademie der Landw. Sitzungsberichte* **12**, 39.
Thorbek, G. (1970). *Publs Eur. Ass. Anim. Prod.* no. 13, p. 129.
Walker, D. M. & Norton, B. W. (1971). *J. agric. Sci., Camb.* **77**, 363.

A sex difference in scorbutic guinea-pig survival. By A. ODUMOSU and C. W. M. WILSON, *Department of Pharmacology, University of Dublin, Trinity College, Dublin* 2, *Republic of Ireland*

Odumosu & Wilson (1971) have shown that in times of ascorbic acid deficiency some female guinea-pigs can readjust their ascorbic acid metabolism and so compensate for the influence of the defective gene responsible for ascorbaemia (Stone, 1966), although males appear to be incapable of doing so. The extent to which the availability of gulono-lactone is the determining factor for this readjustment is unknown.

Twelve male and twelve female guinea-pigs, after receiving a normal supplemented diet for 6 weeks (Odumosu & Wilson, 1970*a*, *b*), were transferred to scorbutogenic diets with saline, intraperintoneal injection, and water *ad lib.* for 12 d (♂), and 20 d (♀), when the maximum points in the scorbutic weight curves are attained. Both groups were then subdivided into one subgroup receiving gulono-lactone supplement (30 mg/kg daily intraperitoneal injection) (GS), whereas the other

Table 1. (1) *Mean weights and alterations from mean* (*g*) *at days* 12 (♂) *and* 20 (♀), (2) *numbers losing weight and* (3) *nos remaining alive in groups of six* ♂ *and* ♀ *guinea-pigs fed on scorbutogenic diets and receiving daily supplements of saline* (*SS*) *or gulono-lactone* (*GS*)

	Males						Females					
Time on diet (d)	SS			GS			SS			GS		
	1	2	3	1	2	3	1	2	3	1	2	3
0	484·8±11·5	0	6	467·0±15·6	0	6	460·8±21·7	0	6	459·5±11·5	0	6
6	+29·2	0	6	+25·8	0	6	+7·2	0	6	+3·0	0	6
12	+31·4	3	6	+31·7	2	6	+9·7	1	6	+14·0	0	6
18	−5·6	6	3	−12·8	5	4	+11·0	2	6	+24·0	1	6
20	−64·0	3	3	−13·7	2	4	−7·3	5	6	+37·0	1	6
24	−107·0	3	3	−15·0	2	4	−13·5	4	6	−9·8	3	6
30	—	−	0	+30·5	1	2	−62·6	4	6	−21·3	4	6
36	—	−	0	+68·5	2	2	−12·0	2	2	−12·0	3	5
42	—	−	0	+108·0	2	2	−72·0	1	1	+93·0	3	3
52	—	−	0	+63·0	2	1	−25·0	1	1	+87·0	1	3
60	—	−	0	+35·0	1	0	—	−	0	+87·0	2	3
80	—	−	0	—	−	0	—	−	0	+39·0	3	2
84	—	−	0	—	−	0	—	−	0	+80·0	0	1
90	—	−	0	—	−	0	—	−	0	+86·0	0	1

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continued to receive saline (SS). All the subgroups continued on the scorbutogenic diet, and were weighed at 3 d intervals. Table 1 shows the alterations in weight and survival times in the different subgroups. All the unsupplemented males lost weight and the last one died on day 29, as in previous experiments (Odumosu & Wilson, 1970*a*, *b*, 1971). The last unsupplemented female finally died on day 58, having survived for 29 d longer than her male counterpart. The last GS male was losing weight, and died on day 60. The second of the surviving GS females finally died on day 82 and the third one continued to gain weight and survive up to day 90 when she was returned to the normal supplemented diet.

It has been confirmed that male and female guinea-pigs react differently to the stress of ascorbic acid deficiency, females having a shorter initial weight gain, and surviving longer than males. This can be attributed to an increase in tissue ascorbic acid values after the initial fall in the surviving females (Odumosu & Wilson, 1971). Administration of gulono-lactone can more than double life expectancy in scorbutic male guinea-pigs. In female guinea-pigs gulono-lactone administration prolongs their life expectancy; the longer life of the female in comparison with the male is maintained.

REFERENCES

Odumosu, A. & Wilson, C. W. M. (1970*a*). *Br. J. Pharmac.* **40**, 171P.
Odumosu, A. & Wilson, C. W. M. (1970*b*). *Br. J. Pharmac.* **40**, 548P.
Odumosu, A. & Wilson, C. W. M. (1971). *Br. J. Pharmac.* **42**, 637P.
Stone, I. (1966). *Perspect. Biol. Med.* **10**, 133.

The effect of level of feeding and protein concentration on disappearance of protein in different segments of the gut in sheep. By E. R. ØRSKOV and C. FRASER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

When increments of soya-bean meal (Ørskov, Fraser & McDonald, 1971*a*) or fish meal (Ørskov, Fraser & McDonald, 1971*b*) were given as supplements to a barley-based diet, substantial increases in the amount of protein passing the abomasum were noted. The increases paralleled those in N retention observed when young ruminants were given diets with increasing concentrations of crude protein (Andrews & Ørskov, 1970). These results differed from those of Hogan & Weston (1967) who did not find any differences in the amount of protein passing the abomasum with diets containing 78 or 198 g crude protein/kg. There were, however, large differences in level of food intake, which was about 450 g dry matter/d in the experiment of Hogan & Weston (1967) and about 1000 g/d in our experiments.

The experiments described here were conducted to investigate the effect of level of feeding on the quantity of protein escaping degradation in the rumen.

Two barley-based diets with differing amounts of soya-bean meal containing 119 and 192 g crude protein/kg dry matter were used. They were given to four sheep weighing approximately 45 kg live weight in four amounts, namely 325, 650, 975 and 1300 g/d.

The sheep were fitted with cannulas in the abomasum and terminal ileum. The technique of sampling was similar to that used by Ørskov *et al.* (1971*a*) except that

a mixture of Cr-EDTA and Cr_2O_3 was used as an indigestible marker. The results relating to the digestion of protein are given in Table 1.

Table 1. *Crude protein intake and disappearance in rumen, small and large intestine, and excretion in the faeces of sheep*

(Each value is the mean of four observations)

Diet	Dry-matter intake (g/d)	Crude protein		Non-ammonia crude protein disappearing in small intestine (g/d)	Crude protein	
		Intake (g/d)	Disappearing in rumen (g/d)		Disappearing in large intestine (g/d)	Excreted in faeces (g/d)
Low-protein 1	276	33	−14	21	11	13
Low-protein 2	562	66	−22	50	14	22
Low-protein 3	838	100	−33	73	27	32
Low-protein 4	1106	132	−37	105	15	42
High-protein 1	280	54	3	18	17	13
High-protein 2	559	108	0	64	17	24
High-protein 3	835	161	4	104	12	37
High-protein 4	1118	214	4	134	26	45
SE of means			11	10	3	2

Level of feeding was found to affect the extent of degradation of dietary protein. When the two lower amounts of the diets were given there were no significant differences between the two diets in the amount disappearing in the small intestine. Differences became apparent ($P<0.05$) at the two higher feeding levels. There were no differences between the two diets either in the rate of disappearance of crude protein in the large intestine or in the rate of excretion in the faeces.

The results show that the level of feeding and presumably the rumen retention time influenced greatly the extent of degradation of dietary protein. The results may help to explain differences between the results obtained in different laboratories, though other sources of protein are likely to give different results (Schoeman, deVet & Burger, 1972). In this respect chemical protection of dietary protein was shown by Hughes & Williams (1970) to have a much more pronounced effect on wool growth when the level of feeding was low.

REFERENCES

Andrews, R. P. & Ørskov, E. R. (1970). *J. agric. Sci., Camb.* **75**, 19.
Hogan, J. P. & Weston, R. H. (1967). *Aust. J. agric. Res.* **18**, 973.
Hughes, J. G. & Williams, G. L. (1970). *Anim. Prod.* **12**, 366.
Ørskov, E. R., Fraser, C. & McDonald, I. (1971*a*). *Br. J. Nutr.* **25**, 225.
Ørskov, E. R., Fraser, C. & McDonald, I. (1971*b*). *Br. J. Nutr.* **25**, 243.
Schoeman, E. A., deVet, P. J. & Burger, W. J. (1972). *Agroanimalia* **4**, 35.

An interaction between organic sulphur and molybdenum affecting copper metabolism in sheep. By N. F. SUTTLE, *Moredun Research Institute, Edinburgh EH1 7JH*

The interrelationship between dietary sulphur, molybdenum and copper is at present thought to involve only the sulphate component of dietary S, the presence

of adequate dietary SO_4 being required for Mo to inhibit the storage of Cu in the liver of sheep (Dick, 1953*a*, *b*; Underwood, 1971). The organic component of dietary S has not been studied in this context, however. Most of the S in herbage is in the organic form as a constituent of proteins which are hydrolysed to yield amino acids in the rumen. The ability of organic S to interact with Mo has, therefore, been studied, using a S-amino acid as the organic S source.

Four groups of five hypocupraemic ewes were repleted with a Cu-supplemented, semi-purified diet (8 mg Cu/kg) containing one of two levels of dietary S, 1 or 4 g/kg and of Mo, 0·5 or 4·5 mg/kg, in a 2×2 factorial experiment. S was added as methionine and Mo as ammonium molybdate (Expt 1). In a similar 2×2 experiment (Expt 2) inorganic sulphate, as $Na_2SO_4.10H_2O$, was substituted for methionine. The increases in plasma Cu concentration after 35 d were used to assess the efficiency of Cu utilization and the results are given in Table 1.

Table 1. *Effects of dietary sulphur and molybdenum on the recovery in plasma copper shown by initially hypocupraemic ewes given a Cu-supplemented diet* (8 *mg/kg*)

	Dietary treatment			
	None	S	Mo	Mo+S
Dietary concentration				
S(g/kg)	1	4	1	4
Mo(mg/kg)	0·5	0·5	4·5	4·5
Increase in plasma Cu after 35 d (μg/l):				
Expt 1 (organic S) (five ewes/group)	405±77	367±117	522±114	50±33
Expt 2 (inorganic sulphate S) (seven ewes/group)	562±53	387±90	497±67	−35±38

There was an equally marked interaction between the two forms of dietary S and Mo. Mo *per se* had no effect on plasma Cu, and S *per se* depressed the recovery in plasma Cu to only a small extent. The combined Mo+S treatment inhibited the recovery almost completely, however, giving significant Mo × S interactions ($P<0·05$, Expt 1; $P<0·01$, Expt 2). Past emphasis on the inorganic sulphate component of the dietary S has probably been misplaced and it is suggested that total S rather than inorganic sulphate should be measured in experimental and field survey work relating to clinical Cu deficiency in ruminants. The nutritional inadequacy of a diet containing 8 mg Cu/kg when dietary S and Mo were increased to commonly encountered levels suggests that the Cu × Mo × S interrelationship may have a more widespread involvement in hypocuprosis than has previously been realized.

REFERENCES

Dick, A. T. (1953*a*). *Aust. vet. J.* **29**, 18.
Dick, A. T. (1953*b*). *Aust. vet. J.* **29**, 233.
Underwood, E. J. (1971). *Trace Elements in Human and Animal Nutrition* p. 66. New York: Academic Press.