The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications. These are published as received from authors.*

ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Sixty-sixth Scientific Meeting of the Nutrition Society (One Hundred and Sixth of the Scottish Group) was held in the Assembly Hall of the School of Agriculture, University of Aberdeen, King Street, Aberdeen, on Friday, 22 March 1974, *at* 09.00 *hours, when the following papers were read :*

The simultaneous measurement of digesta flow into and out of the small intestine of the sheep using an automatic sampler. By M. V. Tas, N. W. Offer, R. A. Evans and R. F. E. Axford, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor*

Automatic sampling from the duodenum of the sheep has been described (Evans, Axford & Offer, 1971). The technique has been further developed to take and retain representative samples from the total digesta passing through re-entrant cannulas in the proximal duodenum and terminal ileum. This has been applied to a 30 kg Welsh Mountain wether for a continuous sampling period of 34 d. The animal was fed on a ration of hay and concentrate pellets at different levels of intake, giving daily intakes of dietary nitrogen of 12·8, 9·6, 6·4, 12·8 g for the four periods indicated on Fig. 1 by arrows at the changeover points.

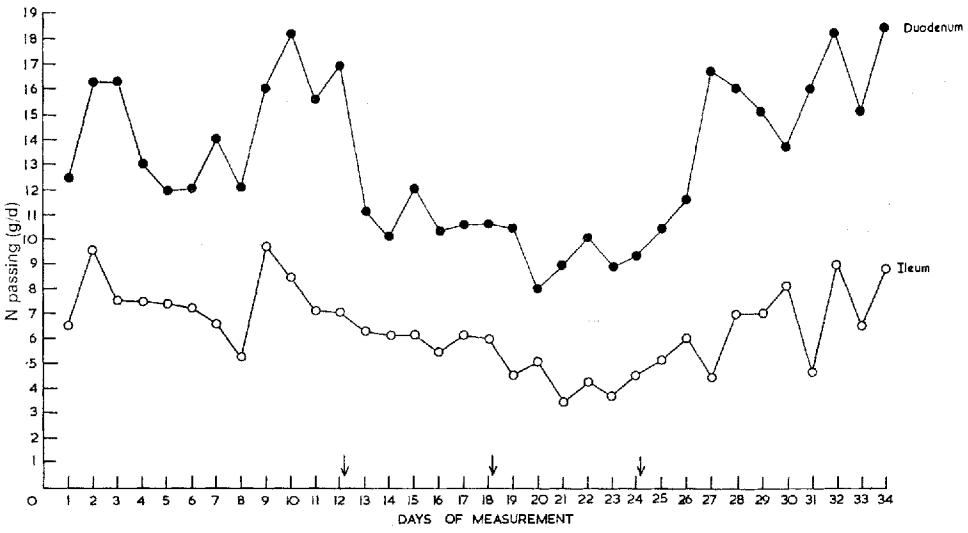


Fig. 1. Flow of digesta nitrogen (g/d) through small intestine of sheep.

The figure illustrates the relationship between the flows of N at the two sampling sites. When the apparent absorption from the small intestine was compared with the flow of N into it on a daily basis, the correlation coefficient was 0·85 ($P<0·001$).

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

The relationship was represented by the equation:

$$\text{N absorption (g)} = -1{\cdot}55 + 0{\cdot}63\ (\text{N flow at duodenum (g)})$$

REFERENCE

Evans, R. A., Axford, R. F. E. & Offer, N. W. (1971). *Proc. Nutr. Soc.* **30**, 40A.

Determination of portal and hepatic metabolite production rates in the adult dairy cow. By G. D. BAIRD, H. W. SYMONDS and R. ASH, *ARC Institute for Research on Animal Diseases, Compton, Newbury RG16 0NN, Berks.*

Dairy cows in which polyvinyl cannulae were implanted surgically have been used for the measurement of rates of blood flow in the portal vein and the liver, and also for the measurement of metabolite production rates in the portal bed and liver. The techniques used for implanting the cannulas have been described (Symonds & Baird, 1973). Blood flow rate was determined by the indicator-dilution method using *p*-aminohippuric acid (Katz & Bergman, 1969). Metabolite production rates were calculated from the measured blood flow rates, and from the metabolite concentrations determined in blood samples taken as nearly simultaneously as possible from a carotid artery, the portal vein and an hepatic vein.

Table 1 records some metabolite concentrations that were measured in whole-blood samples obtained from a lactating dairy cow. The concentrations of the volatile fatty acids were determined by gas–liquid chromatography, performed essentially as described by Weigand, Young & McGilliard (1972), and the concentrations of the remaining metabolites by spectrophotometric techniques.

Table 1. *Metabolite concentrations and portal and hepatic production rates in a lactating cow in vivo*

(The values were obtained by averaging from three to five separate observations. Over the period of sampling, the animal had been cannulated between 30 and 60 d and the milk yield amounted to between 13 and 18 l/d)

Metabolite	Metabolite concentrations (mmol/l)			Metabolite production rates (mmol/min)	
	Carotid artery	Portal vein	Hepatic vein	Portal*	Hepatic†
Glucose	2·84	2·79	3·03	−1·25	+7·72
Lactate	0·38	0·45	0·32	+1·80	−3·58
Hydroxybutyrate	0·86	1·02	1·16	+4·10	+5·97
Acetate	3·13	4·52	5·14	+34·75	+31·68
Propionate	0·05	0·45	0·06	+10·15	−9·69
Butyrate	0·05	0·21	0·09	+3·90	−2·84
Alanine	0·16	0·19	0·17	+0·68	−0·51
Ethanol	—	0·07	0·04	Not calculable	

*Positive portal production indicates net uptake from gut; negative production indicates net utilization by gut.

†Positive hepatic production indicates net production by liver; negative production indicates net utilization by liver.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

The blood flow rates that were obtained for this same animal were 25 l/min and 33 l/min for portal and hepatic flow rates respectively. Using these values for flow rate, and the metabolite concentration data, portal and hepatic production rates were calculated for each of the metabolites except ethanol, and these production rate values are also included in Table 1. The implications of some of these data will be discussed.

REFERENCES

Katz, M. L. & Bergman, E. N. (1969). *Am. J. Physiol.* **216**, 946.
Symonds, H. W. & Baird, G. D. (1973). *Res. vet. Sci.* **14**, 267.
Weigand, E., Young, J. W. & McGilliard, A. D. (1972). *Biochem. J.* **126**, 201.

Metabolism of propionate by the portal-drained viscera in sheep. By T. E. C. WEEKES and A. J. F. WEBSTER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Direct measurements were made of the extent of propionate metabolism, L-lactate formation and glucose uptake by the portal-drained viscera of conscious sheep at low or high rates of propionate infusion into the rumen. Five sheep were prepared with rumen cannulas and indwelling catheters in the portal vein, anterior mesenteric vein and the aorta (Webster & White, 1973). After recovery, the sheep were usually given 1 kg dried grass/24 h, but were fasted overnight before each trial. The volatile fatty acids, acetate, propionate and butyrate, were administered to the rumen by giving first a priming dose of 54, 162 and 69 mmol (low-propionate) or 54, 200 and 87·5 mmol (high-propionate), respectively. This was followed by infusion for 6 h of 69·2, 40·0 and 30·0 mmol/h (low-propionate) or 69·2, 79·9 and 36·0 mmol/h (high-propionate), respectively. Rumen liquor volatile fatty acid concentrations reached a plateau by 4 h after the start of the infusion. Portal and aortic blood was sampled every 30 min between 4 and 6 h after the start of the infusion and portal blood flow estimated by continuous thermal dilution (Webster & White, 1973). Blood propionate concentrations were determined by freeze-transfer and gas–liquid chromatography (P. J. Barker & D. B. Lindsay, unpublished results). Results are presented in Table 1.

Table 1. *The net uptake or production of propionate, lactate and glucose* (μmol/*min per kg* $W^{0.75}$) *in the portal-drained viscera of sheep during intraruminal infusion of volatile fatty acids*

(Mean values with their standard errors; number of experiments shown in parentheses)

	Infusion	
	Low-propionate	High-propionate
Rate of propionate infusion	27·3±2·1 (6)	61·4± 3·1 (11)
Net uptake of propionate	23·4±3·7 (3)	55·8±12·6 (7)
Net production of lactate	7·7±0·4 (6)	6·9± 0·9 (11)
Net utilization of glucose	6·9±1·1 (6)	9·7± 1·4 (11)

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

The net amounts of propionate appearing in the portal blood averaged 86% (low-propionate) and 91% (high-propionate) of the intraruminal propionate infusion rates. The net amounts of lactate formed and glucose utilized by the portal-drained viscera were not significantly affected by the propionate infusion rate. Glucose utilization was always sufficient to account for the amounts of lactate formed. These results suggest that only a very limited direct conversion of propionate into lactate occurs in the rumen mucosa of sheep, in agreement with in vitro incubation studies (Weekes, 1973) and in vivo experiments in cattle (Weigand, Young & McGilliard, 1972).

REFERENCES

Webster, A. J. F. & White, F. (1973). *Br. J. Nutr.* **29**, 279.
Weekes, T.E.C. (1973). Observations on the metabolic role of the rumen epithelium. PhD Thesis, University of Aberdeen.
Weigand, E., Young, J. W. & McGilliard, A. D. (1972). *Biochem. J.* **126**, 201.

The measurement and prediction of the digestibility of mixed diets containing silage. By F. G. PALMER, T. B. MILLER and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Apart from the work of Griffiths, Spillane & Bath (1973), there is little published information on the apparent digestibility of mixed diets based on silage. The digestibility of diets containing either all silage or 80, 60 or 40% of the dry matter from silage, with the remainder supplied from either barley or hay, was determined using Friesian steers weighing approximately 400 kg. The silage and hay contained (g/kg) 187 and 855 dry matter and 33 and 63 crude protein, respectively. Several chemical determinations were made on the diets and the accuracy of the prediction of digestibility from these values examined.

Regression analysis showed that for those diets containing barley, the apparent digestibility of organic matter declined by 0·013 ± 0·004 for every 20% increase in silage content but for those diets containing hay, the apparent digestibility of

Table 1. *Apparent digestibility ratios of organic matter, gross energy and crude protein and the organic matter digestibility ratios measured in vitro of the eight diets*

		Digestibility determined in vivo			Organic matter digestibility
Diet	Composition (w/w)	Organic matter	Gross energy	Crude protein	measured in vitro
Silage–barley	100:0	0·756	0·758	0·698	0·677
	80:20	0·757	0·742	0·668	0·727
	60:40	0·780	0·749	0·606	0·755
	40:60	0·799	0·769	0·577	0·788
SE		0·014	0·008	0·037	0·031
Silage–hay	80:20	0·729	0·717	0·621	0·676
	60:40	0·710	0·677	0·567	0·674
	40:60	0·688	0·648	0·368	0·667
	0:100	0·649	0·615	0·309	0·611
SE		0·023	0·031	0·093	0·016

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

organic matter and gross energy increased by 0·021 ±0·001 and 0·029 ±0·001 respectively for a corresponding increase in silage content. Organic matter digestibility determined in vivo could be accurately calculated from the digestibility of the two components for those diets containing hay and was significantly correlated ($P<0·01$) with digestibility determined in vitro. Measurement of the fibre or lignin content of the diets did not give values which accurately predicted the digestibility of organic matter or gross energy.

REFERENCE

Griffiths, T. W., Spillane, T. A. & Bath, I. H. (1973). *J. agric. Sci., Camb.* **80**, 75.

The digestion of fresh, frozen and dried perennial ryegrass. By D. E. Beever, S. B. Cammell and Annie Wallace, *Grassland Research Institute, Hurley, Maidenhead, Berkshire*

The validity of using herbage preserved by deep-freezing to represent the fresh material as harvested, for intake, digestion and metabolism has been questioned by ourselves and other workers (MacRae & Ullyatt, 1974).

To investigate this matter further, S23 perennial ryegrass was harvested daily between 8 May and 25 May 1973, and batches of herbage, containing approximately 750 g dry matter, were prepared and fed in two equal feeds during that day to four mature sheep fitted with re-entrant cannulas at the proximal duodenum and terminal ileum. Similar batches were either dried for 18 h at 103° in a forced-draught oven or frozen (−21° for 48 h) and stored at −5° prior to feeding to the same sheep in later periods.

After 10 d on each diet, a 24 h collection of ileal digesta was made, followed 2 d later by a 24 h measurement of volatile fatty acid (VFA) production (Weller, Gray, Pilgrim & Jones, 1967). Finally, 4 d later, a 24 h collection of duodenal digesta was made in conjunction with an intraruminal infusion of $Na_2{}^{35}SO_4$ to measure rumen microbial protein synthesis (Harrison, Beever & Thomson, 1972).

Table 1. *Nitrogen content of food given to sheep, and total N entering and leaving the small intestine of sheep given different diets*

(Mean values with their standard errors where shown)

	Diet			
Total N present (g/24 h)	Fresh	Frozen	Dried	SEM
In food	20·74	22·21	21·03	
At proximal duodenum	18·66	20·61	28·65	1·52
At terminal ileum	5·94	5·82	9·04	0·47

The quantities (g/24 h) of nitrogen consumed and entering and leaving the small intestine are shown in Table 1. Both the fresh and frozen diets had similar net losses of N across the rumen (2·1 and 1·6 g/24 h respectively), while the dried diet showed a net gain of 7·6 g/24 h ($P<0·01$). Similarly, losses of N within the small intestine

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

were unaffected by freezing (fresh 12·7, frozen 14·8 g N/24 h) but were significantly lower than the value recorded with the dried diet (19·6 g N/24 h; $P<0·05$).

The mean VFA production rates were comparable for all diets (fresh 3·53, frozen 3·33, dried 3·88 ($\pm$0·20) mol/24 h; $P>0·05$) reflecting the similarity in total ruminal digestion of soluble carbohydrates and cellulose noted on the three diets. However, when the energy contents of the total VFA were expressed as a percentage of ruminally digested energy (fresh 60%, frozen 58%, dried 73%) the results indicated little difference due to freezing of the herbage, but a substantial increase due to drying.

Compared with fresh herbage, the frozen herbage showed a 15% increase on the amount of amino acids entering the small intestine and a 26% increase in microbial protein synthesis. The corresponding values for the dried herbage (51% and 57% respectively) support the previous results of Beever, Thomson, Pfeffer & Armstrong (1969) and suggest the possible mechanisms of such.

The authors are indebted to Dr P. S. Bramley and I. R. A. D. Compton for the surgical preparation of the sheep.

REFERENCES

Beever, D. E., Thomson, D. J., Pfeffer, E. & Armstrong, D. G. (1969). *Proc. Nutr. Soc.* **28**, 26A.
Harrison, D. G., Beever, D. E. & Thomson, D. J. (1972). *Proc. Nutr. Soc.* **31**, 60A.
MacRae, J. C. & Ullyatt, M. J. (1974). *J. agric. Sci., Camb.* (In the Press.)
Weller, R. A., Gray, F. V., Pilgrim, A. F. & Jones, G. B. (1967). *Aust. J. agric. Res.* **18**, 107.

The estimation of body composition in beef cattle by deuterium oxide dilution. By R. M. CRABTREE, R. A. HOUSEMAN and M. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Deuterium oxide (D_2O) was used to estimate total body water (TBW) in six Friesian and six Hereford$\times$Friesian steers given diets which were intended to produce differences in body composition at slaughter. Three animals of each breed were slaughtered at 340 kg empty body-weight and the remaining six animals at 420 kg empty body-weight. D_2O was infused intravenously 24 h before slaughter and blood samples taken every 2 h for 12 h. The half carcass of each animal, together with the non-carcass parts and internal organs, was chemically analysed and the composition of the whole animal calculated.

Results indicated that D_2O over-estimated TBW by 9·6%, a value similar to that obtained in cattle by Carnegie & Tulloh (1968) using tritiated water. The concentration of D_2O in the rumen water was the same as that in the rest of the body after 5 h. Linear and multiple regression analyses were used to obtain equations to estimate body composition (Table 1). D_2O more accurately estimated empty body water (EBW) than TBW. The inclusion of live weight in the regression analyses increased the accuracy with which both TBW and EBW could be estimated. Total fat-free mass was calculated to within $\pm$3·40% and empty body nitrogen to within $\pm$6·91%. Live weight in conjunction with total D_2O space (DS) gave the best prediction of lipid in the empty body.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Table 1. *Regression equations for estimating the weight of body components from deuterium oxide space (DS): lipid in the empty body is estimated from DS and live weight (W)*

Body component	Coefficient	Intercept	RSD	RSD as % of mean	r^2	Mean value of component (kg)
Total body water	0·59	+87·14	10·53	4·25	0·85	247·6
Empty body water	0·53	+66·64	6·68	3·16	0·92	211·6
Total nitrogen	0·03	+3·09	0·75	6·91	0·74	10·9
Fat-free mass	0·75	+90·68	9·99	3·40	0·91	293·7
Lipid in empty body	0·86 W–0·68 DS	−99·69	12·99	13·99	0·87	92·2

RSD, residual standard deviation.

The DS was adjusted by the amount of D_2O present in the gastrointestinal tract to allow an estimate of EBW to be made. Using this as the independent variable, a more accurate estimation of the various body components could be made.

REFERENCE

Carnegie, A. B. & Tulloh, N. M. (1968). *Proc. Aust. Soc. Anim. Prod.* 7, 38.

Chemical composition of body-weight changes in lactating beef cows.

By T. E. Trigg, T. B. Miller and J. H. Topps, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Recent experiments in this laboratory have shown that lactating beef cows have widely varying responses when given a maintenance level of feeding or less. Some cows decline rapidly in milk yield, some maintain yield but lose considerable weight, while others are intermediate with respect to these characteristics. An attempt has been made to measure, using deuterium oxide (D_2O), the composition of body-weight changes in such cows and some results of this work are now presented.

Six Hereford × Friesian cows in mid-lactation were fed on a diet which provided approximately 460 kJ metabolizable energy (ME)/kg $W^{0.75}$ for 71 d and then on a diet giving 753 kJ ME/kg $W^{0.75}$ for 55 d. D_2O space was measured at the start and at the end of each feeding period after a 36 h fast on each occasion. To calculate some of the following results it was assumed that at each measurement rumen volume did not change appreciably and that the ratios of protein tissue:water and ash:water were constant at 1:3·4 and 1:13·8 respectively (McDonald, Edwards & Greenhalgh, 1973).

Tissue specific energy losses ranged from 30·0 to 38·7 MJ/kg and were two or more times higher than the corresponding gains, except for animal 6. The high- and medium-yield cows, when underfed, lost both considerable amounts of fat and significant quantities of protein. On refeeding, all animals showed appreciable gains in body protein but the four higher-yielding animals recovered only a little of the fat used during undernutrition (Table 1).

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Table 1. *Changes in body-weight, deuterium oxide space (DS) and body tissue (g/d) in six Hereford × Friesian lactating cows which were subjected to a period of undernutrition followed by refeeding*

		Undernutrition				Refeeding			
Animal No.	Milk yield	Weight loss	DS decrease	Protein loss	Fat loss	Weight gain	DS increase	Protein gain	Fat gain
3	High	1368	171	51	1134	382	236	70	59
8	High	1487	289	86	1091	436	345	102	−36
2	Medium	842	92	27	716	582	400	119	34
7	Medium	1000	176	52	759	791	509	151	94
4	Low	421	−100	−30	558	927	491	145	255
6	Low	329	−26	−8	365	854	127	38	680

REFERENCE

McDonald, P., Edwards, R. A. & Greenhalgh, J. F. D. (1973). *Animal Nutrition* 2nd ed. Edinburgh: Oliver & Boyd.

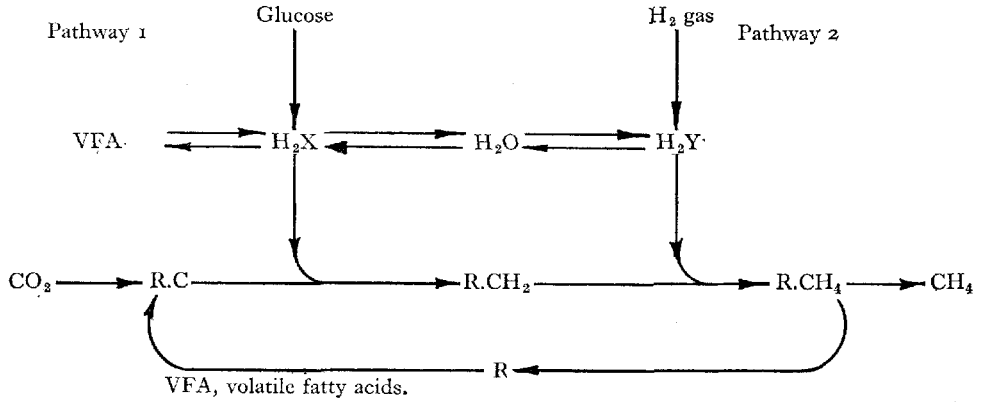
Use of tritium compounds in the study of methanogenesis. By J. W. CZERKAWSKI and GRACE BRECKENRIDGE, *Hannah Research Institute, Ayr KA6 5HL*

When diluted rumen contents are incubated under hydrogen gas (e.g. Czerkawski, Harfoot & Breckenridge, 1972), each mol of methane formed is associated with an uptake of 4 mol H_2, according to the equation: $CO_2+4H_2 \rightarrow CH_4+2H_2O$. However, as the concentration of rumen contents is increased, the ratio decreases and approaches a value of 2 with undiluted rumen contents. It can readily be shown that CO_2 is a substrate, by incubating rumen contents with [^{14}C]carbonate, and showing that the specific radioactivity of CH_4 formed is the same as that of CO_2.

When undiluted rumen contents were incubated under [^{3}H]hydrogen, CH_4 was formed at 0·5 mol/mol H_2 used, but the CH_4 was not labelled. The radioactivity in H_2 gas disappeared rapidly and could be recovered in good yield in water, but the specific activity of water was very low and it was unlikely that CH_4 would be labelled even if there was a flow of H from H_2 gas, through water, to CH_4. However, when rumen contents were incubated under H_2 with ^3H_2O in the reaction mixture, the specific activity of CH_4 (per g atom H) was found to be half of that of water. This suggested that half the H in CH_4 was derived from gaseous H_2 through water and that the rest came from the metabolism of substrates such as glucose. This was confirmed by incubating rumen contents under H_2 with [^{3}H]glucose, with ^3H_2O or with both, and showing that the labelling was in general additive. The results were consistent with the simple scheme shown on the next page.

Further experiments with chloroform (a potent inhibitor of methanogenesis) showed that the specific activity of the residual CH_4 did not change when [^{3}H]-glucose was incubated, but was lower than control with ^3H_2O in the reaction mixture.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press



This indicated that pathway 2 was more rapidly affected than pathway 1. This supports the previous conclusion that vitamin B_{12} derivatives might participate in pathway 2. In a similar experiment with [^{3}H]glucose, chloroform caused an increased incorporation of ^{3}H into butyric and valeric acids. The increased synthesis of these acids from acetate and propionate by the malonyl pathway would require NADPH, and suggests that this cofactor could participate in pathway 1.

A more detailed scheme involving various cofactors will be discussed.

REFERENCE

Czerkawski, J. W., Harfoot, C. G. & Breckenridge, G. (1972). *J. appl. Bact.* **35**, 537.

Lack of effect of intraruminal loading on short-term intake of a concentrate feed by sheep. By J. M. Forbes and T. Blair, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Experiments in which animals are accustomed to eating various foods before rate of eating is measured (e.g. Lofgren & Warner, 1972) are not necessarily appropriate for studying the short-term chemical control of feeding, because the animals might have learnt to associate the sensory qualities of each food with its eventual nutrient value (Booth, 1972).

We have fed rumen-fistulated sheep with a standard concentrate *ad lib.* for either 2 h in the morning and 0·5 h in the afternoon or 7 h each day, and made intraruminal additions of food (100 g), dried faeces (100 g) or glucose (0, 50, 100 or 200 g) in 500 ml water at the time of offering fresh food in the morning. Feed buckets were weighed 15, 30, 60 and 120 min later. In seven experiments, each with three sheep, involving a total of twenty-six treatments, there were no significant differences between weights of food eaten at any time in the first 2 h after offering food.

There was thus no evidence from these experiments that relatively large intraruminal additions of normal (food), bulky (faeces) or readily fermentable (glucose) material affected rate of eating in the short term. The amount of food eaten at a meal appears to be controlled by factors other than immediate absorption of products of the food eaten earlier in the current bout of feeding.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

REFERENCES

Booth, D. A. (1972). *J. comp. physiol. Psychol.* **81**, 457.
Lofgren, P. A. & Warner, R. G. (1972). *J. Anim. Sci.* **35**, 1239.

Changes in voluntary food intake, body-weight and metabolic rate with thyroxine treatment in sheep. By T. BLAIR and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Thyroxine has the well-documented ability to increase the metabolic rate of an animal. It was used, therefore, as a tool to examine the effect of changing metabolic rate on voluntary food intake, as a preliminary to work with other hormones.

Four wether sheep, initial weight range 50–56 kg, each acting as its own control, were given: no treatment, subcutaneous thyroxine injections (8–14 d) and alkaline saline injections. Dose rates were 1 mg monosodium-L-thyroxine/d for two sheep, while the other two were given 2 mg/d, both before and after shearing. Food intake and weight changes were recorded in all four sheep, before the heat production of one from each pair was estimated in a closed-circuit respiration chamber.

The two sheep given the lower level of thyroxine consumed more of a complete pelleted diet compared with their intakes during control periods ($P<0.05$) and whereas they gained weight during thyroxine treatment, they lost weight under control conditions. With the other pair on the higher level, intake was lower during thyroxine treatment than in control periods, both before and after shearing, although not all differences were statistically significant. Weight gains occurred during the control periods and weight losses during the thyroxine treatment. Respiration data were similar for both doses of thyroxine. With 1 mg thyroxine/d, oxygen consumption ($P<0.01$), heat production ($P<0.05$) and urinary nitrogen ($P<0.001$) were increased, the latter due to an accelerated protein catabolism. However, as food intake tended to be higher than for the controls (non-significant), the thyroxine treatment increased energy retention. The intake of the animal receiving 2 mg thryoxine/d was restricted to 28 g/kg live weight while in the chamber. Oxygen consumption, carbon dioxide production, heat production, urinary N (all $P<0.01$) and methane production ($P<0.05$) were higher than for the pretreatment control. Return to the control metabolic rate was not achieved until 7 d after the last thyroxine injection of 2 mg.

Thyroxine injections, therefore, were successful in increasing the metabolic rate of wether sheep. Voluntary food intake was either increased or decreased depending on the level of hormone used and weight changes affected correspondingly. Such a dose-response may help to explain the range of results achieved with thyroxine treatment of ruminants (Baile & Forbes, 1974).

We are grateful to the Agricultural Research Council for a grant to support this work.

REFERENCE

Baile, C. A. & Forbes, J. M. (1974). *Physiol. Rev.* **54**, 160.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

The relation between insulin and glucose and the concentration of amino acids in plasma and erythrocytes in sheep after feeding. By D. M. ANDERSON, J. L. MANGAN and P. C. WRIGHT, *Biochemistry Department, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

Plasma amino acids in sheep decrease significantly after feeding (Mangan & Wright, 1973). This could be due to gluconeogenesis or to stimulation of insulin secretion associated with feeding (Manns & Boda, 1967; Bassett, 1972). The present studies examine the relation between amino acid concentration and insulin secretion after feeding.

Six sheep were fed once daily on a hay–oat diet, 5:1 (w/w). Right atrial blood was obtained through an indwelling catheter for periods of 8 h. During feeding (2 h) there was no significant change in blood glucose concentration, results for twelve expts being $3{\cdot}30 \pm 0{\cdot}23$ to $2{\cdot}97 \pm 0{\cdot}22$ mmol/l ($\pm$SD). Overall insulin concentration increased during the 3 h after feeding, from $22{\cdot}5 \pm 2{\cdot}9$ to $48{\cdot}5 \pm 7{\cdot}5$ μU/ml ($P > 0{\cdot}01$), but the pattern of amino acid concentrations shown by 30 min sampling did not correlate with the insulin concentrations and this was again demonstrated in 3 d experiments (Fig. 1). This would indicate that a considerable proportion of the decrease in amino acids after feeding is not directly related to insulin concentration.

Whole blood concentrations of amino acids follow a similar pattern to plasma,

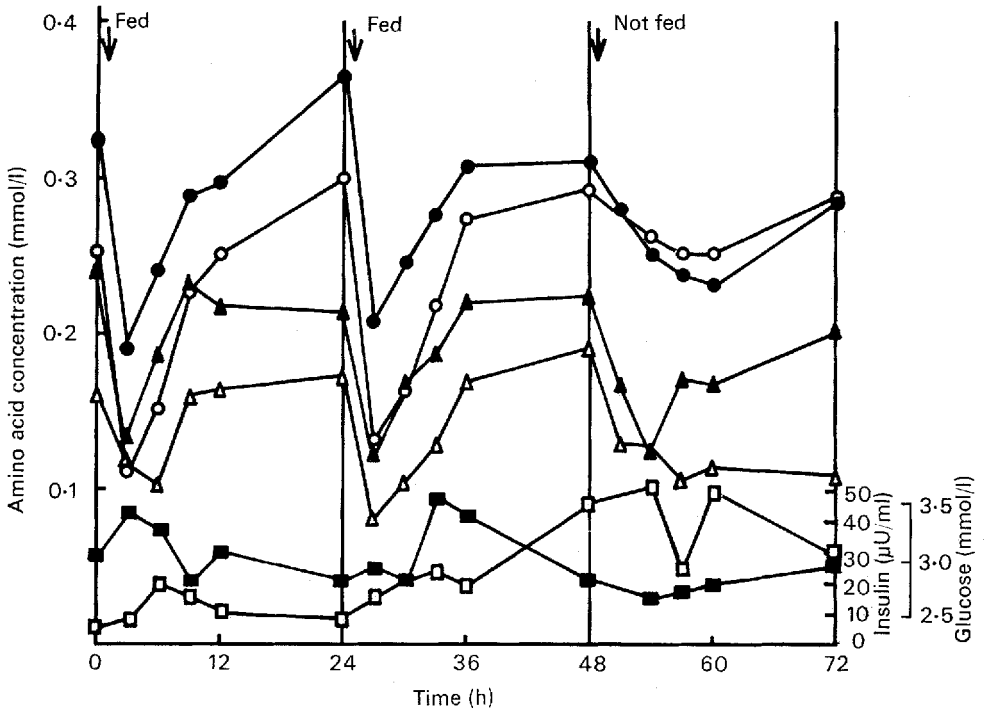


Fig. 1. Plasma concentrations of: total amino acids ($\times 10^{-1}$), —●—; glycine ($\times 0{\cdot}5$), —○—; alanine, —▲—; and leucine, —△—; in a sheep fed once daily at 09.00 hours for 2 d, followed by 1 d in which feeding was omitted. Corresponding values for insulin, —■—; and glucose, —□—.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

but some amino acids, particularly aspartic and glutamic acids and ornithine, were at a higher level due to high concentrations in the erythrocytes. Concentrations of amino acid in the erythrocytes fluctuated rapidly and again showed no relationship with insulin or glucose.

REFERENCES

Bassett, J. M. (1972). *Aust. J. biol. Sci.* **25**, 1277.
Mangan, J. L. & Wright, P. C. (1973). *Proc. Nutr. Soc.* **32**, 52A.
Manns, J. G. & Boda, J. M. (1967). *Am. J. Physiol.* **212**, 747.

Nature of the unusual branched-chain fatty acids in the triglycerides of the barley-fed lamb. By W. R. H. DUNCAN, A. K. LOUGH and G. A. GARTON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and P. BROOKS, *Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol BS8 1TS*

In earlier studies (Garton, Hovell & Duncan, 1972), in which lambs were fed on diets rich in barley, it was shown that the adipose tissue triglycerides contained relatively high proportions of *n*-fatty acids possessing an odd number of carbon atoms and of monomethyl branched-chain fatty acids. The production of these acids is associated with the availability of propionate (produced by ruminal fermentation of barley carbohydrate) in excess of that which can be metabolized normally, resulting in the utilization of propionate and its carboxylation product, methylmalonate, in fatty acid synthesis. Based on analysis of the chemical oxidation products of concentrates of the branched-chain acids, it was concluded that they were mainly monomethyl-substituted acids of chain length 14, 15, 16 and 17 carbon atoms.

Subsequently, more detailed analyses have been made, using high-resolution gas-liquid chromatography in conjunction with mass spectrometry. That the above monomethyl-substituted acids predominated was confirmed and, in addition, single methyl branches were found in acids of chain length 10, 11, 12 and 13 carbon atoms. Within each molecular species a number of positional isomers was identified, notably in respect of methyltetradecanoic acid (substituent in position 2, 4, 6, 8, 10 or 12) and methylhexadecanoic acid (substituent in position 2, 4, 6, 8, 12 or 14). Homologous series could also be recognized, in one of which all eight members ranging from 4-methyldecanoic acid to 4-methylheptadecanoic acid were identified.

Relatively small proportions of long-chain fatty acids with more than one methyl branch per molecule were also detected; these included five members of an homologous series of di-branched components ranging in chain length from 11 to 15 carbon atoms and having the substituent methyl groups at positions 4 and 8, and one tri-branched acid (2, 6, 10-trimethyltetradecanoic acid).

It is thus apparent that methylmalonate can substitute for malonate at any stage of chain lengthening of fatty acids synthesized from either acetate or propionate as terminal 'primer' unit, and that up to three methylmalonate residues can be utilized in this way.

REFERENCE

Garton, G. A., Hovell, F. D. DeB. & Duncan, W. R. H. (1972). *Br. J. Nutr.* **28**, 409.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Effect of different dietary cereals on the occurrence of branched-chain fatty acids in lamb fats. By W. R. H. DUNCAN, E. R. ØRSKOV and G. A. GARTON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Lambs given diets containing large amounts of rolled barley produce soft adipose tissue characterized by the presence, notably in the subcutaneous triglycerides, of abnormally high proportions of odd-numbered *n*-fatty acids and branched-chain fatty acids (many of novel constitution) which arise endogenously as a result of enhanced availability of propionate (Duncan, Ørskov & Garton, 1972; Garton, Hovell & Duncan, 1972*a*, *b*; Duncan, Lough, Garton & Brooks, 1974). Diets containing large amounts of whole barley also lead to the formation of triglycerides of unusual fatty-acid composition, though they contain less propionate-derived fatty acids than when processed barley is included in the diet (Duncan, Ørskov, Fraser & Garton, 1974). It was therefore of importance to determine whether the production of triglycerides of unusual fatty-acid composition was also associated with the feeding to lambs of diets having a high content (900 g/kg) of other whole cereals.

Groups of male lambs were fed to appetite, from weaning until they weighed 35–40 kg, on diets containing whole barley, whole oats, whole maize or whole wheat, and their subcutaneous triglycerides were analysed according to Garton *et al.* (1972*b*) to give the results shown in Table 1.

Table 1. *Proportions of odd-numbered* n*-fatty acids and branched-chain fatty acids in the subcutaneous triglycerides of lambs fed on diets containing whole cereals*

(Values, means for four lambs on each diet, are percentage by weight of total fatty acids)

		Branched-chain acids† (BA)		
Dietary cereal	Odd-numbered *n*-acids* (OA)	Total	Propionate-derived (excluding ante-iso)	Total OA+BA
Barley	7·4	9·1	6·1	16·5
Wheat	9·7	9·6	6·7	19·3
Maize	6·1	6·1	4·4	12·2
Oats	2·8	3·2	1·4	6·0

*Mostly 15:0, 17:0 and 17:1.

†Mostly monomethyl-substituted 14:0, 15:0, 16:0 and 17:0.

Whereas lambs fed on whole maize or whole wheat produced triglycerides containing unusual proportions of odd-numbered acids and branched-chain acids (including those of novel structure) similar to those observed when whole barley was given, the triglycerides of the lambs fed on whole oats contained only very small proportions of such acids. This difference between the effects of whole barley, maize and wheat on the one hand and whole oats on the other is evidently a reflection of the relative amounts of acetate and propionate available for absorption from the rumen (cf. Ørskov, Fraser & Gordon, 1974).

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

REFERENCES

Duncan, W. R. H., Lough, A. K., Garton, G. A. & Brooks, P. (1974). *Proc. Nutr. Soc.* **33**, 80A.
Duncan, W. R. H., Ørskov, E. R., Fraser, C. & Garton, G. A. (1974). *Br. J. Nutr.* **32**, 71.
Duncan, W. R. H., Ørskov, E. R. & Garton, G. A. (1972). *Proc. Nutr. Soc.* **31**, 19A.
Garton, G. A., Hovell, F. D. DeB. & Duncan, W. R. H. (*1972a*). *Proc. Nutr. Soc.* **31**, 20A.
Garton, G. A., Hovell, F. D. DeB. & Duncan, W. R. H. (*1972b*). *Br. J. Nutr.* **28**, 409.
Ørskov, E. R., Fraser, C. & Gordon, J. G. (1974). *Br. J. Nutr.* **32**, 59.

The composition of milk from cows fed on various fat-supplemented diets.

By W. BANKS, J. L. CLAPPERTON and MORAG E. FERRIE, *The Hannah Research Institute, Ayr KA6 5HL*

The physical properties of butter partially reflect the chemical composition of the milk fat and this may be influenced by the diet of the cow (Steele, Noble & Moore, 1971). To produce milk fats of distinct composition in an attempt to establish a relationship with physical properties, four Ayrshire cows in mid-lactation were used in a latin square design; each period lasted 21d and the cows were offered a basal ration of hay and molassed sugar beet pulp and, in proportion to their milk yield, one of four concentrate diets. One diet contained no added fat and the others contained 100 g/kg of, respectively, soya-bean oil, palm oil or tallow.

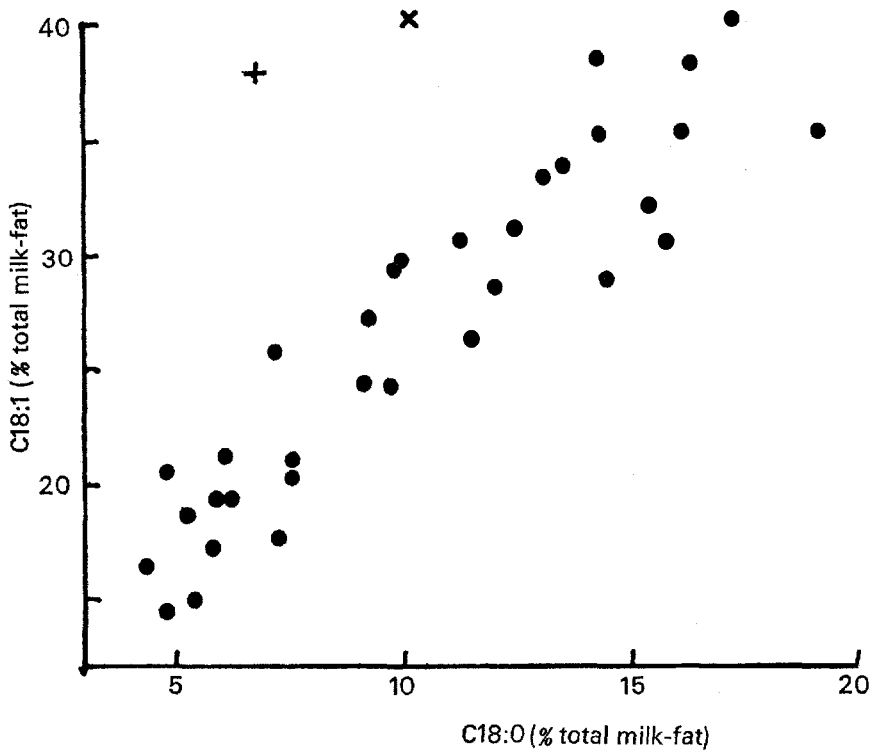


Fig. 1. The relationship of fatty acids C18:0 to C18:1 in milk from cows fed on various oil-supplemented diets; ●, present work; ×, Steele & Moore (1968) and +, Steele, Noble & Moore (1971) represent values obtained when fat-supplemented diets have caused the low milk-fat syndrome.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

All the fat additions increased the yield of butterfat and reduced the proportion of the C6:0 to C14:0 acids in the milk fat. Soya-bean oil reduced the content of C16:0 and C16:1 acids and increased that of C18:0 and C18:1. Palm oil increased the C16:0, C16:1 and C18:1 acids, and the addition of tallow reduced the C16:0 and increased the C18:0 and C18:1 acids.

The presence in the mammary gland of a microsomal desaturase specific for the conversion of C18:0 to C18:1 (Annison, Linzell, Fazakerley & Nichols, 1967) presupposes some relationship between these acids. The values obtained are shown in Fig. 1 and the relationship was:

$$y = 10{\cdot}45 + 1{\cdot}54x \ (r^2 = 0{\cdot}806),$$

where x and y are the weight percentages of C18:0 and C18:1. The values, however, also include varying amounts of C18:1-*trans* fatty acid and the relationship requires further investigation.

Also shown in Fig. 1 are two points taken from experiments (Steele & Moore, 1968; Steele *et al.* 1971) in which milk fat content was depressed and, in these instances, either a greater proportion of C18:1-*trans* is reaching the mammary gland or the equilibrium of the desaturase is displaced in favour of the C18:1-*cis* fatty acids.

REFERENCES

Annison, E. F., Linzell, J. L., Fazakerley, S. & Nichols, B. W. (1967). *Biochem. J.* **102**, 637
Steele, W. & Moore, J. H. (1968). *J. Dairy Res.* **35**, 353.
Steele, W., Noble, R. C. & Moore, J. H. (1971). *J. Dairy Res.* **38**, 49.

The effect of day length on the growth of lambs at two levels of feeding.

By A. EL-SHAHAT, R. JONES, J. M. FORBES and T. G. BOAZ, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

The voluntary intake of food by sheep is lower in winter than in summer (Gordon, 1964; Tarttellin, 1968) and it is possible that decreasing day length is responsible for the deceleration of growth sometimes observed in sheep and cattle in autumn. We have therefore compared the growth rates and carcasses of seventy-two lambs, sired by Down rams out of Finn–Blackface ewes, kept under 16 or 8 h artificial light/d and fed either *ad lib.* or at a restricted level (70 g/kg live weight$^{0.75}$ per d) on a barley-based concentrate feed. They were housed in September, 1973, when 5 months of age and weighing 22 kg, one female and two castrate males to a pen, with twelve pens in each of two controlled-environment rooms. Equal numbers were shorn or left unshorn. The treatments were continued until the animals were slaughtered after 15–17 weeks of light treatment.

At both levels of feeding the animals under the 16 h day length gained weight more rapidly than those under 8 h. The differences became discernible by the 5th week of light treatment and by the 9th week were very highly significant ($P < 0{\cdot}001$). Results for the 1st 14 weeks are shown in Table 1. There was no effect of shearing.

These results and the carcass data will be discussed in relation to the mode of action of the effect of light on growth and the possibility of commercial exploitation of this effect.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Table 1. *Weight gains, food intakes and conversion ratios of lambs under two day lengths and two levels of feeding (25 September to 31 December, 1973)*

Light treatment	Feeding	Gain in live weight (kg)	Food intake (kg fresh wt)	Food intake: live-weight gain
16 h	*ad lib.*	18·7	149·0	8·0
8 h	*ad lib.*	16·4	137·3	8·4
16 h	restricted	14·1	87·8	6·2
8 h	restricted	9·7	84·9	8·8

REFERENCES

Gordon, J. G. (1964). *Nature, Lond.* **204**, 798.
Tarttellin, M. F. (1968). *J. Physiol., Lond.* **195**, 29P.

Effect of undernutrition on nitrogen metabolism in the pregnant ewe.

By J. A. GUADA and J. J. ROBINSON (introduced by A. S. JONES), *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

A lower excretion of urinary nitrogen in the pregnant, compared with the non-pregnant ewe, and a progressive reduction in urea N excretion between 90 d of gestation and parturition are characteristic effects of pregnancy on N metabolism in adequately nourished ewes (Graham, 1964; Robinson, Scott & Fraser, 1973). The effect of undernutrition on N metabolism is less well defined.

An experiment was made in which the food intake of twelve Finnish Landrace × Dorset Horn ewes was abruptly reduced from 1·3× to 0·7× maintenance at 120 d of gestation for a period of 10 d. Glucose and urea concentrations in plasma were determined prior to, and periodically during, the 10 d period of reduced feeding in all twelve ewes; in addition, the concentration of total N, urea N and creatinine

Table 1. *Effect of abrupt reduction in food intake of the ewe from 1·3× to 0·7× maintenance at 120 d of gestation on the concentration of plasma constituents and on the urinary excretion of some nitrogen compounds*

		Days after reduction in food intake						SE of means
		0	2	3	5	7	9	
Urine								
Total N (g/d)	A	10·0	9·3	10·2	9·9	8·3	8·3	0·4
	B	9·0	9·3	10·3	10·6	9·3	8·6	0·8
Urea N (g/d)	A	6·7	6·8	6·9	6·7	5·6	5·3	0·3
	B	5·4	6·6	7·3	7·3	7·0	6·1	0·4
Creatinine N (g/d)	A	0·60	0·57	0·59	0·57	0·54	0·57	0·008
	B	0·60	0·56	0·59	0·52	0·52	0·50	0·022
Plasma								
Urea (mmol/l)	A	3·43	3·11	3·18	3·53	3·36	3·00	0·29
	B	3·39	3·11	3·93	4·46	4·36	3·61	0·21
Glucose (mmol/l)	A	3·33	3·05	2·89	2·61	2·55	2·61	0·22
	B	3·16	2·72	2·28	2·05	2·11	2·11	0·07

A, Ewes with one lamb; B, Ewes with more than one lamb.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

N were estimated in daily urine samples collected via urethral catheters from six ewes on the 2nd, 3rd, 5th, 7th and 9th day of the period when food was restricted.

The effects of the abrupt reduction in food intake on the concentration of plasma constituents and on the excretion of urinary nitrogenous compounds for ewes carrying one or more lambs are indicated in Table 1.

There was a significant correlation between plasma urea concentration and excretion of urea N ($r=0{\cdot}860$), and between the change in plasma urea concentration during the period of reduced feeding and foetal weight ($r=0{\cdot}870$). The increase in urea excretion and plasma urea concentration indicate an increase in the catabolism of maternal body protein to provide glucose for the growing foetus (Graham, 1968). The fact that urea N excretion tended to decrease after the 3rd day of reduced feeding may, however, suggest a reduction in foetal growth or a diminished dependence on protein for gluconeogenesis.

J.A.G. holds the Fundacion Juan March Fellowship, Universidad de Orreda, Leon, Spain.

REFERENCES

Graham, N. McC. (1964). *Aust. J. agric. Res.* **15**, 127.
Graham, N. McC. (1968). *Aust. J. agric. Res.* **19**, 555.
Robinson, J. J., Scott, D. & Fraser, C. (1973). *J. agric. Sci., Camb.* **80**, 363.

Magnesium retention of lactating beef cows given two levels of magnesium with either an adequate or low plane of nutrition. By J. FORDYCE, T. B. MILLER and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

It has been suggested that the adverse effect of low dietary energy on utilization of magnesium as found by Swan & Jamieson (1956*a*, *b*) is a casual factor in hypomagnesaemia occurring in beef cows kept under hill conditions. To examine the likelihood of this possibility, an experiment has been done with eight lactating Hereford × Friesian cows given either a maintenance or 2× maintenance level of dietary energy each with two levels of Mg. Four cows were allocated at random to each energy level for a period of 24 weeks from about the 12th week of lactation. Within each group of four cows, two were given either high or low amounts of Mg. The latter treatments were reversed on five occasions after each period of 4 weeks. The cows were milked

Table 1. *Intake, excretion and retention of magnesium (g/d) by lactating Hereford × Friesian cows*

(Mean values with their standard errors for four animals)

Diet	Mg intake	Mg in Faeces	Mg in Urine	Mg in Milk	Mg retention
High-energy					
high Mg	20·8	12·2	3·52	0·68	4·4
low Mg	11·4	8·1	2·35	0·64	0·3
Low-energy					
high Mg	23·0	15·3	1·05	0·41	6·2
low Mg	14·1	11·1	0·81	0·43	1·8
SE		1·1	0·29	0·16	0·3

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

twice daily and collection of faeces and urine was made in the last week of each monthly period. Mean values for the six mineral balances are given in Table 1.

Similar amounts of Mg were apparently absorbed from the high- and low-energy diets, but since urinary loss was significantly greater ($P<0.001$) and output of milk Mg a little higher from those animals given the high-energy diet, the retention of Mg by these animals was significantly less ($P<0.01$) than that of cows receiving the low-energy diet. These results, therefore, do not support the concept of a low dietary energy intake being associated with a poorer utilization of Mg in the lactating beef cow.

REFERENCE

Swan, J. B. & Jamieson, N. D. (1956*a*). *N.Z. Jl Sci. Technol.* (A) **38**, 137.
Swan, J. B. & Jamieson, N. D. (1956*b*). *N.Z. Jl Sci. Technol.* (A) **38**, 316.

The effect of oxalate on calcium absorption in growing goats. By M. S. CAMPOS, G. VARELA and A. MURILLO, *Department of Animal Physiology, University of Granada, Granada, Spain*

Oxalate severely decreases the absorption of calcium in rats (Varela & Murillo, 1966). Oslage, Farries & Keppel (1960) showed a low absorption of Ca oxalate in sheep. To study the effect of oxalate anion in goats, we have determined Ca absorption in (a) conscious goats, (b) conscious goats with a duodenal fistula, (c) anaesthetized goats whose complete small intestine was perfused and (d) conscious goats with a Mann-Bollman fistula to perfuse an isolated jejunal loop.

The diet contained 2·3 g Ca/kg and 6·5 g Ca (chloride or oxalate)/kg diet was added to the diet or introduced thorugh the duodenal fistula. The solutions perfused contained 1 g $CaCl_2$ and 1·2 g sodium oxalate/l.

Table 1. *Calcium absorption in goats under various conditions*

(Mean values with their standard errors where given)

Experimental conditions	No. of trials	Ca absorption (%)		
		$CaCl_2$	$CaCl_2$+ Na oxalate	Ca oxalate
(a) Intact conscious goats	12	35·7±5·4	28·4±2·6	15·5±0·8
(b) Conscious goats with a duodenal fistula	24	35·8±2·1	Negative	2·5±1·8
(c) Anaesthetized goats with the complete small intestine perfused	2	28·9	11·3	—
(d) Conscious animal with an isolated jejunal loop perfused	9	13·2±1·0	Negative	—

The addition of Na oxalate to the diet produced a non-significant decrease in Ca absorption. However, if Ca was given as oxalate its absorption was significantly lower ($P<0.01$). When Na or Ca oxalate was directly introduced through the duodenal fistula, Ca absorption was greatly decreased ($P<0.01$). These results are consistent with the destructive action of the rumen flora on the oxalate, which is less marked on the Ca salt, as it is insoluble.

The absorption of Ca by perfused goat intestine was significantly reduced, as in the rat, when Na oxalate was present. Although the duodenal pH is low in the

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

goat (2·6–4·3), the goat's ability to absorb Ca from diets containing oxalate must be mainly attributed to the destructive action of the ruminal flora on this anion, as the goat's intestine itself behaves like that of non-ruminants.

REFERENCES

Oslage, H. J., Farries, F. E. & Keppel, H. (1960). *Arch. Tierernähr.* **10**, 374.
Varela, G. & Murillo, A. (1966). *Proc. Nutr. Soc.* **25**, xxvii.

The effect of sodium chloride supplementation on the mineral nutrition in sheep. By G. MOSELEY and D. I. H. JONES, *Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Cards.*

It has been suggested (Payne, 1972) that the low blood sodium levels observed in dairy cows on summer pasture are indicative of an inadequate Na intake and that Na supplementation is necessary. However, there is considerable variation in mineral content between grass species and between varieties of the same species, particularly with regard to Na (Lehr, 1960; ap Griffith, Jones & Walters, 1965; ap Griffith & Walters, 1966), and supplementation of some grasses may prove excessive and detrimental to animal performance.

In this experiment four groups of wethers were fed on a diet of chopped hay *ad lib.* (50:50 mixture of S23 and S321 ryegrass) with a supplement of 200 g barley/d for 70 d. This basal diet was adequate in Na (5 g Na/kg dry matter) and was fed unchanged to the control group. The other groups received increasing amounts of NaCl mixed and pelleted with the barley to give dietary Na concentrations (g/kg) of 17, 25 and 31 respectively.

Dry matter intake, dry organic matter intake, dry organic matter digestibility and live-weight gain all decreased with increasing concentration of NaCl and were significantly reduced ($P<0.01$) at the highest level.

There were no significant or consistent changes in the serum concentrations of Na or potassium following NaCl supplementation, but serum calcium and magnesium levels were significantly lowered as shown in Table 1.

Mineral and nitrogen balances showed that the apparent availability of Na, K, Ca and Mg increased with increasing dietary NaCl while N and phosphorus decreased.

Table 1. *Effect of increasing dietary sodium chloride levels on serum mineral concentrations (mmol/l)*

Diet	Sodium		Potassium		Calcium		Magnesium	
1 (5 g Na/kg)	146	147	6·77	7·03	2·48	2·45	0·774	0·827
2 (17 g Na/kg)	150	147	7·46	7·28	2·34	2·25	0·753	0·687**
3 (25 g Na/kg)	150	147	6·95	7·21	2·54	2·37**	0·774	0·741*
4 (31 g Na/kg)	151	149	6·90	7·23	2·48	2·33*	0·765	0·724*
Standard error	1·3		0·37		0·06		0·020	

Values significantly different from diet 1: *$P<0.05$, **$P<0.01$.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

The retention of N and all minerals except Ca decreased significantly with increasing NaCl intake and was due mainly to an increasing urinary output of minerals and N.

The supplementation of high-Na grasses could result in dietary Na levels of the order of 17 g/kg. It appears that under such conditions undesirable nutritional effects may occur, particularly in regard to Ca and Mg metabolism.

REFERENCES

ap Griffith, G., Jones, D. I. H. & Walters, R. J. K. (1965). *J. Sci. Fd Agric.* **16**, 94.
ap Griffith, G. & Walters, R. J. K. (1966). *J. agric. Sci., Camb.* **67**, 81.
Lehr, J. J. (1961). *Proc. 8th int. Grassld Congr.* 1960, *Reading*, p. 101.
Payne, J. M. (1972). *Jl R. agric. Soc.* **133**, 69.

Lead metabolism in lambs and the effect of phosphate supplements. By J. N. MORRISON, J. QUARTERMAN and W. R. HUMPHRIES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Toxic symptoms or death occur in non-pregnant sheep when the food contains more than about 200 μg lead/g (Allcroft & Blaxter, 1950; Stewart & Allcroft, 1956; Butler, Nisbet & Robertson, 1957) and at lower levels in pregnant sheep (Allcroft & Blaxter, 1950). The extent of absorption and retention of ingested Pb can be modified in rats by changes in dietary phosphate content (Sobel & Burger, 1955; Quarterman, Morrison & Carey, 1974). We wished to examine the effect of dietary phosphorus on Pb metabolism in sheep.

Eight lambs were given 77 mg lead acetate in aqueous solution by mouth once per week from birth to weaning. They were then given dried-grass cubes containing 0·2 g Pb/kg until slaughtered at about 1 year old. The cubes given to four lambs contained 6·9 g calcium and 3·3 g P/kg. The other four lambs received cubes supplemented with P to contain 12·6 g P/kg. Four control lambs received no Pb while suckling and later received cubes but no supplement of Pb or P.

Control lambs gained 39·0 ± 2·0 kg during the experiment compared with a mean gain of 29·2 ± 1·7 kg for all animals receiving Pb ($P < 0·01$). The phosphate supplement had no significant effect upon weight gain.

Blood Pb concentration rose to a maximum of 0·61 ± 0·05 mg/l blood at 7 months of age in the groups receiving Pb but no P supplement, and decreased thereafter. Both the maximum blood Pb concentration (0·55 ± 0·02 mg/l) and subsequent values were lower in the group receiving both Pb and P supplements. There were no effects of Pb or P on haemoglobin concentration, packed cell volume, erythrocyte or white cell numbers or erythrocyte fragility.

P supplementation had no effect on the Pb content of livers and kidneys at slaughter; the mean content (μg/g fresh weight) was 5·8 ± 0·3 for liver and 7·9 ± 1·5 for kidney. The corresponding values in lambs not given Pb were 1·1 ± 0·2 and 0·5 ± 0·2. Urine Pb concentration did not reflect dietary Pb intake.

Bone samples were taken from the tails of two animals/group during the experiment and from the radius-ulna of four carcases in each group at slaughter. The tail vertebrae and large bone samples contained 3·05 and 2·62 μg Pb/mg Ca respectively

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

from lambs receiving Pb alone; 2·14 and 1·86 μg Pb/mg Ca from lambs receiving Pb and P, and 0·10 and 0·10 μg Pb/mg Ca in the controls.

REFERENCES

Allcroft, R. & Blaxter, K. L. (1950). *J. comp. Path.* **60**, 209.
Butler, E. J., Nisbet, D. I. & Robertson, J. M. (1957). *J. comp. Path.* **67**, 378.
Quarterman, J., Morrison, J. N. & Carey, L. (1974). *Proc. 7th Ann. Conf. on Trace Substances in Environmental Health, Univ. Missouri, Columbia, Missouri.* (In the Press).
Sobel, A. E. & Burger, M. (1955). *J. biol. Chem.* **212**, 105.
Stewart, W. L. & Allcroft, R. (1956). *Vet. Rec.* **68**, 723.

The effects of trace elements and ethylenediaminetetraacetic acid on the survival of chicks inoculated with *Salmonella gallinarum*. By R. HILL and I. M. SMITH, *Royal Veterinary College, Boltons Park, Potters Bar, Herts.*

There are several strands of information leading to the possibility that certain trace elements or chelating agents, or both, may influence the outcome of some experimental infections (Sword, 1966; Bullen, Cushnie & Rogers, 1967; Fletcher & Goldstein, 1970). This is being studied using 2-week-old chicks inoculated orally with *Salmonella gallinarum*.

In previous reports the superiority of meat over fish meal in promoting the survival of chicks inoculated with *S. gallinarum* was described (Hill & Smith, 1969; Smith & Hill, 1970). There are large differences in the selenium and iron contents of meat and fish meals: fish meal is much richer than meat in Se and meat is much richer than fish in Fe, but the addition of Se (1 mg/kg) to a meat-meal diet did not depress survival and the addition of Fe (200 mg/kg as ferric ammonium citrate or as haemoglobin) to a fish-meal diet did not increase survival. However, as the inclusion of 1 g disodium ethylenediaminetetraacetate (Na_2H_2EDTA)/kg in the meat or fish-meal diet significantly increased survival, an interaction between mineral metabolism and the disease was still possible.

A similar increase in survival occurred when Na_2H_2EDTA was added to a mixed-protein diet (MPD), and to reduce the number of treatments to be studied this single basal diet was used in most subsequent experiments. The addition of 200 mg Fe/kg to diet MPD as $FeSO_4$ gave a small increase in survival but the same Fe supplement plus 1 g Na_2H_2EDTA/kg gave a marked and significant increase in survival, appreciably greater than either alone. These comparisons were made in five experiments and the results, given as total number surviving of 175 inoculated per treatment, were: thirty-five for the basal diet (MPD), forty-nine with added Na_2H_2EDTA, forty-four with $FeSO_4$, and eighty with both supplements. It is not clear at present how specifically Fe contributed to this result, since the inclusion of 200 mg copper/kg as $CuSO_4$ with Na_2H_2EDTA gave sixty-eight survivors and of 200 mg zinc/kg as $ZnCO_3$ with Na_2H_2EDTA gave seventy survivors. Both these survival rates were significantly greater than that given by the basal diet, and neither was less than that given by $FeSO_4$ plus Na_2H_2EDTA.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

REFERENCES

Bullen, J. J., Cushnie, G. H. & Rogers, H. J. (1967). *Immunology* **12**, 303.
Fletcher, J. & Goldstein, E. (1970). *Br. J. exp. Path.* **51**, 280.
Hill, R. & Smith, I. M. (1969). *J. comp. Path.* **79**, 469.
Smith, I. M. & Hill, R. (1972). *J. comp. Path.* **82**, 209.
Sword, C. P. (1966). *J. Bact.* **92**, 536.

The hydrolysis and absorption of thioglucosides of rapeseed meal. By A. MARANGOS and R. HILL, *Royal Veterinary College, Boltons Park, Potters Bar, Herts.*

The goitrogenic activity of rapeseed meal lies partly in the quantity of thioglucoside present and partly in the type of thioglucoside, but also on the degree of hydrolysis that occurs before absorption, as the products of hydrolysis rather than the thioglucosides themselves are goitrogenic. It is believed that the destruction of myrosinase, a thioglucosidase present in rapeseed, during the extraction of oil from the seed and preparation of the residual meal, is important in reducing the goitrogenic activity of the meal (Clandinin, Slinger & Bell, 1972). This may be so, but if it is, the magnitude of the effect is quite small, because rapeseed meals used in recent experiments with poultry, though without myrosinase or free hydrolysis products, were all goitrogenic, some markedly. Clearly, hydrolysis of the thioglucosides must have occurred in some degree after ingestion of the meal.

The myrosinase activity of the digestive tract and contents were determined in mature fowl given a control, soya-bean meal diet. No activity was found in the wall of the digestive tract, and none in the contents except that of the caecum. Activity here was strong, though the rate of hydrolysis in vitro from the whole of the caecal contents was less than that of 15 mg of an enzyme preparation from white mustard seed.

The myrosinase activity was in the particulate fraction of caecal contents, not in the supernatant liquid, and could be destroyed by adding neomycin. The enzyme was evidently produced by bacteria and did not function extracellularly.

In birds given a rapeseed meal of high potential goitrogenic activity at 200 g/kg diet, myrosinase was active in the contents of ileum and colon as well as of caecum, though the activity was much greater in caecum than ileum or colon. Caecectomy of 10-week-old birds followed by a period of feeding with 200g rapeseed meal/kg diet caused enlargement of the thyroid glands; thus the low myrosinase activity of the contents of ileum and colon, which was similar to that of intact birds given rapeseed meal, released sufficient isothiocyanate and oxazolidinethione to produce thyroid enlargement.

Using chromium sesquioxide as a marker, estimates were made of the percentage absorption of thioglucosides and their hydrolysis products. Among eight birds values varied widely: the mean with SD was $77{\cdot}8 \pm 35{\cdot}8$. The high SD was given partly by one bird for which the value was 3. These data give no indication of the proportions absorbed as thioglucoside or as products of hydrolysis.

REFERENCE

Clandinin, D. R., Slinger, S. J. & Bell, J. M. (1972). *Rapeseed Ass. of Canada, Winnipeg, Manitoba.* Publication no. 16, p. 8.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

The absorption of glucose from the gut and the amount removed by the liver in young pigs. By D. M. ANDERSON* and A. J. NORTHROP, *Department of Biochemistry, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

The pig lays down large amounts of fat from digested carbohydrate and the role which the liver plays in this is not clear. The present experiments were designed to study this by direct measurement of plasma flow in the veins of the liver and the glucose concentration differences in them. Fifteen pigs weighing 28·6±1·0 kg (±SE) were used, and they were given 40 g standard diet/kg body-weight. The hepatic plasma flow was determined using sulphobromothalein, while portal flow was assumed to be 80% of this. The ratio of flows was determined by infusing *p*-amino-hippuric acid into the coeliac artery, which gives the ratio of portal:hepatic flow but not absolute values. The pigs were fasted for 18 h before the experiments began.

The mean hepatic plasma flow in the 75 min before feeding was 1197±100 ml/min (±SE) and this increased gradually to a peak 1767±133 ml/min 150 min after feeding and then declined to 1604 ml/min 255 min after feeding. The experiments stopped at this point. In the 75 min before feeding the gut removed on average 1·9 mg glucose/min from the arterial plasma supplying it, while the liver put out 47·4 mg glucose/min. After feeding, the absorption of glucose from the gut increased in 45 min to a plateau of 420 mg/min while the liver only accumulated glucose from 45 min to 135 min and from 195 min until 255 min at mean rates of 96 mg/min and 61 mg/min respectively. During the 255 min following feeding, the liver apparently removed only 11% of the glucose absorbed by the gut. This suggests that in pigs of this age, the glucose absorbed by the gut passes directly to the muscle and the adipose tissue to be metabolized.

*Present address: Organon Laboratories Ltd, Newhouse, Lanarkshire ML1 5SH.

Progressive supplementation of barley with amino acids in a pig diet. By M. F. FULLER, R. M. LIVINGSTONE and I. MENNIE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Fifteen female pigs, weighing initially about 40 kg, were given for 7 d a basal diet of ground barley with added vitamins and minerals. For a further 7 d, L-lysine, as the monohydrochloride, was added at graded levels between 1·2 and 6·0 g/kg, three pigs being randomly allocated to each level. Two successive 48 h collections of urine were made in the last 4 d of each 7 d period by means of urethral catheters. Preliminary work had shown that alteration of the rate of urine nitrogen excretion was complete within 3 d of adding amino acid. The greatest reduction in daily urine N excretion was obtained when 3·6 g L-lysine/kg was added (Table 1). In the 3rd 7 d period, 3·6 g L-lysine/kg was added to the basal diet for all pigs and in the 4th period the pigs received additionally (g/kg): 0·5 L-lysine, 1·0 DL-methionine, 0·6 L-threonine, 1·5 L-isoleucine, or 0·3 L-tryptophan, three pigs being randomly allocated to each amino acid. The daily urine N excretion of pigs given the threonine

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Table 1. *Changes in daily urine nitrogen excretion in pigs with amino acid supplementation of the basal diet*

(Mean values for three animals, except those marked †, which are for two)

Period	Mean weight (kg)	Additions to basal diet (g/kg)	Change in urine N (g/kg$^{0.75}$ per d) after further addition of (g/kg):					SE of mean based on: Three animals	SE of mean based on: Two animals
1	45	—	1·2 L-lysine	2·4 L-lysine	3·6 L-lysine	4·8 L-lysine	6·0 L-lysine		
2	49		−0·13	−0·17	−0·24	−0·19	−0·12†	0·020	0·024
3	53	3·6 L-lysine	0·5 L-lysine	1·0 DL-methionine	0·6 L-threonine	1·5 L-isoleucine	0·3 L-tryptophan		
4	57		−0·03†	−0·01	−0·18†	−0·04†	−0·02	0·028	0·033
5	61	3·6 L-lysine 0·6 L-threonine	0·5 L-lysine	1·0 DL-methionine	0·4 L-threonine	1·5 L-isoleucine	0·3 L-tryptophan		
6	65		+0·06†	0·00	+0·01	+0·01	0·00†	0·012	0·015
7	70	3·6 L-lysine 0·6 L-threonine	0·5 L-lysine	1·0 DL-methionine	0·4 L-threonine	1·5 L-isoleucine	0·3 L-tryptophan		
8	75		+0·03	−0·07†	+0·04	+0·01	+0·01	0·045	0·055

supplement was significantly reduced; the other amino acids had smaller effects, which were not significant. In period 5, 3·6 g L-lysine and 0·6 g L-threonine/kg were added to the basal diet, and in period 6 the same supplements of the five amino acids were given as in period 4, except that only 0·4 g L-threonine/kg was added. None of the supplements significantly affected daily urine N excretion. The procedures of periods 5 and 6 were repeated in two further periods when again there was no significant response to any of the supplements. The most probable explanation of the absence of any response to amino acid supplements in the last four periods is the declining requirements for amino acids (per kg diet) of pigs at the higher weights. It appears that, for the pig of 60–80 kg, only lysine and threonine, of the amino acids used, are capable of improving the utilization of barley protein.

Protein synthesis in the rat liver after fracture of the femur. By S. N. KHAN, W. J. TILSTONE, A. FLECK and I. BROOM, *Departments of Pathological Chemistry and of Biochemistry, University of Glasgow, and Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow*

Moderate to severe trauma is accompanied by two characteristic responses in plasma protein concentration, namely an increase in levels of acute phase reactants and a fall in albumin (Cuthbertson & Tompsett, 1935; Owen, 1967). Attempts have been made to correlate these changes in plasma concentration with changes in protein metabolism in general after trauma, and in particular to the extent to which nutritional factors are causative. Very recently Ballantyne, Tilstone & Fleck (1973) measured albumin synthesis directly in the rabbit and showed that fracture produced a 60% decrease, about half of which was due to reduced food intake. The present communication is a report of the effect of fracture of femur on protein synthesis in the rat liver, as measured by profiling and amino acid incorporation into free and bound polyribosomes. Pair-fed male Wistar rats of 150–250 g bodyweight were used in all experiments.

Parallel changes both in polyribosome profiles and in amino acid incorporation (AA), measured relative to ribosomal RNA, in free and in bound polysomes were noted. Using the proportion of total polysome area represented by monomer and dimer (MD%) as an index of protein synthesis, an increase in synthesis of about 100% was found at 12 h post-fracture and of about 30% at 48 h post-fracture. At 24 h post-fracture, however, a decreased synthesis of about 20% was found. The results are summarized in Table 1. They are given as ratio of injured to pair-fed controls; at least four animals, analysed in pairs, were in each group and SE are given for groups with more than two pairs.

It is of interest that free and bound polysomes behaved similarly, since the former are thought to synthesize protein for intracellular use and the latter to synthesize protein for extracellular use (Redman, 1969). Nutrition as a causal agent can largely be discounted as a factor in the cyclic changes described above, but the

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

nature and magnitude of these changes is relevant to those seeking to formulate optimal nutrition for recovery in the injured.

Table 1. *Protein synthesis in rat liver following fracture of femur, in terms of proportion of total polysomes as monomer and dimer (MD%), and amino acid incorporation in vivo measured relative to ribosomal RNA (AA), expressed as ratios of injured to pair-fed controls*

(Mean values with their standard errors where given)

Time after fracture (h)	Free polysomes		Bound polysomes	
	MD%	AA	MD%	AA
3	0·71		0·61	
12	0·82±0·06	1·2	0·53	1·46
15	0·96		0·53±0·03	
18	1·21		0·93	
24	1·06	0·75	1·08	0·70
36	0·84	1·15	0·80	
48	0·90±0·07		0·79±0·09	1·20
72	1·24±0·08		1·0	

REFERENCES

Ballantyne, F. C., Tilstone, W. J. & Fleck, A. (1973). *Br. J. exp. Path.* **54**, 409.
Cuthbertson, D. P. & Tompsett, S. L. (1935). *Br. J. exp. Path.* **16**, 471.
Owen, J. A. (1967). *Adv. clin. Chem.* **9**, 1.
Redman, C. M. (1969). *J. biol. Chem.* **244**, 4308.

Changes in the distribution of fat in pigs given fattening or slimming dietary regimens. By V. R. FOWLER and W. ROSS*, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Five female and five castrated male pigs of similar age and breeding were selected from a larger group of *ad lib.*-fed pigs when they weighed about 75 kg. They were fed individually on a standard (160 g crude protetn (CP)/kg) diet until each weighed 81·5 kg. At this weight, one pig of each sex was slaughtered as a control. The remaining pigs were split into two balanced groups and were subjected to either a slimming regimen (S) or a fattening regimen (F). The S regimen provided 500 g/d of a diet containing 290 g CP/kg and supplied about 6 MJ/d metabolizable energy (ME), which was estimated to be about 50% of maintenance; the F regimen was 3 kg/d of a diet with 40 g CP/kg supplying 45 MJ/d ME (about 375% of maintenance). A male and a female were killed after 4 and 8 weeks on each regimen. Carcasses were dissected into major adipose depots and muscle groups, and the lipid content of each was determined using the method of Atkinson, Fowler, Garton & Lough (1972).

The results for each category, for individual pigs, are given in Table 1.

Interpretation is complicated by the large reductions in the weight of fat-free body (FFB) in the slimmed group (Table 1a). This group lost fat also, but the fat: FFB ratio (Table 1b) remained relatively constant, with the possible exception of the

*Present address: North of Scotland College of Agriculture, School of Agriculture, 581 King Street, Aberdeen AB9 1UD.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Table 1. *Weights and proportions of chemical fat and fat-free body (FFB) of pigs*

			Regimen				
		Sex	Control	4 weeks fattened	8 weeks fattened	4 weeks slimmed	8 weeks slimmed
(a)	Weight of fat-free body (kg)	M	60·0	57·5	60·8	53·5	52·7
		F	59·4	61·1	61·8	57·1	52·5
	Weight of chemical fat (kg)	M	18·4	35·1	49·6	16·8	15·9
		F	18·5	34·5	45·7	17·9	12·9
(b)	Chemical fat (g/kg FFB)						
	Total body fat	M	307	621	816	314	302
		F	313	566	739	313	246
	Subcutaneous fat	M	157	367	516	159	157
		F	157	314	453	163	112
	Perirenal fat	M	15	38	51	17	10
		F	15	33	49	17	8
	Mesenteric fat	M	8	21	25	11	6
		F	7	18	24	7	6
	Intermuscular fat	M	15	20	20	13	10
	(limbs only)	F	14	19	20	13	11
	Intramuscular fat	M	17	22	21	16	16
	(limbs only)	F	19	21	21	16	15

female slimmed for 8 weeks. The total chemical lipid of the fattened group increased by a little more than twofold over 8 weeks, with mesenteric and perirenal fat increasing at a higher rate than the total (about threefold) and intermuscular fat at a much lower rate. Intramuscular fat in the limbs was remarkably stable, only increasing by about 10% in the fattened group. It is concluded that the amount of fat in various depots is not proportional to the total chemical fat and that, even on a high-protein diet, submaintenance feeding can result in a considerable loss of the FFB.

REFERENCE

Atkinson, T., Fowler, V. R., Garton, G. A. & Lough, A. K. (1972). *Analyst* **97**, 562.

A survey of user-experience with automated amino acid analysers.

By A. A. WOODHAM, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Since the original description of an automated method for the estimation of amino acids in protein hydrolysates using ion-exchange chromatography (Spackman, Stein & Moore, 1958) a variety of commercially produced automated analysers have become available. Users of these experience difficulties, some of which fall into the category of 'teething-troubles', while others are of a recurrent nature or may develop even after considerable experience with the equipment. Further, the procedures employed by individual analysts may differ considerably.

During 1970 a survey was conducted among users within the ARC Institutes and Units and also among members of the General Analytical Panel of the ARC Protein Evaluation Group. A questionnaire compiled with the assistance of Dr G. M.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press

Ellinger and Dr J. Davidson of this Institute was circulated to the participants, and the replies were collated in 1971.

Of thirty-six analysers surveyed, twenty were of one type, the Technicon NC1. The remaining sixteen instruments included one or two examples of three Beckmann modifications—120, 120C and 121; the Jeolco 5AH; the EEL 'original', 193 and multi-channel; the Locarte; and the BioCal BC200. The Technicon TSM and 2-column instruments were also represented.

Questions asked covered general matters such as the number and previous experience of the operators, sample throughput and type, i.e. pure proteins, feeding-stuffs, or physiological materials. Details of equipment and technique were requested, including column dimensions, hydrolysis conditions, duration of run, reproducibility, methods used for estimating sulphur-containing amino acids and the extent to which manufacturers recommendations were followed. Finally, users were asked to list their problems.

Although most users did adhere fairly closely to the manufacturers recommendations, modifications in resin type, buffer conditions, column dimensions and duration of run were all evident, particularly among the Technicon users. Hydrolysis conditions showed considerable variation. A minority of operators determined methionine and cystine on oxidized samples and less than one-third regularly replicated hydrolyses. Most users reported trouble from break-downs, some claiming up to 45% of working time lost. Causes of break-down were varied in the extreme and criticisms were mainly directed at the difficulties of servicing and spare parts supply. An unfortunate aspect of the survey was the preponderance of one type of instrument compared with small numbers of each of the more recent types, and conflicting verdicts on some of the latter probably reflect to some extent the experience of the operator.

More detailed information on the results of the survey can be obtained from the author.

REFERENCE

Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.

https://doi.org/10.1079/PNS19740058 Published online by Cambridge University Press