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# ABSTRACTS OF COMMUNICATIONS

*The Two Hundred and Eighteenth Meeting of the Nutrition Society was held at the Edinburgh School of Agriculture, King's Buildings, West Mains Road, Edinburgh, on Friday,* 20 *February* 1970, *at* 11.00 *hours, when the following papers were read :*

**Effects on chick growth of adding glutamic acid or diammonium citrate to diets containing crystalline essential amino acids.** By D. W. F. SHANNON, R. BLAIR, J. M. MCNAB and D. J. W. LEE, *ARC Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh,* 9

There is a divergence of opinion on the utilizability of ammonium nitrogen by the chick. A number of authors have shown beneficial effects from dietary additions of urea, diammonium hydrogen citrate and ammonium acetate while others were unable to demonstrate any utilization of ammonium or urea nitrogen or of biuret. Ammonium nitrogen, if utilizable by the chick, is only likely to be useful in meeting the non-essential amino acid requirement and it seemed appropriate, therefore, to use diets limiting only in non-essential amino acids. Two experiments were carried out to investigate the ability of diammonium hydrogen citrate and glutamic acid to meet the non-essential amino acid requirement.

Seven-day-old male broiler chicks were given diets containing as the only sources of nitrogen, essential amino acids (A) or this diet supplemented with 11·1% diammonium hydrogen citrate (B) or 12·0% glutamic acid (C). Diet C in addition had a higher level of glycine and contained 1·0% proline. Each diet was given *ad lib.* to five pairs of male broiler chicks for an experimental period of 11 d.

Chicks given diet B gained weight significantly faster ($P<0{\cdot}001$) than those given diet A but significantly more slowly than those given diet C ($P<0{\cdot}001$). Food conversion efficiency was significantly better for diets B and C than for diet A. Carcass analysis revealed no significant treatment differences in carcass nitrogen or dry-matter content, confirming that diammonium hydrogen citrate can be utilized to meet the non-essential amino acid requirement of the chick. The results of plasma and liver free amino acids analyses will be discussed.

**Glutamic dehydrogenase levels and formation of glutamic acid in the livers of chicks fed synthetic diets.** By D. J. W. LEE, J. M. MCNAB, D. W. F. SHANNON and R. BLAIR, *ARC Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh,* 9

The growth response of diammonium hydrogen citrate (DAHC), when given to chicks fed a synthetic diet containing crystalline essential amino acids but no non-

essential amino acids, has been shown in the previous paper (Shannon, Blair, McNab & Lee, 1970). This suggests that chicks can utilize non-amino acid nitrogen in the form of DAHC. In vitro studies were also made on the ability of the livers from the chicks used in this growth experiment to form free non-essential amino acids from ammonium ions, and to see if the dietary treatments, A, B and C of the previous paper had any effect on this ability. This was done in two ways: by the assay of glutamic dehydrogenase and by incubating liver homogenates with [$\alpha$-$^{14}$C]ketoglutaric acid and DAHC.

Glutamic dehydrogenase was assayed according to Schmidt (1963) using the conditions described for chick liver. The results obtained showed that the activity of glutamic dehydrogenase in the livers from birds of group A was significantly higher than the activity of this enzyme in the liver from birds of groups B and C, both when the activities were expressed as units/g wet weight liver ($P<0.05$) and as units/mg protein ($P<0.01$). There was no significant difference between the activities of groups B and C.

The incubation of [$\alpha$-$^{14}$C]ketoglutaric acid with liver homogenates used basically the same conditions as were used for the assay of glutamic dehydrogenase with the exception that DAHC was used instead of ammonium chloride and that no NADH was added. After incubating at 37° for 30 min, the reaction was stopped using ice-cold ethanol. The protein-free extract was chromatographed on a column of Dowex-1-formate to separate any glutamic acid formed from any remaining $\alpha$-ketoglutaric acid. The amount of radioactivity recovered as [$^{14}$C]glutamic acid and as [$\alpha$-$^{14}$C]-ketoglutaric acid was calculated and expressed as a percentage of total radioactivity added to the incubate. From the results obtained, it appears that the amount of glutamate recovered from the livers of group B was lower than that recovered from group C which in turn was lower than that recovered from group A.

The results of these studies were discussed with respect to each other and to the utilization of ammonium nitrogen.

Thanks are due to Mrs M. Blanchard for technical assistance.

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**The incorporation of ammonium nitrogen into amino acids by chick liver homogenates.** By J. M. McNab, D. J. W. Lee and D. W. F. Shannon, *ARC Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh, 9*

The possible utilization by the chick of non-protein nitrogen in the form of ammonium salts has been discussed in a previous paper by Shannon, Blair, McNab & Lee (1970). It was shown that chicks which were given a diet containing diam-

monium hydrogen citrate were able to convert this nitrogen-source into compounds necessary for healthy growth. The evidence of others is based almost exclusively on growth responses to the diet and little effort has been directed at determining the biochemical nature of the transformations involved in such conversions. It has been illustrated in the preceding paper (Lee, McNab, Shannon & Blair, 1970) that enzymes are present in chick livers capable of transferring ammonium nitrogen from the salt to α-ketoglutaric acid.

In this paper, we report a study of the reactions of different ammonium salts with α-ketoglutaric acid in the presence of chick liver homogenates. These were prepared by removing the livers from 3-week-old male broiler chickens: after weighing, the livers were added to 1·0 M-tris–hydrochloride buffer (pH=7·6) and a slurry produced in a Potter-Elvehjem homogenizer. For each g of tissue, 2 ml of buffer were added and the mixture ground until an even homogenate was produced. All the tissue preparation steps were carried out in an ice-bath. Incubations were carried out at 37° for 30 min. The reaction mixtures comprised 0·4 ml ammonium salt (0·56 M with respect to the ammonium moiety), 0·5 ml α-ketoglutaric acid (0·033 M) and 0·1 ml ion-free water to which 0·5 ml liver homogenate was added. Each reaction contained approximately 166 mg wet tissue.

The reactions were terminated by the addition of 3 ml sulphosalicylic acid (4%, w/v) to which a known amount of norleucine had been added. The precipitated protein was removed by centrifugation and the supernatant solution analysed for amino acids, each being measured by reference to norleucine. Ammonium acetate, formate, sulphate and diammonium hydrogen citrate with and without α-ketoglutaric acid were examined. The changes in concentration of free amino acids which occurred after reaction indicated that the citrate salt was the best nitrogen donor to α-ketoglutaric acid. The possibility of transamination and the use of other keto acids as acceptor molecules was discussed.

We are grateful to Mr R. K. Scougall for the amino acid analyses.

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**Intake and digestibility of silage by sheep affected with a gut parasite.**

By A. E. Reveron R. and J. H. Topps, *School of Agriculture, University of Aberdeen* and G. C. Hunter, *Rowett Research Institute, Bucksburn, Aberdeen*

Eight wether sheep, aged about 1 year, were treated twice with an antihelminthic (Nilverm; ICI) to rid them of nematodes. Five days after the second treatment, four of the animals were each infested with 70 000 larvae of *Trichostrongylus colubriformis*. Voluntary intake of a good-quality grass silage by each sheep was measured

daily over a period of 6 weeks during which time three determinations of digestibility were made. Two animals were then reinfested with 140 000 larvae of *T. colubriformis* and two of the sheep not originally infested were given the same number of parasites. Intake and digestibility of the silage were measured during a further 3 weeks. The numbers of worm eggs in the sheep's faeces were frequently counted and at the end of the experiment the infested animals were subjected to a post-mortem examination.

Table 1. *Intake (kg dry matter/$W^{0.73}$) and digestibility of silage by sheep following infestation with* T. colubriformis (*mean values for four animals*)

| | Non-infested sheep | | | | Infested sheep | | | |
|---|---|---|---|---|---|---|---|---|
| | | Digestibility (%) | | | | Digestibility (%) | | |
| Week after infestation | Intake | Dry matter | Organic matter | Crude protein | Intake | Dry matter | Organic matter | Crude protein |
| 1 | 51·7 | | | | 53·3 | | | |
| 2 | 55·7 | 72·3 | 73·9 | 63·9 | 53·9 | 72·6 | 74·1 | 64·0 |
| 3 | 56·5 | | | | 48·2 | | | |
| 4 | 57·9 | 74·9 | 77·6 | 68·4 | 47·7 | 74·9 | 74·8 | 68·1 |
| 5 | 55·2 | | | | 42·3 | | | |
| 6 | 55·2 | 68·6 | 71·0 | 54·1 | 44·1 | 66·8 | 70·5 | 50·2 |
| Mean | 55·4 | 71·9 | 74·2 | 62·1 | 48·3 | 71·4 | 73·1 | 60·8 |

From the 3rd week after infestation, the intake of dry matter by the infested sheep was significantly less ($P<0.05$) than the intake by non-infested animals. During the 6th week the digestibility of protein in the infested sheep was significantly less ($P<0.05$) than in the non-infested.

After the 6th week there appeared to be no further decreases in either intake or digestion resulting from reinfestation or a heavier infestation with 140 000 larvae. However, all animals except the two not infested began to lose weight at the end of the experiment. Neither faecal egg counts during the experiment nor intestinal worm counts at post-mortem appeared to be related to decreases in intake and digestibility in the infested sheep.

**The effect of change of rations on the rate of flow of liquid digesta from the rumen of sheep.** By MARIA DEL CARMEN ALVAREZ HERRERO,* J. L. CLAPPERTON and J. W. CZERKAWSKI, *Hannah Dairy Research Institute, Ayr*

The usual response of sheep to a relatively rapid change in ration is to reduce their food intake and this effect can persist for a considerable time. As part of a larger investigation of this, measurements have been made of the flow of liquid digesta from the rumen using polyethylene glycol (PEG) as a marker.

Two 6-year-old Romney Marsh wether sheep fitted with rumen cannulas were gradually introduced to one of two rations. The first ration was 300 g flaked maize given at 10.00 hours, and 300 g flaked maize and 100 g chopped hay at 22.00 hours,

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and in the second, maize was replaced by 350 g dried grass cubes. A solution of PEG, molecular weight 4000, containing 5 g/l, was infused into the rumen at the rate of 500 ml/d. The concentration of PEG in the rumen was determined on 3 d of each week at 10.00 hours (before feeding), at 12.00 hours and at 16.00 hours using the method of Hydén (1961). When steady values were obtained, the rations of the sheep were interchanged over a period of 2 d during which time the animals were fed mixed rations. Measurements of the PEG concentration were also made during the 5 d immediately following the change of ration.

Immediately after the change of ration, the concentration of PEG obtained on the maize diet was 65% higher than with the sheep given dried grass. There was no change in PEG concentration throughout the experiment in the sheep given dried grass but the level of PEG in the rumen of the sheep given maize fell until, after about 6 weeks, it reached the same value as with the sheep given dried grass. There was no significant change in PEG concentration in relation to time after feeding.

The results during the changes of ration showed that there was a fall in the PEG concentration on the 2nd day followed by a rise. These results are consistent with an increased rate of flow during the change of ration.

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**Experimental diabetes mellitus in the pig.** By D. M. Anderson and R. W. Ash, *ARC Institute of Animal Physiology, Babraham, Cambridge*

Minkowski (1893) and Carlson & Drennan (1912) reported that following pancreatectomy, pigs survived for as long as 66 d without insulin, which was not then available. Lukens (1937) also found that the diabetes was comparatively mild, although his pigs survived only 8–15 d.

In the present series of experiments, eleven pigs, weighing 2–26 kg, were anaesthetized with halothane and the pancreas removed. Recovery from the operation was usually rapid; glucose was excreted in the urine within 3 h of pancreatectomy. Subsequently, one piglet was suckled by the sow, and the remainder were kept in cages and fed restricted amounts of either a liquid milk substitute (Amvilac No. 1) or a mixture of Amvilac powder and dry meals. Two days after the operation protamine zinc insulin and regular insulin (3:1) were administered twice daily to control the diabetes. The urinary excretion of glucose was kept below 0·3 moles/d by 2·2 units of insulin/100 g food when food consumption was in the range 0·3–2·6 kg/d. Signs of hypoglycaemia were more frequently observed in those pigs which received the liquid diet, although they were fed four to five times a day, than in the animals fed the dry mixed diet. Mild hypoglycaemia responded to glucagon but it

was necessary to administer glucose by stomach-tube to pigs which were comatose or convulsive. An apparent steatorrhoea developed in the one suckled piglet but the administration of pancreatic enzymes (Cotazym) appeared unnecessary in the other pigs.

When insulin administration was discontinued, the plasma glucose concentration increased within 24 h to 20–30 mM and there was a concurrent polydipsia and polyuria. The urinary glucose concentration was 55–275 mM and after 2–3 d the output reached a plateau of 1·4–2·2 moles/d. Ketones were detected in the urine after 4–7 d and thereafter the output of urinary free acetone increased either slowly or rapidly from 3 m-moles to 14–44 m-moles/d; in one experiment, the peak excretion was 56 m-moles/d. During the early stages of an induced diabetes, fasting resulted in an immediate and substantial decrease in glucose excretion and an increase in urinary free acetone after 24 h.

After 5–19 d without insulin, either food consumption ceased or the appetite became capricious and there was a severe decrease in body-weight. A decreased food consumption and lassitude appeared to be associated with an increase in urinary excretion of ketones. However, other signs that could be attributed to a marked change in acid–base balance were not observed; a reversal of the diabetic state occurred within 2–3 d of the resumption of insulin administration.

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**The utilization of salts of acetic acid by growing lambs.** By F. D. DEB. HOVELL and J. F. D. GREENHALGH, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

The utilization of acetates by growing lambs was investigated in a comparative slaughter trial in which the barley of a barley–soya-bean meal–fish-meal control diet was partly replaced by a mixture of sodium, calcium and potassium acetates. The ratio of protein to metabolizable energy (ME) was kept constant in all diets, the control diet containing 19% crude protein in dry matter.

Three groups of eight entire male lambs were given one of three diets containing 0, 14 or 21% of ME as acetates (calculated as the heat of combustion of their acetic acid equivalent). All lambs were individually penned and fed, ME intake being either 180 or 200 kcal/kg$^{0.73}$ per 24 h. The lambs were killed at 40 or 45 kg live weight and the carcass and non-carcass remainder were minced and analysed. The wool was treated separately, being simply washed and dried. Initial composition was determined from a group of five animals killed at about 18 kg live weight.

The main results were as follows:

| | Acetate in diet (%) | | | SE and significance of difference, | |
|---|---|---|---|---|---|
| | 0 | 14 | 21 | 0 *v.* 21% | acetate |
| Time on expt (d) | 120 | 123 | 122 | 5 | NS |
| ME intake (kcal/d) | 2260 | 2283 | 2272 | 53 | NS |
| Gain (g/d) in: | | | | | |
| Live weight | 206 | 201 | 203 | 9 | NS |
| Carcass weight | 108 | 107 | 101 | 5 | NS |
| Remainder of body | 46 | 41 | 39 | 2 | ** |
| Wool | 6 | 6 | 6 | 1 | NS |
| Whole body N × 6·25† | 26 | 25 | 25 | 2 | NS |
| Whole body fat | 50 | 44 | 40 | 2 | ** |
| Whole body gain of energy (kcal/d)‡ | 648 | 585 | 551 | 25 | ** |

†Excluding wool. ‡Including wool.
**$P<0.01$. NS, not significant.

These results suggest that high levels of acetate are utilized inefficiently for body-fat synthesis. However, it would be premature to relate the results obtained here to the utilization of volatile fatty acids resulting from normal rumen fermentation.

**The human taste threshold to alcohol and its implications.** By C. W. M. Wilson (introduced by G. L. S. Pawan), *Department of Pharmacology, Trinity College, University of Dublin*

The taste threshold to ethanol has been measured in humans by adaptation of the method described by Harris & Kalmus (1949) for measurement of the taste threshold of phenylthiocarbamate. Measurement of the alcohol taste threshold of a sample of 163 subjects, mean age 27 years, during the afternoon, established that a sweet taste threshold is first detected at 4·2 ± SE 0·24% alcohol and that the burning threshold is appreciated at 21·2 ± SE 1·22% alcohol (O'Brien, Rolfe & MacAirt, 1968). If the alcohol concentration is further raised after the point at which the burning taste is appreciated, it becomes more intense and finally the subject refuses to accept higher concentrations. The sweet and burning frequency distribution histograms depart significantly from the unimodal normal shape (Wilson, 1969). The taste thresholds undergo a definite circadian rhythm. When the taste thresholds are measured at 6-hourly intervals from midday, the threshold was found to be at its lowest, that is, alcohol was detected at its lowest concentration for sweet and burning tastes, at 18.00 hours in the evening. The threshold to sweet and burning tastes was at its highest at 06.00 hours.

When 30% alcohol is introduced direct into the stomach at a dose of 0.8 mg/kg, the sweet and burning taste thresholds are raised significantly during the following 2 h. They return towards their original levels as the blood alcohol diminishes. While the blood alcohol is elevated, alcohol appears in the saliva. The sweet and burning taste thresholds are significantly raised after oral administration of 800 mg of metronidazole (Wilson, 1969). The alcohol taste thresholds are also significantly

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raised by mixing metronidazole with the test solutions of alcohol at the same concentrations of metronidazole at which the drug is secreted in the saliva.

The human taste threshold to alcohol has physiological characteristics. This is demonstrated by the fact that it can be measured in a normal population sample, that it has a circadian rhythm, that it can be affected by changing blood alcohol concentrations and is altered by administration of the drug metronidazole. The effect of the drug on the alcohol taste threshold indicates the taste threshold is detected by peripheral taste receptors and through central mechanisms. The evidence obtained from the sample survey suggests that there may be a genetic basis to variations in the alcohol taste threshold.

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**The automated estimation of chromic oxide.** By J. MATHIESON (introduced by J. DAVIDSON), *Rowett Research Institute, Bucksburn, Aberdeen*

In digestion studies with ruminants, chromic oxide is frequently used as a reference marker. A modification of the automated colorimetric method of Stevenson & Clare (1963) for estimating chromic oxide using a Technicon AutoAnalyzer system has been compared with the manual colorimetric method of Stevenson & de Langen (1960). On fourteen duplicate samples of sheep faeces, containing between 0·1 and 5 mg $Cr_2O_3$ per g dry matter, estimations by the automated method were about 1·5% higher (1·4±0·5%) than by the manual.

*Method.* The dried material containing 1–5 mg $Cr_2O_3$ was ashed at 550° and digested with a $H_2SO_4$–$H_3PO_4$ mixture in the presence of $MnSO_4$ and $KBrO_3$ as described by Stevenson & de Langen (1960) before diluting with water to 100 ml.

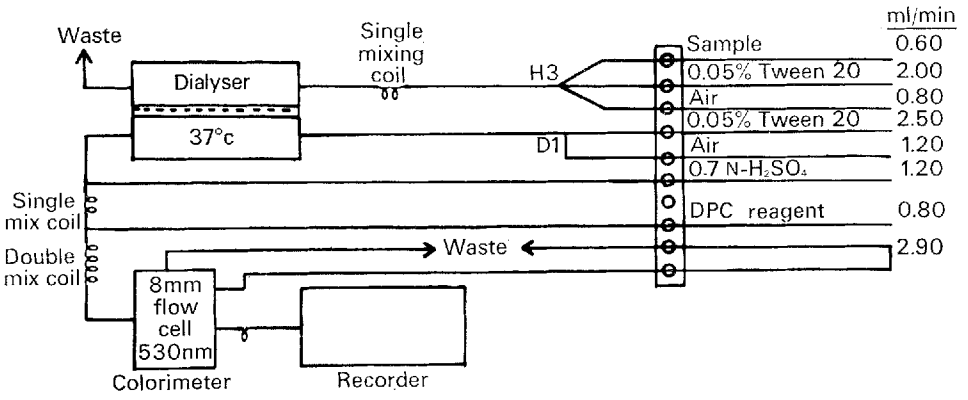


Fig. 1. Flow diagram for automated estimation of $Cr_2O_3$.

Samples in the AutoAnalyzer were analysed at thirty per h using a Mk II sampler having a sample to wash ratio of 1 to 2.

The flow diagram with reagents is shown in Fig. 1. In the modified method, 0·25% diphenylcarbazide reagent was dissolved in 20% acetone rather than in ethanol because this improved the base-line and definition of peaks on the record chart. 'Spot test' diphenylcarbazide reagent from British Drug Houses was found to be more reliable than 'Analar' reagent. $K_2Cr_2O_7$ standards were made up according to Stevenson & Clare (1963) in a blank solution prepared by digesting an appropriate amount of chromium-free ash from faeces or other test material.

By this procedure about 2000 estimations have now been made without difficulty.

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**The effect of increasing the concentration of urea or fish meal in a rolled-barley diet on protein absorption from the small intestine of sheep.**

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

The results of previous work (Ørskov & Fraser, 1969) showed that, when various levels of soya-bean meal were included in a rolled-barley diet for sheep, the amount of non-ammonia crude protein apparently absorbed from the small intestine increased markedly. The increase showed a curvilinear trend so that more protein was available when the diet contained 18 rather than 20% crude protein in the dry matter. This suggests that deamination in the rumen increased progressively as dietary crude protein increased. Since the microbial nitrogen production is likely to be related to the amount of carbohydrate fermented (Hungate, 1966) and since the dry-matter intake was held constant, the increase was assumed to be dietary protein which had escaped degradation in the rumen.

To provide additional information, an experiment was conducted in which increments of urea or fish meal were added to a rolled-barley diet. Dry-matter intake was approximately 1025 g dry matter/d. The design was that of two combined $4 \times 4$ Latin squares using four female Cheviot or Suffolk crosses with cannulas in abomasum and terminal ileum. Four diets were given with different concentrations of urea or fish meal. In a control period at the end of the test feeding, the rolled-barley diet was given without supplements. The control diet contained 9·4%, the four urea diets 12·0, 15·3, 18·9 and 23·4%, and the four fish-meal diets 11·3, 14·4, 18·9 and 21·4% $N \times 6{\cdot}25$ in dry matter.

The periods were 14 d and, during the last 24 h, samples were taken at 2 h intervals from abomasal and ileal content and from the rectum. Chromic oxide was used as an indigestible marker.

With urea, the amounts of non-ammonia crude protein apparently disappearing between the abomasum and terminal ileum were 77 g for the control, and 76, 67, 83

and 80 g for the four urea levels respectively. None of the differences approached significance. The standard error of urea levels was ±11 g. With fish meal, there was a highly significant ($P<0.001$) linear increase in the amount of non-ammonia crude protein disappearing between the abomasum and terminal ileum, ranging from 77 g on the basal diet to 132 g with the highest supplementation. The relationship can be described by the equation $Y=0.37X+43.8$ (RSD ±21 g) where Y is g/d of non-ammonia crude protein disappearing between the abomasum and terminal ileum, and X the protein intake in g.

It is concluded that the level of crude protein in the control diet was sufficient for the microbial requirement and that urea did not spare the microbial utilization of dietary protein. With fish-meal supplementation, substantial quantities of protein were escaping rumen fermentation but more than half of the protein supplement was apparently destroyed in the rumen.

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**Voluntary intake of roughage diets by ruminants.** By S. BEN-SAUD, *Department of Agricultural Science and Applied Biology, University of Cambridge*

A number of hypotheses on intake control in ruminants have been suggested (Blaxter, Wainman & Wilson, 1961; Campling, Freer & Balch, 1961; Crampton, 1957; Egan & Moir, 1965; Owen, Davies & Ridgman, 1969; Pearce, 1966; Troelsen & Campbell, 1966). None of the existing hypotheses is applicable throughout the whole range of roughages of different forms and some clear discrepancies occur. A comprehensive hypothesis applicable to the full range of roughages is suggested:

(1) Protein deficiency, acting as a chemostatic regulator, is the primary limiting factor to voluntary intake of poor-quality roughage. This results in low intake, low distention in the digestive tract and slower rate of passage. Grinding such diets is unlikely to provide significant improvement.

(2) The rate of breakdown of food particles and onward passage through the reticulo-omasal orifice is the limiting factor to the intake of medium-quality roughage, where protein is not limiting. Here, grinding leads to faster passage from the rumen, higher intake and lower mean retention time.

(3) The intake of highest-quality roughages may not be limited by specific protein deficiency nor by the rate of breakdown in the rumen, resulting in maximum intake regulated through the central nervous system. In such cases, grinding would not increase intake and rate of passage.

(4) The rate of passage itself is more the result rather than the cause of variation in voluntary food intake.

The above hypothesis is in good agreement with published results. Further support to it comes from two recent experiments:

In the first, shredded barley straw (19 mm screen) and a simulated straw, based on semi-purified ingredients with fine polyethylene as a diluent, were used in experiments with sheep.

These two diets were given alone or supplemented to the level of 10% crude protein. The intake of straw and simulated straw increased by 57% and by 69% respectively due to protein supplementation.

In the second experiment pelleted, shredded barley straw and pelleted, ground lucerne were given with and without a balloon containing 3 l of water suspended in the rumen. At a restricted level of feeding, the mean retention time was lower for straw than for lucerne whereas under *ad lib.* feeding the position was reversed. The inclusion of the balloon decreased the mean retention time at the restricted level of feeding.

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**Effects of dietary calcium and phosphorus concentrations on the faecal excretion of copper, manganese and zinc in sheep.** By N. F. SUTTLE and A. C. FIELD, *Moredun Research Institute, Gilmerton Road, Edinburgh, 9*

Although the effects of high dietary concentrations of calcium and phosphorus on the metabolism of trace-elements by non-ruminants are well documented (Suttle, 1968), their effects on ruminants have received little attention. In an experiment designed to study the effects of dietary Ca and P concentrations on Mg metabolism in sheep, conditions were also suitable for estimating their effects on the faecal excretion of zinc, manganese and copper. Six adult castrated sheep were given a semi-purified basal diet (Suttle & Field, 1969) containing 0·06, 1·05 or 1·98% Ca with 0·06 or 0·59% P in a 6×6 Latin square experiment. Calcium carbonate and trisodium phosphate provided the supplementary Ca and P and the basic food allowance, 0·8 kg/d, was increased for supplemented diets so that the intake of nutrients other than Ca and P remained constant. Treatments were applied for consecutive 14 d periods and faeces were collected for the last 5 d of each. The results are summarized in Table 1.

Increasing the dietary Ca from 1·0 to 2·0% produced increases in the faecal excretions of Mn ($P<0·1$) and Zn ($P<0·01$), but changes in dietary P were without effect. Faecal Cu was only increased by Ca at the lower dietary P level ($P<0·05$); the Ca×P interaction was not, however, significant. The urinary excretion of Cu, Mn and Zn is small relative to intake, and changes in faecal excretion probably reflect changes in balance which are the net effects of changes in absorption and metabolic

faecal excretion. The reductions in apparent availabilities of Cu, Mn and Zn to 23·9, 3·3 and −10·5%, respectively, at the highest Ca level, are indicative of predominant effects on one of these processes. Although faecal trace-element excretion was not affected at the intermediate Ca level, changes in absorption and metabolic faecal excretion may have counteracted each other. The fact that significant effects on faecal excretion were only observed at unnaturally high dietary Ca concentrations does not, therefore, mean that trace element metabolism was unaffected at the lower levels.

Table 1. *Effect of dietary Ca and P concentrations on the mean faecal excretion of Cu, Mn and Zn (mg/d) by six adult male sheep*

| | | Mean faecal excretion | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | | Mn | | Zn | |
| Dietary P level (% DM) | | 0·06 | 0·59 | 0·06 | 0·59 | 0·06 | 0·59 |
| Dietary Ca level (% DM) | 0·06 | 2·72 | 2·64 | 33·7 | 28·7 | 15·3 | 14·2 |
| | 1·05 | 2·76 | 2·51 | 31·4 | 29·4 | 15·6 | 14·6 |
| | 1·98 | 3·45 | 2·51 | 34·6 | 34·5 | 18·6 | 18·1 |
| SE of means | | ±0·31 | | ± 3·1 | | ± 1·3 | |
| Intake | | 4·53 | | 35·8 | | 16·6 | |

DM, dry matter

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**Mineral excretion by three pairs of monozygotic cattle twins.** By A. C. FIELD and N. F. SUTTLE, *Moredun Research Institute, Edinburgh* 9

The use of monozygotic twins (MZ) in balance trials may increase sensitivity by reducing the large between-animal variation which occurs in mineral studies with ruminants. A comparison of the variation in mineral excretion between and within MZ twins on the same diet has not been reported hitherto.

Data on the mineral excretion of three pairs of non-lactating MZ twins were obtained from an experiment designed to study the effect of dietary potassium on magnesium metabolism (Table 1). It was a $3 \times 2$ factorial design within a $6 \times 6$ Latin square with three dietary levels of Mg and two levels of K. The cows were reared together and were of the same breed and age and of similar weight.

The variation was greater between than within twins for excretion of Mg ($P<0{\cdot}10$), Ca ($P<0{\cdot}10$) and P ($P<0{\cdot}001$) in urine, and of Na ($P<0{\cdot}05$) and K ($P<0{\cdot}10$) in faeces. An indirect measure of the availability of dietary Mg was obtained from the regression coefficient of urinary Mg on dietary Mg. The values for the individual pairs were 22·8, 7·4 and 18·6%, and the differences between pairs were greater ($P<0{\cdot}05$) than those within pairs.

Table 1. *Mean excretion of minerals in urine and faeces (g/d) by three pairs of monozygotic cattle twins*

| | | Pair | | | Variance ratios Between MZ / Within MZ | Twin efficiency value |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| Urine | Mg | 0·82 | 0·32 | 0·65 | 6·46[a] | 3·7 |
| | Ca | 0·21 | 0·32 | 0·07 | 5·47[a] | 3·2 |
| | P | 1·92 | 7·39 | 0·62 | 172[c] | 86 |
| | Na | 37·7 | 38·6 | 37·4 | 1·26 | 1·1 |
| | K | 127 | 128 | 129 | 0·51 | — |
| | | Pair | | | Variance ratios Between MZ / Within MZ | Twin efficiency value |
| | | 1 | 2 | 3 | | |
| Faeces | Mg | 4·05 | 4·45 | 4·43 | 1·00 | 1.0 |
| | Ca | 19·2 | 19·3 | 19·8 | 0·11 | — |
| | P | 9·81 | 6·45 | 10·93 | 5·76 | 3·4 |
| | Na | 2·73 | 2·59 | 4·27 | 31·7[b] | 16.3 |
| | K | 7·42 | 4·51 | 5·81 | 7·37[a] | 4·2 |

[a], $P<0.10$; [b], $P<0.05$; [c], $P<0.001$.

The twin efficiency values, i.e. the number of sets of randomly selected animals which a set of identical twins can replace without loss of sensitivity, obtained in the present experiment will be relatively low because of the close contemporaneity of the pairs. The large values found for P in urine strongly suggest genetic control, but any conclusions about the excretion of other minerals and hereditability are precluded by the small number of MZ twins used.

**The estimation of the body composition of living pigs by $^{42}K$ dilution.**

By M. F. Fuller, R. A. Houseman and A. Cadenhead, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

The potassium content of the body has been estimated by $^{42}K$ dilution in man (Corsa, Olney, Steenburg, Ball & Moore, 1950), in rats (Talso, Miller, Carballo & Vasquez, 1960) and in pigs (Pfau, 1966).

Using a variety of diets, and feeding rates, we produced pigs weighing 90 kg which contained from 16 to 38% of total fat. They were all injected intravenously with 0·4 mCi of $^{42}K$ as KCl in isotonic saline (PES1P; The Radiochemical Centre, Amersham, Bucks, England). The specific activities (SA) of potassium in plasma and urine were measured at intervals after the injection. Plasma SA reached equilibrium in 10–12 h. Satisfactory estimates of equilibrium SA were obtained for eighteen of the twenty pigs, either from plasma or from urine voided several hours after the plasma had reached equilibrium. The total exchangeable potassium ($K_e$) was calculated according to the equation:

$$K_e = \frac{I-L}{SA},$$

where I = the total activity injected, and L = the total activity lost in urine and faeces.

Immediately afterwards, the animals were slaughtered, and the alimentary tracts emptied. The carcasses were minced and their water, fat and N contents determined.

The relation between $K_e$ (g) and fat-free weight (FFW; kg) could be expressed by the equation:

$$FFW = 15{\cdot}0 + 0{\cdot}279\ K_e;\ \text{RSD} = \pm 1{\cdot}65\ \text{kg},\ r = 0{\cdot}94.$$

Alternatively, the regression of % fat in the empty body (F%) on $K_e$ per 100 kg empty body-weight ($K_e$/W) was:

$$F\% = 85{\cdot}8 - 0{\cdot}299\ K_e/W;\ \text{RSD} = \pm 2{\cdot}3\%,\ r = 0{\cdot}93.$$

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**Vitamin E, training and performance in adolescent swimmers.** By I. M. SHARMAN, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*, and M. G. DOWN and R. N. SEN,* *Department of Ergonomics and Cybernetics, University of Technology, Loughborough, Leicestershire*

Vitamin E has for long attracted interest as a possible ergogenic aid to athletic performance (Prokop, 1960). In animals, deficiency of the vitamin has caused muscular dystrophy, while sufficiency has increased resistance to hypoxia. It has also been claimed to have beneficial effects in the treatment of circulatory diseases in humans.

In this study, a related experimental group design was employed to observe the effects of dietary supplementation with a vitamin E preparation and training on the physical function and performance of adolescent swimmers. Two matched groups of thirteen subjects each, one taking the vitamin, the other placebo, were tested at the beginning and end of a 6-week training programme.

The effects of a daily intake of 400 mg α-tocopheryl acetate were evaluated on a battery of tests of anthropometric status, cardio-respiratory efficiency, motor fitness and performance. The subjects were recruited from the swimming club at a boys' boarding school. They led similar communal lives and undertook a comparable training programme, in terms of both frequency and effort, graduated according to individual ability. The subjects, on average, took part in four swimming sessions per week. These combined continuous endurance swimming at constant speed with controlled interval training geared to the individual's target racing pace. Neither knowledge of results nor their meaning was conveyed to the subjects, to minimize the chance of any added motivational stimulus at the final testing. The double blind method of treatment ensured that neither subjects nor investigators knew to which group any individual belonged.

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The 5% level of confidence, using Student's *t* test, was accepted as a statistically significant difference between the effects of the two treatments, while all differences up to the 10% level were noted, since experiments of this type, by definition, introduce varying degrees of motivation, training and dietary regimen.

No significant group differences were observed on any of the tests undertaken before treatment. After treatment, significant differences were observed within both groups as a result of the training with regard to anthropometric status, cardio-respiratory efficiency and motor fitness tests. No significant differences in the effects of vitamin E as such were observed at the 5% confidence level in the results on anthropometric status, cardio-respiratory efficiency or motor fitness, and no evidence was therefore obtained of the suggested ergogenic properties of vitamin E.

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**Effect of loss of incisors on the excretion of faecal dry matter by grazing sheep.** By A. C. FIELD and C. I. HARRIS, *Moredun Research Institute, Edinburgh* 9

'Broken-mouth' is generally thought to lower productivity by interfering with grazing, but no attempts have been made to measure the intake of herbage by broken-mouthed ewes under either hill or lowland conditions. In the present preliminary experiment, the relative herbage intakes of broken- and sound-mouthed ewes were measured indirect from the faecal dry matter (FDM) and faecal nitrogen concentration (FN).

Twelve 6-year-old Blackface ewes were used; one group (broken-mouthed, live weight 44·7 ± 1·55 kg) of six ewes had lost all incisors and in the other (sound-mouthed, live weight 46·9 ± 1·32 kg) dentition was complete. The experiment consisted of three periods: in the first, the ewes grazed a bare ryegrass–wild white pasture; in the second, a mixed sward comprising good growths of ryegrass, wild white clover, meadow fescue and timothy; and in the third, the ewes were housed in separate pens and given 1 kg/d chopped hay in two equal feeds. Each period lasted 14 d and a complete collection of faeces excreted over the last 5 d was made. Faeces from individual sheep were dried, ground and analysed for N by the macro-Kjeldahl method.

In period 1, the mean values with their standard errors for broken- and sound-mouthed ewes were respectively 2·36 ± 0·105 and 2·63 ± 0·283 g/100 g DM for FN, 231 ± 9·72 and 214 ± 24·1 g/d for FDM. The corresponding figures for period 2 were 3·15 ± 0·131 and 3·28 ± 0·152 for FN, 289 ± 20·7 and 279 ± 29·2 for FDM, and for period 3 were 2·04 ± 0·090 and 1·94 ± 0·060 for FN, and 243 ± 5·67 and 254 ± 5·58 for FDM. There was a negative correlation between FDM and FN in period 1 ($r = -0{\cdot}61$, $P < 0{\cdot}05$) and in period 3 ($r = -0{\cdot}71$, $P < 0{\cdot}01$). The mean

digestibilities of the dry matter in hay were 73·0 and 71·1% for the broken- and sound-mouthed ewes, respectively.

Evidence for greater selection of herbage by the sound-mouthed ewes on the bare pasture in period 1 was obtained; the variation in FDM and in FN within groups was greater ($P<0.05$) for the sound-mouthed ewes. There were no significant differences in FDM and FN between the groups in period 1 (Behrens–Fisher test) and in period 2, indicating that the complete loss of incisors had no effect on the intake of herbage dry matter by the grazing ewe. No significant differences in dry-matter digestibility, FDM and FN between groups occurred when the ewes were on a constant diet in period 3.

*The Two Hundred and Nineteenth meeting of The Nutrition Society was held in the Nutrition Department, The Atkins Building, Queen Elizabeth College, Campden Hill, London W8, on Friday, 20 March 1970, at 15.00 hours, when the following papers were read:*

**Influence of the addition of molybdenum on the digestibility and palatability of a diet for sheep.** By G. VARELA, J. J. ESCRIVA and J. BOZA, *Department of Animal Physiology Experimental Station of Zaidin, Granada, Spain*

With the object of discovering the effect of the addition of molybdenum, at non-toxic levels, on the nutritive value and acceptability of a diet for sheep (castrated lambs 1 year old), digestibility and palatability tests have been carried out, using a diet rich in fibre composed of barley and wheat straw with the addition of calcium carbonate. The molybdenum content of this diet was 0·27 ppm and was supplemented by sodium molybdate up to the following five levels of molybdenum: 2·27, 4·27, 6·27, 8·27 and 10·27 ppm.

Six digestibility experiments designed on a 'Latin square' following the 'direct method', have been carried out, with the results shown in Table 1. Addition of 8·27 and 10·27 ppm of molybdenum significantly ($P<0.001$) increased the digestibility of the fibre and, to a lesser extent, the total digestible nutrient (TDN) content; no statistically valid differences in the digestibility of protein, fat and nitrogen-free extracts (NFE) were observed.

| Mo content (ppm) | Digestibility (%) | | | | TDN content (%) |
|---|---|---|---|---|---|
| | Protein | Fat | Fibre | NFE | |
| 0·27 | 58·4 | 74·7 | 35·7 | 80·5 | 67·8 |
| 2·27 | 58·8 | 74·0 | 36·2 | 80·1 | 67·6 |
| 4·27 | 58·9 | 72·9 | 36·1 | 79·0 | 66·8 |
| 6·27 | 59·3 | 71·3 | 36·4 | 78·6 | 66·5 |
| 8·27 | 59·9 | 74·2 | 40·8 | 80·2 | 68·4 |
| 10·27 | 58·5 | 73·7 | 43·7 | 81·0 | 69·2 |