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# PROCEEDINGS OF THE NUTRITION SOCIETY

## ABSTRACTS OF COMMUNICATIONS

*The Three Hundred and Eighty-eighth Meeting of the Nutrition Society was held at the Faculty of Letters Lecture Theatre, University of Reading, on Wednesday and Thursday,* 13/14 *July* 1983, *when the following papers were read:*

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**No effect of β-carotene on the response of an inbred mouse strain to the bladder carcinogen *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN).** By R. M. HICKS, J. A. TURTON, C. N. TOMLINSON, J. GWYNNE, E. CHRYSOSTOMOU, K. NANDRA and M. PEDRICK, *Department of Cell Pathology, School of Pathology, Middlesex Hospital Medical School, Riding House Street, London W1P 7LD*

There is good experimental and epidemiological evidence that high intakes of vitamin A and some of its analogues (retinoids) will reduce the relative risk for developing cancer in both man and experimental animals. In man, high-vitamin-A diets usually include large amounts of green vegetables which contain the nontoxic vitamin A precursor, β-carotene. It has been suggested that β-carotene itself may be protective and that β-carotene prophylaxis should be attempted in clinical trials (Peto *et al.* 1981).

We previously compared the potential of three synthetic retinoids to inhibit the development of bladder cancer induced in the B6D2F1 mouse by the specific bladder carcinogen BBN. One significantly ($P<0.01$) reduced the age-related incidence of bladder cancer over a 5-month period. The effective compound, *N*-(4-hydroxyphenyl)-retinamide (HPR), at 1 mM in the diet, reduced both the grade and stage of carcinomas. The effect of β-carotene has now been investigated in the same model. Groups of 24±1 B6D2F1 mice were given either the alcoholic vehicle or ten portions of BBN by stomach tube over 10 weeks to give a total dose of either 15 or 30 mg of the carcinogen. They were then maintained for 6 months, either on a standard laboratory diet or on a diet supplemented with 5 mM-β-carotene. After this time the animals were killed and their bladders examined histologically.

No significant inhibitory effect of the β-carotene diet was observed on the incidence at 6 months of either hyperplasias or carcinomas produced in response to either dose of BBN (see Table).

*Histology of urothelium*

(Number of animals with percentage incidence in parentheses)

| Group | Number of usable animals | Normal urothelium | Hyperplastic urothelium | Total carcinomas |
|---|---|---|---|---|
| Vehicle + control diet | 24 | 24 (100) | 0 | 0 |
| Vehicle + β-carotene | 23 | 16 (70) | 7 (30) | 0 |
| 15 mg BBN + control diet | 25 | 3 (12) | 16 (64) | 6 (24) |
| 15 mg BBN + β-carotene | 24 | 2 (8) | 18 (75) | 4 (16) |
| 30 mg BBN + control diet | 25 | 0 | 13 (52) | 12 (48) |
| 30 mg BBN + β-carotene | 25 | 2 (8) | 12 (50) | 11 (42) |

These results indicate that, in contrast to the retinoid HPR, high dietary levels of β-carotene are unable to reduce the carcinogenic response of this hybrid mouse to previous exposure to BBN. If these results were to be extrapolated to man, they would suggest that β-carotene would be less effective than certain retinoids in treating populations previously exposed to carcinogens. These experiments were not designed to investigate whether *pre-treatment* with β-carotene could prevent carcinogenesis after subsequent exposure to a carcinogen.

Peto, R., Doll, R., Buckley, J. D. & Sporn, M. B. (1981). *Nature*, **290**, 201–208.

**Skeletal damage as an early sign of retinoid toxicity in the rat.** By J. A. TURTON, R. M. HICKS, J. GWYNNE, *Department of Cell Pathology,* and D. KATZ, *Department of Histopathology, Bland-Sutton Institute and School of Pathology, Middlesex Hospital Medical School, Riding House Street, London W1P 7LD*

We have previously reported that skeletal changes appear in rats and mice as early as 7 weeks after feeding with retinoids (Turton *et al.* 1983). Further studies have been undertaken to determine how soon after exposure to retinoid these and other indicators of toxicity can be observed in young, rapidly growing rats. Weanling female F344 rats were given diets containing 2·5 mM-*N*-(ethyl)-retinamide (NER) or placebo-retinoid and killed at 2, 4, 6, 9, 12 and 15 weeks. Body-weights and measurements of spontaneous activity were recorded. At post-mortem the liver, kidney, spleen, salivary gland, lymph nodes, thymus, hind-limb, humerus and femur were weighed and the length of the body, tail, hind-limb, humerus and femur measured. Humeral and femoral diameters were recorded. The femur was sectioned and cortical bone thickness and medullary cavity diameter measured. Femora were examined histologically.

Feeding NER did not affect body-weight and all rats given placebo or NER diets grew well, increasing from 60 to 190 g at 15 weeks. As observed previously, the most significant changes were seen in the femur and humerus of retinoid-fed rats. At 2 and 4 weeks minor changes were seen but by 6 weeks the long-bone weights and periosteal diameters were significantly reduced in NER-fed animals. This trend continued throughout the experiment. Sectional measurements demonstrated increased cortical bone thickness, but the reduction in periosteal diameter was largely due to a significant decrease in medullary cavity diameter from week 6. Long bone, hind-limb, body and tail lengths were not significantly changed by feeding NER. There were no obvious behavioural changes in retinoid-fed animals as measured by spontaneous activity. Rats given diets containing 2·5 mM-*N*-(4-hydroxyphenyl)-retinamide (HPR) or *N*-(tetrazol-5-yl)-retinamide (TZR) and killed at 6 weeks had similar skeletal changes to those given NER. In NER-fed animals histological examination of femora showed an increased number of osteoblasts scattered throughout the cortical bone without localization in periosteal or endosteal regions. Osteoclastic activity was not a feature of tissue from NER or control groups. The marrow cavity in NER-fed animals contained normal haematopoietic tissue, but there was an increased proportion of cells with round chromatic nuclei and scanty cytoplasm, suggesting more erythropoietic precursors in NER-fed rats.

It is concluded that 6 weeks was the earliest time at which significant signs of skeletal damage can be detected in weanling rats. This appears to be a rapid in vivo method of detecting retinoid toxicity in this species.

Turton, J. A., Hicks, R. M., Gwynne, J., Hunt, R., Palmer, L., Medawar, P. B. & Hawkey, C. M. (1983). *Proceedings of the Nutrition Society* **42**, 12A.

**The role of insulin and corticosterone in the increase in muscle protein synthesis in refed fasted rats.** By P. C. BATES, B. ODEDRA and D. J. MILLWARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

Protein synthesis in muscle increases markedly during feeding in animals and man (Rennie *et al.* 1982). To investigate the relative importance of insulin and glucocorticoids in this response, changes in muscle protein synthesis and the levels of insulin and corticosterone were measured during the first hour of refeeding of 4-d fasted rats treated with corticosterone, anti-insulin serum or untreated prior to refeeding. Protein synthesis was measured in vivo by the large dose phenylalanine method (Garlick *et al.* 1980). As shown in the Table, increases in protein synthesis were observed as early as 40 min and individual increases were highly correlated with the increase in insulin and the fall in corticosterone. Treatment with corticosterone or anti-insulin serum partially but not completely inhibited the restoration of protein synthesis. This suggests that elevated corticosterone can only partially block any feeding-induced activation of protein synthesis and that there may be other factors apart from insulin involved in the activation.

| | | Protein synthesis (% per day) | | Insulin (μunits/ml) | | Corticosterone (ng/ml) | |
|---|---|---|---|---|---|---|---|
| Group and treatment | *n* | Mean | SD | Mean | SD | Mean | SD |
| A. Fasted | 11 | 2·49 | 1·31 | 7·9 | 2·9 | 481 | 162 |
| Fed 20 min | 6 | 2·98 | 1·8 | 7·25 | 5·2 | 490 | 135 |
| Fed 40 min | 6 | 4·22 | 1·71 | 12·5 | 8·8 | 240 | 134 |
| Fed 60 min | 7 | 5·88 | 2·03 | 9·0 | 5·0 | 184 | 52 |
| B. Fasted | 6 | 4·30 | 1·0 | 4·3 | 2·2 | 621 | 78 |
| Fed 60 min | 5 | 8·02 | 0·49 | 25·0 | 8·0 | 28 | 9 |
| Fed 60 min + corticosterone | 5 | 6·27 | 0·7 | 12·7 | 4·32 | 832 | 71 |
| C. Fasted | 6 | 2·20 | 0·7 | 1·6 | 1·8 | 758 | 191 |
| Fed 60 min | 7 | 4·86 | 1·09 | 19·7 | 8·5 | 151 | 88 |
| Fed 60 min + anti-insulin serum | 7 | 3·48 | 0·3 | ND | | 374 | 297 |

ND, non-detected.

Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.
Rennie, M. J., Edwards, R. H. T., Halliday, D., Matthews, D. F., Wolman, S. L. & Millward, D. J. (1982). *Clinical Science* **63**, 519–523.

**Effects of anabolic agents (trenbolone acetate + oestradiol-17β) on the excretion of 3-methylhistidine by beef steers.** By C. I. HARRIS, G. MILNE, RUTH M. MCDIARMID and A. C. BREWER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The improved weight gain resulting from the use of anabolic implants is well-known in the beef industry but the mechanism by which the anabolic response is achieved in vivo is not understood. Studies with rats suggest that rates of protein synthesis and breakdown are reduced although the response may vary with the chemical implanted (Vernon & Buttery, 1978). This report is part of a study of the response of protein metabolism to Revalor implants (Hoechst, Hounslow, Middlesex; 140 mg trenbolone acetate + 20 mg oestradiol-17β) when changes in protein breakdown were monitored by the urinary excretion of 3-methylhistidine (3MH). The influence of Revalor implants on protein synthesis, amino acid oxidation and heat production have been detailed previously (Lobley *et al.* 1982).

Three Hereford × Friesian steers were implanted at 300 kg body-weight and compared with three non-implanted control animals (see Lobley *et al.* 1982). Measurements were made at 2, 5, 8 and 11 weeks after implantation (Expt 1). No change in the rates of whole-body protein synthesis was found although the fractional rate of amino acid oxidation decreased (Lobley *et al.* 1982). No significant decrease in the urinary excretion of 3MH was found. A second experiment, essentially similar to the first, was carried out except that heat production and urine excretion were measured at 1, 4, 7 and 10 weeks after implantation but protein synthesis and amino acid oxidation were not determined. Live-weight gains (1·56 *v.* 1·04 kg/d, $P<0·01$) and N retentions (48·2 *v.* 18·4 gN/d, $P<0·001$) were significantly greater in the Revalor-implanted animals, but no significant differences in heat production were found. Excretion of 3MH was decreased in implanted animals and reached statistical significance at 4 and 7 weeks after implantation (Expt 2) (see Table).

*Mean urinary excretion of 3MH (μmol/d per kg) before and after implantation*

| | | | | | | |
|---|---|---|---|---|---|---|
| Expt 1 | | | | | | |
| Period | (weeks) | −1 | +2 | +5 | +8 | +11 |
| Control | (*n*3) | 3·16 | 2·90 | 3·14 | 3·03 | 2·82 |
| Implant | (*n*3) | 3·09 | 3·12 | 2·84 | 2·81 | 3·14 |
| Expt 2 | | | | | | |
| Period | (weeks) | −2 | +1 | +4 | +7 | +10 |
| Control | (*n*2) | 3·04 | 3·15 | 2·90 | 2·89 | 2·93 |
| Implant | (*n*3) | 3·04 | 2·88 | 2·49** | 2·38** | 2·64 |

Significantly different from control: **$P<0·01$.

These findings, and those of Lobley *et al.* (1982), suggest that the anabolic response in steers is due to a decreased rate of muscle protein breakdown and amino acid oxidation, while the rate of protein synthesis is not detectably altered.

Lobley, G. E., Smith, J. S., Mollison, G., Connel, A. & Galbraith, H. (1982). *Proceedings of the Nutrition Society* **41**, 28A.

Vernon, B. G. & Buttery, P. J. (1978). *Animal Production* **26**, 1–9.

**Phytic acid and heavy-metal uptake and depletion.** By H. E. ROSE and J. QUARTERMAN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The risk of exposure to heavy metals may be reduced by dietary alteration. Phytic acid (myoinositol-1,2,3,4,5,6-hexakis dihydrogen phosphate) is known to reduce the absorption of several essential minerals particularly if the diet has a high calcium content. In vitro studies have shown that calcium phytate binds both lead and cadmium (Wise & Gilburt, 1981) and dietary addition of calcium phytate has resulted in decreased blood lead levels in mice consuming Pb (Wise, 1982). We have studied the effects of dietary phytate on both the uptake and the depletion rates of Pb and Cd in rats.

6 g Ca/kg (as $CaCO_3$) or 10 g phytic acid/kg (as sodium phytate) or both Ca and phytate were added to a semi-purified diet (basal Ca and phytate, 6 and 5 g/kg respectively) containing either Pb (200 mg/kg from lead acetate) or Cd (5 mg/kg from cadmium hydroxide) and given to weanling rats for 4 weeks. Addition of phytate or Ca reduced the accumulation of Pb in both blood and bone (see Table). The greatest inhibition of tissue Pb retention was evident when both Ca and phytate were added. Cd accumulation was measured in the liver and kidneys and was significantly increased by the addition of Ca, in agreement with the report of Sasser *et al.* (1978). Phytate, either alone or with increased dietary Ca, had no effect on tissue Cd levels.

| | Pb analyses | | Cd analyses | |
|---|---|---|---|---|
| Addition to Pb/Cd supplemented diet | Femur (μg/g dry ash) | Blood (μg/100 ml) | Liver (μg/g wet weight) | Kidney (μg/g wet weight) |
| None | 332 | 25·6 | 0·30 | 0·62 |
| 6 g Ca/kg | 150 | 15·2 | 0·49 | 0·98 |
| 10 g phytic acid/kg | 124 | 14·5 | 0·36 | 0·49 |
| Ca + phytic acid | 39 | 7·5 | 0·40 | 0·44 |
| Log SE* | 0·214 | 0·339 | 0·118 | 0·144 |

*Analyses of variance were performed on the logarithmically transformed data.

In a second experiment weanling rats were given Cd (5 mg/kg diet) and Pb (200 mg/kg diet) for 4 weeks and the accumulation of Cd and Pb in tissues was estimated in some of the animals. Phytate was then added to the basal diet which contained no Cd or Pb and the tissue Cd and Pb concentrations were measured after a further 2 or 4 weeks. Phytate had no significant effect on the rate of depletion of Pb or Cd from tissue samples.

Dietary phytate was thus shown to have a greater effect on Pb compared with Cd retention despite in vitro work showing that both metals form insoluble complexes with calcium phytate that should, presumably, decrease the absorption of either from the gut.

The research was financed by Rio Tinto Zinc Services Ltd.

Sasser, L. B., Chertok, R. J., Callaham, M. R. & Jarboe, G. E. (1978). In *Trace Element Metabolism in Man and Animals*—3, pp. 562–565 [M. Kirchgessner, editor]. Freising-Weihenstephan: ATW.
Wise, A. (1982). *Bulletin of Environmental Contamination and Toxicology* **29**, 550–553.
Wise, A. & Gilburt, D. J. (1981). *Toxicology Letters* **9**, 45–50.

**Influence of dietary fats on the rat caecal microflora.** By A. K. MALLETT and I. R. ROWLAND, *British Industrial Biological Research Association, Carshalton, Surrey SM5 4DS* and A. WISE, *Medical Research Council Laboratories, Carshalton, Surrey SM5 4EF*

Epidemiological evidence suggests a causal relationship between dietary fat intake and the incidence of certain cancers in man. Modification by dietary fat of the metabolism of potential carcinogens by the intestinal microflora has been postulated as a mechanism to explain the correlation. Here we report a study in rats of the influence of dietary fats of different extents of saturation on the caecal microflora and some of its enzymic activities (Wise *et al.* 1983).

Male Sprague-Dawley rats (3 weeks old) were fed for 30 d on a basal, purified, low-fat (10 g/kg) diet, or that diet with iso-energetic quantities of starch replaced by 350 g of beef fat (dripping), cocoa butter, virgin olive oil or safflower oil/kg diet.

| | Control | Beef fat | Cocoa butter | Olive oil | Safflower oil |
|---|---|---|---|---|---|
| Caecal contents (g) | 2·1 | 2·4 | 2·7 | 1·8 | 1·5$^{**}$ |
| Bacteria × $10^{10}$/caecum | 15 | 5·2$^{***}$ | 11 | 19 | 9·3$^{**}$ |
| Aerobes/$10^4$ bacteria | 32 | 7·1$^{**}$ | 8·6$^{*}$ | 7·5$^{**}$ | 10$^{**}$ |
| Azoreductase | 11 | 4·2$^{**}$ | 3·6$^{**}$ | 6·7 | 2·1$^{**}$ |
| Nitrate reductase | 6·9 | 3·9$^{*}$ | 2·8$^{*}$ | 4·0$^{*}$ | 3·2 |
| β-Glucosidase | 18 | 10$^{**}$ | 11$^{*}$ | 8·9$^{***}$ | 5·5$^{***}$ |
| β-Glucuronidase | 38 | 78$^{***}$ | 49 | 60$^{*}$ | 43 |

Median enzyme activities (μmol/h per caecum) and bacterial numbers were determined using eight rats in each dietary group.

Mann Whitney U test by comparison to the control: $^{*}P<0·05$, $^{**}P<0·01$, $^{***}P<0·001$.

Beef fat and safflower oil significantly reduced the number of caecal bacteria and all fats decreased the proportion of aerobes within the total bacterial population. In general, feeding of high-fat diets decreased most of the microbial enzyme activities in the caecum with the exception of β-glucuronidase, the activity of which was increased, particularly by beef fat and olive oil. Although there were significant differences among the fats in the extent to which they affected the enzymes, these differences could not be explained on the basis of the extent of saturation of their constituent fatty acids. Previous studies have shown that humans or rats given diets high in animal protein and fat have elevated faecal β-glucuronidase activity (Reddy *et al.* 1974; Goldin & Gorbach, 1976). Our results suggest that it is the fat component that is responsible for this increased enzyme activity.

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We thank Carol Bearne and David Gilburt for technical assistance.

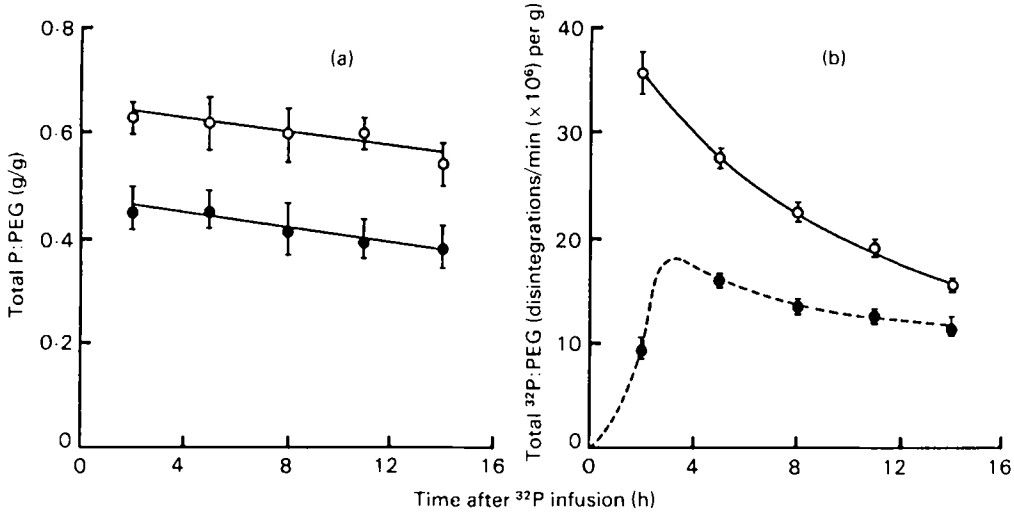
Goldin, B. R. & Gorbach, S. L. (1976). *Journal of the National Cancer Institute* **57**, 371–375.

Reddy, B. S., Weisburger, J. H. & Wynder, E. L. (1974). *Science* **183**, 416–417.

Wise, A., Mallett, A. K. & Rowland, I. R. (1983). *Nutrition and Cancer* **4** (In the press.)

**True and apparent absorption of phosphate between reticulum and duodenum of the ruminating calf.** By J. N. BANKS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT*

Net absorption of P between reticulum and duodenum of the calf has been demonstrated (Banks & Smith, 1984). To investigate true absorption three calves with rumen and duodenal cannulas were automatically fed, at 3 h intervals, diets of sugar beet pulp, flaked maize and alkali-treated straw with a P supplement and polyethylene glycol (PEG) and $^{103}$Ru-phenanthroline as markers. For an experiment $Na_2H^{32}PO_4$ was infused into the rumen with a feed at 05.00 hours. No further feeds were given until 20.00 hours but marker additions continued at 3 h intervals. Reticulum and duodenum samples were taken at 07.00 hours and subsequently at 3 h intervals until 19.00 hours. At the reticulum all $^{32}$P was present initially as inorganic P but the proportion fell to level out at 0·70 at 16.00 and 19.00 hours. Digesta samples, and fluid- and solid-rich fractions prepared from them, were analysed; $^{32}$P was counted at a window setting of 500–1000 keV to eliminate the effects of $^{103}$Ru which was itself counted after 2 months. Calculated mean corrected flows relative to PEG (*n* 3, SEM as vertical bars) at the reticulum (○) and duodenum (●) of (a) total P and (b) $^{32}$P are shown in the figure.




True and apparent absorption of P between reticulum and duodenum of the ruminating calf.

Assuming a mean delay of 3 h (different reasonable assumptions did not affect the main conclusions) between samples leaving the reticulum and reaching the duodenum and comparing flows at 16.00 and 19.00 hours respectively, mean proportions (with SEM) of total P and $^{32}$P absorbed were 0·34 (0·05) and 0·37 (0·03) respectively. It appeared that true and apparent absorption of P between reticulum and duodenum (presumably primarily in the omasum) did not differ substantially.

Banks, J. N. & Smith, R. H. (1984). *Proceedings of the Nutrition Society* **43**, 9A.

**Sites of absorption of magnesium and phosphate in the stomach of the ruminating calf.** By J. N. BANKS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT*

Differing views exist on the main site of Mg absorption within the ruminant stomach. Some results have favoured the reticulo-rumen (Martens, 1983). Contrary conclusions were reached from analyses of digesta from different sites in ruminating calves equipped with rumen and abomasal cannulas and omasal sleeves and fed twice daily (Edrise & Smith, 1979). These experiments indicated that both Mg and P were absorbed substantially from the omasum. Experiments have now been done using four calves similarly cannulated but with duodenal cannulas also and fed automatically at 3 h intervals. Diets consisted of flaked maize and dried grass (2:1) with polyethylene glycol and $^{103}$Ru-phenanthroline as markers and (A) no further supplements, (B) supplementary Mg, or (C) supplementary Mg + K. Each diet was given in turn until essentially steady-state conditions were achieved. Samples were taken 2 h after a morning feed from reticulum, omasal outflow and duodenum. These samples, and fluid- and solid-rich fractions prepared from them, were analysed and daily flows (g) of Mg and P at the different sites calculated (Edrise, 1979). Values were as follows:

| Diet ... | A | | B | | C | | Pooled SEM | |
|---|---|---|---|---|---|---|---|---|
| | Mg | P | Mg | P | Mg | P | Mg | P |
| Intake | 2·14 | 6·86 | 8·06 | 6·86 | 8·06 | 6·89 | 0·093 | 0·113 |
| Reticulum flow | 2·40$^{\bullet}$ | 22·8$^{\bullet\bullet}$ | 6·00$^{\bullet\bullet}$ | 23·9$^{\bullet\bullet}$ | 8·73 | 25·8$^{\bullet\bullet}$ | 0·187 | 1·14 |
| Omasal outflow | 1·83$^{\bullet}$ | 16·4$^{\bullet\bullet}$ | 4·61$^{\bullet\bullet}$ | 14·9$^{\bullet\bullet}$ | 5·80$^{\bullet}$ | 15·8$^{\bullet\bullet}$ | 0·202 | 1·36 |
| Duodenal flow | 1·95 | 15·8 | 4·71 | 14·1 | — | — | 0·286 | 1·73 |

Significant differences from the immediately preceding site are indicated: $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.01$.

Results agreed with those of Martens (1983) in showing appreciable absorption of Mg up to the reticulum for diet B when the molar ratio of Na:K in reticulum contents was high (5·1) although even then at least as much Mg was absorbed in the omasum. A lower Na:K value in the reticulo-rumen (3·3) for diet C appeared to abolish Mg absorption up to the reticulum but not in the omasum. The results, like those of Edrise & Smith (1979), showed substantial net absorption of P as well as Mg in the omasum but neither element showed appreciable net exchange in the abomasum.

Edrise, B. M. (1979). PhD Thesis, University of Reading.
Edrise, B. M. & Smith, R. H. (1979). *Annales de Recherches Veterinaires* **10**, 354–355.
Martens, H. (1983). *British Journal of Nutrition* **49**, 153–158.

**Tryptophan requirement of rainbow trout (*Salmo gairdneri*).** By M. J. WALTON, C. B. COWEY, R. M. COLOSO and J. W. ADRON, *NERC Institute of Marine Biochemistry, St. Fittick's Road, Aberdeen AB1 3RA*

There are wide disparities in the estimated dietary requirements of different species of fish for tryptophan (Cowey, 1979). In rainbow trout (*Salmo gairdneri*) a deficiency leads to poor growth, scoliosis and abnormal calcium deposits in the kidney (Ketola, 1982), but there is little quantitative data on tryptophan requirement in this species.

Six groups of rainbow trout of mean weight 14 g were given diets (containing fish meal, gelatin and an amino acid mixture) that provided graded levels (0·8, 1·3, 2·0, 3·0, 4·0 and 6·0 g/kg diet) of tryptophan. The crude protein content of the diet was 550 g/kg and the fish were fed at the rate of 20 g/kg body-weight/d. The food was eaten without wastage. The experiment lasted for 12 weeks. Plots of weight gain against dietary tryptophan level showed a breakpoint at 2·5 g tryptophan/kg diet (50 mg/kg biomass per d). Those fish given diets with low levels of tryptophan had low weight gains, poor feed conversion ratios and increased mortalities.

Blood and liver concentrations of tryptophan increased proportionally with dietary level and dose-response plots did not show a well-defined breakpoint. Tryptophan pyrrolase activity was detected only in the liver; the fish enzyme appears to be unlike the rat enzyme (which is inducible by hormones and substrate) but resembles more that of the sheep and cat (Badawy & Evans, 1976). The activity of the trout enzyme did not vary significantly between dietary treatments.

Radioactivity in expired carbon dioxide, collected over a 20 h period following intraperitoneal injection of a tracer dose of [$^{14}$COOH]-tryptophan, was very low in those fish given the three diets of lowest tryptophan content but increased sharply in fish given the other three diets. A dose-response curve was obtained with a breakpoint at 2·0 g tryptophan/kg diet—a value lower than that obtained from the growth results.

Many of the fish given the deficient diets (less than 2 g tryptophan/kg) suffered from cataracts and from scoliosis. X-ray pictures of these fish revealed prominent curvatures of the spine in both lateral and vertical planes. On the basis of the weight-gain results, the tryptophan requirement of rainbow trout in this experiment was met by diets containing 2·5 g/kg.

Badawy, A. A. B. & Evans, M. (1976). *Biochemical Journal* **158**, 79–88.
Cowey, C. B. (1979). In *Finfish Nutrition and Fishfeed Technology*, vol. 1, pp. 1–16 [J. E. Halver and K. Tiews, editors]. Berlin: H. Heenemann GmbH & Co.
Ketola, H. G. (1982). *Comparative Biochemistry and Physiology* **73B**, 17–24.

**Effect of three different rumen environments on the rate and extent of the rumen degradability of untreated straw, ammonia-treated straw and hay.** By AYONA T. SILVA and E. R. ØRSKOV, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Recent advances in rumen microbial biology have revealed the importance of many nutritional factors in the growth and activity of cellulolytic microflora inhabiting the rumen.

The main objective of the experiment described here was to study the effects of different rumen environments on the rate and extent of fibre degradation. Hay, barley straw and ammonia-treated barley straw were selected as feeds to create three rumen environments in which rumen pH and ammonia level were not considered to be limiting factors for cellulolysis. Each feed was given to eight cannulated sheep. The diets were supplemented with urea, sodium sulphate, vitamins and minerals according to recommendations of the Agricultural Research Council (1980). Rumen degradability of the same three roughages were studied using the nylon-bag technique (Ørskov *et al.* 1980). Untreated straw incubated in all three rumen environments was studied under the electron microscope to assess the differences in colonization of bacteria.

The degradation of both untreated straw and hay was found to be highest in the animals given ammonia-treated straw ($P<0.001$) and the electron microscopy study revealed that the density of bacterial colonization of untreated straw was greater in the rumen of animals given ammonia-treated straw than in the rumen of animals given untreated straw. Rumen pH and ammonia concentration were similar for the three environments and were not considered to limit cellulosis (see Table).

*Effect of different rumen environments on the disappearance of dry matter from nylon-bags incubated in the rumen for* 48 *h* (n 8)

| Feed | Rumen pH | Rumen ammonia (mg/l) | Disappearance of dry matter from nylon-bags (mg/g) | | |
|---|---|---|---|---|---|
| | | | Untreated straw | Ammonia--treated straw | Hay |
| Untreated straw | 6·9 | 268 | 443 | 607 | 605 |
| Ammonia-treated straw | 6·8 | 244 | 541 | 648 | 652 |
| Hay | 6·5 | 212 | 495 | 629 | 638 |
| SEM | 0·1 | 19 | 18 | 19 | 21 |

The implication of these findings on supplementation of straw diets is at present being investigated.

Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock.* Commonwealth Agricultural Bureaux: Slough.

Ørskov, E. R., Hovell, F. D. Deb. & Mould, F. (1980). *Tropical Animal Productions* 5, 193–213.

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**Tissue incorporation and excretion of $^{14}C$ in pigs after injection of [U-$^{14}C$]sodium acetate into the caecum.** By EVA A. LATYMER and A. G. LOW, *National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT*

The quantitative importance of products of bacterial fermentation in the large intestine in the nutrition and hence production of animals is not known. However, the absorption of acetate from the large intestine of pigs and the presence of its metabolites in different fractions of blood plasma has been shown (Latymer & Woodley, 1984). It was thus of interest to identify where the products of the absorbed acetate were deposited in the body and to measure the amounts in faeces and urine.

A balance experiment was carried out on two pigs of 70 and 78 kg fed on a barley–soya diet. [U-$^{14}C$]sodium acetate was injected through a cannula into the caecum of the pigs as described by Latymer & Woodley (1984). The pigs were kept in metabolic cages where urine and faeces were collected separately for 4 d. The animals were then killed, the blood collected and, in addition to the tissues mentioned in the Table, samples of back fat, kidney fat, rump muscle and brain were taken. The rest of the carcass, the tissue samples and the faeces and urine were weighed and frozen. Representative samples were then minced, mixed and homogenized as appropriate and portions solubilized in NCS (Amersham International, Amersham, Bucks).

It was found that the loss of $^{14}C$ via urine and faeces was greatest during the first 2 d after the acetate injection; this represented 85·3 and 94·6% of the amount excreted in 4 d from the 70 and 78 kg pigs respectively. The brain contained only a minute concentration of $^{14}C$. The distribution of the recovered radioactivity can be seen in the Table (expressed as % of injected dose).

| Live weight (kg) | Carcass weight (kg) | Excreted (%) | Liver (%) | Kidney (%) | Small intestine and contents (%) | Large intestine (%) | Large intestinal contents (%) | Blood (%) | Carcass (%) | Recovered (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 70 | 57 | 8·7 | 0·5 | — | 0·4 | 2·2 | 0·1 | 0·1 | 22·8 | 34·7 |
| 78 | 63 | 7·0 | 0·8 | 0·1 | 1·1 | 0·7 | 0·1 | 0·1 | 24·4 | 34·2 |

In the carcass the highest concentration of $^{14}C$ (μCi/g) was found in the back fat of both pigs (0·009 and 0·012) and in the kidney fat (0·006 and 0·013) while the lean rump muscle contained only 0·002 and 0·003. Assuming that the carcass contained 220 g/kg back fat, it can be calculated that 60–80% of the total carcass $^{14}C$ was stored there.

The relatively high proportion of $^{14}C$ still found stored in the body 4 d after administration implies that the exogenous acetic acid served as a source of energy to the pigs.

Latymer, E. A. & Woodley, S. C. (1984). *Proceedings of the Nutrition Society* **43**, 22A.

**Modification of thyroid hormone-induced adaptation of muscle protein turnover in malnutrition by glucocorticoid treatment.** By B. ODEDRA, M. COX and D. J. MILLWARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

Although muscle protein synthesis falls markedly in malnutrition, protein is not markedly lost because of an adaptive fall in degradation (Millward *et al.* 1981). The role of 3,5,3′-triiodothyronine ($T_3$) in mediating this fall in degradation has been investigated in adrenalectomized rats given a low-protein diet (50 g/kg) and treated with various levels of corticosterone which alters thyroid status. As seen in the Table, after 9 d on the diet protein synthesis and degradation fell as did food intake, insulin and free $T_3$. This fall in protein turnover was partially prevented in all of the corticosterone-treated groups, since food intake, insulin and $T_3$ levels were all higher than in the untreated malnourished rats. While treatment with 10 or 20 mg corticosterone/kg per d maintained protein balance, the highest dose (100 mg/kg per d), or a marginal restriction of the food intake combined with the moderate dose (20 mg/kg per d), induced a loss of protein with the rate of protein synthesis depressed below the rate of protein degradation. This demonstrates that the rate of muscle protein wasting in malnutrition will depend on the extent to which protein degradation falls to match synthesis and that this is related to thyroid status.

| Treatment | Protein synthesis (%/d) Mean | SE | Protein degradation (%/d) | Food intake (g/d) | Insulin (μ units/ml) Mean | SE | Free $T_3$ (pg/ml) |
|---|---|---|---|---|---|---|---|
| Low-protein diet for: | | | | | | | |
| 3 d | 12·9 | 0·6 | 12·9 | 12·5 | 7 | 3 | 5·25 |
| 9 d | 6·3 | 0·8 | 6·3 | 8·9 | 4 | 2 | 3·6 |
| + Corticosterone (mg/kg per d) | | | | | | | |
| 10 | 10·1 | 1·1 | 10·1 | 12·5 | 21 | 7 | 7·2 |
| 20 | 10·1 | 0·7 | 10·1 | 13·2 | 25 | 6 | 10·4 |
| 100 | 8·0 | 0·4 | 9·5 | 11·5 | 48 | 12 | 9·7 |
| 20 (restricted food intake) | 7·7 | 0·6 | 10·7 | 10·0 | 15 | 8 | 7·5 |

Millward, D. J., Brown, J. G. & Odedra, B. (1981). In *Nitrogen Metabolism in Man*, pp. 475–494 [J. C. Waterlow and J. M. L. Stephen, editors]. London: Applied Science Publishers.

**Effect of the interaction between two bacterial species on volatile fatty acid production in the chicken caecum.** By O. SZYLIT[1], P. RAIBAUD[1], M. DURAND[2], C. DUMAY[2] and M. BENSAADA[1], [1]*Laboratoire d'Ecologie Microbienne* and [2]*Station de Recherches de Nutrition, INRA-CNRZ, 78350-Jouy-en-Josas, France*

Volatile fatty acids (VFA) present in the caecum of holoxenic (conventional) chickens, comprise mainly acetic, propionic and butyric acids, essentially of microbial origin (Annison *et al.* 1968). To determine the ecological conditions necessary for VFA production in the caecum, we studied the interaction between one amylolytic *Lactobacillus* strain (LEM 220) producing DL-lactic acid in the crop and caecum (Szylit *et al.* 1980) and two strictly anaerobic strains giving in vitro acetate plus either butyrate (*Clostridium butyricum*) or propionate (*Veillonella alcalescens*) partly from lactate.

Groups of four chickens each reared in isolators remained axenic (germ-free) or were inoculated with either one strain of LEM 220 (L220) and of the anaerobic bacteria (mono-associated chickens) or with each of the latter in association with L220 (di-associated chickens). The chickens were given a semi-purified diet (maize starch and fish meal plus 40 g of lactose/kg) and were killed 6 h after the meal at 5 weeks of age. Bacterial establishment and VFA (gas chromatography) were determined in the caecal contents.

The establishment of the three bacterial strains was approximately $10^8$/g of caecal contents in mono- and di-associated animals.

*Volatile fatty acid concentration in caecal contents*

| | Axenic | | *Lactobacillus* L220 | | *Veillonella* Alone | | *Veillonella* + L220 | | *Clostridium* Alone | | *Clostridium* + L220 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Total VFA (mmol/kg) | 1·1 | 0·5 | 0·54 | 0·05 | 1·3 | 0·2 | 10·0 | 1·1 | 9·9 | 3·1 | 15·2 | 0·9 |
| Molar proportions | | | | | | | | | | | | |
| Acetate | 0·89 | | 0·84 | | 0·62 | | 0·43 | | 0·44 | | 0·57 | |
| Propionate | 0·07 | | 0·12 | | 0·30 | | 0·57 | | 0·00 | | 0·00 | |
| Butyrate | 0·04 | | 0·04 | | 0·08 | | 0·00 | | 0·56 | | 0·44 | |

In both anaerobic strains, the VFA composition in caecal contents resembled that obtained in vitro. However, it was necessary to associate L220 to obtain noticeable VFA production by *Veillonella* and a considerable increase of VFA production by *Clostridium*.

Annison, E. F., Hill, K. J. & Kenworthy, R. (1968). *British Journal of Nutrition* **22**, 207–216.

Szylit, O., Champ, M., Ait-Abdelkader, N. & Raibaud, P. (1980). *Reproduction, Nutrition and Development* **20** (5B), 1701–1706.

**Digestibility of carbohydrate from bread and lentils using a breath hydrogen technique and a human ileostomy model.** By THOMAS M. S. WOLEVER, MARY-JANE THORNE, LILIAN U. THOMPSON, ZANE COHEN and DAVID J. A. JENKINS, *Departments of Nutritional Sciences and of Surgery, Faculty of Medicine, University of Toronto, Canada*

Bacterial fermentation of carbohydrate (CHO) in the large intestine of man results in the production of hydrogen gas. This is transported in the blood and appears in the breath in concentrations proportional to the amount of carbohydrate fermented. We have measured the concentration of H in the alveolar air of eight normal subjects hourly for 12 h after the consumption of 120 g CHO portions of wholewheat bread or cooked split lentils. The CHO malabsorbed was estimated by comparison of the sum of the H concentrations after these meals with that after consumption of the bread plus 30 g of the unavailable CHO lactulose. The results (mean with SE) suggested that 9·4 (1·6) and 20·2 (2·2) g CHO were malabsorbed after wholewheat bread and lentils respectively, representing 8 and 17% of the available CHO (see Table).

| | Breath hydrogen studies | | | | | Ileostomy studies | |
|---|---|---|---|---|---|---|---|
| | Total breath hydrogen concentration (ppm) | | Carbohydrate malabsorbed (g) | | % Available carbohydrate malabsorbed | Ileal loss available carbohydrate (g) | % Ileal loss available carbohydrate |
| | Mean | SEM | Mean | SEM | | | |
| Wholewheat bread plus lactulose | 662 | 62 | — | — | — | — | — |
| Lactulose* | 487 | 64 | 30 | — | 100 | — | — |
| Wholewheat bread | 135 | 17 | 9·4 | 1·6 | 7·8 | 7·3 | 7·3 |
| Lentils | 312 | 42 | 20·2 | 2·2 | 16·8 | 17·6 | 17·6 |

*Total hydrogen concentration for lactulose alone estimated by the difference between that for the bread plus lactulose meal and that for the bread alone.

It is not known, however, how much of the H produced was due to the fermentation of available CHO and how much to the fermentation of the dietary fibre present in the meals. To determine this, we gave breakfast test meals of wholewheat bread and lentils containing 100 g available CHO to two individuals with ileostomies. The previous evening they took intestinal washouts of lactulose 6 h after their evening meal. Ileal effluent was collected for 14 h after breakfast, during which time only clear fluids were consumed. Analysis was performed on ileal effluent to determine total CHO loss, and dietary fibre estimated by an enzyme modification of the neutral detergent fibre method. The mean available CHO recovered in ileal effluent after wholewheat bread and lentils was 7·3 and 17·6 g or 7 and 18% respectively (see Table). The studies indicate that breath H evolution relates well to the measured available CHO losses from the small intestine. In that increased stool losses of CHO and volatile fatty acids have not been reported after these foods, support is also given to the hypothesis that, by absorption of the products of bacterial fermentation, the available CHO energy which enters the colon is able to be conserved.

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**Microbiological studies on food intolerance.** By C. E. BAYLISS[1], A. P. HOUSTON[1], V. ALUN JONES[2], S. HISHON[3] and J. O. HUNTER[2] (Introduced by D. A. T. SOUTHGATE), [1]*ARC Food Research Institute, Norwich;* [2]*Department of Gastroenterology, Addenbrookes Hospital, Cambridge;* [3]*District General Hospital, Great Yarmouth*

Specific foods have been implicated in irritable bowel syndrome (Alun Jones *et al.* 1982). The association between food and symptoms of pain, flatulence and occasionally diarrhoea has been tested. Many patients date the onset of symptoms from a course of antibiotics, some of which are known to cause changes in the microbial flora of the gut (Lewis *et al.* 1977), and the delayed response (16–72 h after challenge) suggests a bacterial rather than an immunological mechanism of induction.

In the experiments reported here six patients were hospitalized and microbiological analysis of the faecal flora carried out for 48 h before and up to 72 h after challenge with wheat (five patients; challenge in the form of white or wholemeal bread) or sugar (one patient). Higher numbers of aerobes were found in faecal samples from patients than those from matched age/sex controls and in two patients a 100-fold increase in the number of aerobes occurred during challenge.

*Excretion of aerobic bacteria in faeces*

(Viable bacteria/g dry weight faeces)

| | *n* | Samples | Mean | Range |
|---|---|---|---|---|
| Patients | 6 | 30 | $2{\cdot}2\times10^{9}$ | $2{\cdot}9\times10^{7}$–$1{\cdot}1\times10^{10}$ |
| Controls | 6 | 6 | $9{\cdot}8\times10^{7}$ | $3{\cdot}9\times10^{6}$–$2{\cdot}7\times10^{8}$ |

There were noticeable changes in the predominant aerobic flora of four patients and anaerobic flora of three patients as judged by colony morphology and microscopy. Much lower numbers of bifidobacteria were isolated than expected from previous work (Croucher *et al.* 1983).

The results suggest that in certain individuals ingestion of specific foods may lead to changes in bacterial populations in the large bowel, and that both this and consequent changes in metabolism may be major factors in irritable bowel syndrome.

Alun Jones, V., McLaughlan, P., Shorthouse, M., Workman, E. & Hunter, J. O. (1982). *Lancet* no. 8308, 1115–1117.

Croucher, S. C., Houston, A. P., Bayliss, C. E. & Turner, R. J. (1983). *Applied Environmental Microbiology* **45**, 1025–1033.

Lewis, R. P., Widerman, P., Sutter, V. L. & Finegold, S. M. (1977). In *Metronidazole* pp. 307–319 [S. M. Finegold, editor]. Amsterdam: Excerpta Medica.

**Effects of supplementation with high-selenium wheat bread on glutathione peroxidase and related enzymes in women.** By M. F. ROBINSON, C. D. THOMSON, LAY K. ONG and P. HUEMMER, *Department of Nutrition, University of Otago, Dunedin, New Zealand*

Selenomethionine was more effective than selenite in raising blood Se levels in man but both raised equally the levels of whole blood glutathione peroxidase (GSHPx; *EC* 1.11.1.9) (Robinson *et al.* 1978; Thomson *et al.* 1982). The forms of Se in foods are not known but half that in wheat may be as selenomethionine.

The bioavailability of Se in high-Se wheat from USA has been studied in four women who supplemented their normal daily diet, which contained no fish, liver or kidney, with 200 μg Se as high-Se wheat bread for 8–13 weeks. Se (Watkinson, 1966) and GSHPx (Paglia & Valentine, 1967) were measured in weekly samples of whole blood, erythrocytes, plasma and platelets. Other antioxidant enzymes were assayed in erythrocytes or plasma or both: glutathione-S-transferase (GST; *EC* 2.5.1.18) (Habig *et al.* 1974); superoxide dismutase (SOD; *EC* 1.15.1.1) (McCord & Fridovich, 1969) and catalase (Beers & Sizer, 1952). Plasma vitamin E (Bieri *et al.* 1979) and protein (Lowry *et al.* 1951) were measured.

| Subject ... | MR | | JM | | LC | | SM | |
|---|---|---|---|---|---|---|---|---|
| | C• | D† | C | D | C | D | C | D |
| Supplementation (weeks) | 13 | | 8 | | 9 | | 9 | |
| Se (ng/ml) | | | | | | | | |
| Whole blood | 77 | 166 | 66 | 144 | 58 | 140‖ | 63 | 168‖ |
| Erythrocytes‡ | 78 | 151 | 80 | 126 | 71 | 119‖ | 72 | 139‖ |
| Plasma | 76 | 176 | 57 | 156 | 49 | 154‖ | 56 | 191‖ |
| GSHPx (units/g haemaglobin) | | | | | | | | |
| Whole blood | 19 | 23 | 22 | 28 | 21 | 28 | 22 | 30 |
| Erythrocytes | 17 | 21 | 21 | 27 | 19 | 30 | 21 | 27 |
| Plasma (units/g protein) | 2·3 | 3·6 | 1·6 | 3·5 | 1·6 | 2·0 | 3·1 | 3·8 |

•Control, mean of four values.
†End of dosing, single value.
‡Calculated using packed cell volume.
‖Values at 8 weeks.

Se concentrations and GSHPx activities increased in whole blood, erythrocytes and plasma of all subjects. Platelet GSHPx also increased from 50–80 to 125–150 units/g protein or 2·3–3·6 to 4·2–6·4 units/$10^{10}$ cells.

Activities of GST, SOD or catalase in erythrocytes or plasma did not change, nor did levels of plasma vitamin E.

Beers, R. S. & Sizer, I. W. (1952). *Journal of Biological Chemistry* **195**, 133.
Bieri, J. G., Tolliver, T. J. & Catignani, G. L. (1979). *American Journal of Clinical Nutrition* **32**, 2143–2149.
Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974). *Journal of Biological Chemistry* **249**, 7130–7139.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. H. (1951). *Journal of Biological Chemistry* **193**, 265–275.
McCord, J. M. & Fridovich, I. (1969). *Journal of Biological Chemistry* **244**, 6049–6055.
Paglia, O. E. & Valentine, W. N. (1967). *Journal of Laboratory and Clinical Medicine* **70**, 158–169.
Robinson, M. F., Rea, H. M., Friend, G. M., Stewart, R. D. H., Snow, P. G. & Thomson, C. D. (1978). *British Journal of Nutrition* **39**, 589–600.
Thomson, C. D., Robinson, M. F., Campbell, D. R. & Rea, H. M. (1982). *American Journal of Clinical Nutrition* **36**, 24–31.
Watkinson, J. H. (1966). *Analytical Chemistry* **38**, 92–97.

**Post-weaning changes in the piglet gastrointestinal tract.** By D. J. HAMPSON and D. E. KIDDER, *Department of Veterinary Medicine, University of Bristol, Langford House, Langford, Avon BS18 7DU*

Diarrhoea and growth-checks are often major problems in piglets during the first fortnight after weaning. Changes in small intestinal structure and reductions in enterocyte brush-border enzyme activities have been recorded (Gay *et al.* 1976; Kenworthy, 1976) and it is known that diarrhoea is often associated with proliferation of enterotoxigenic *Escherichia coli* in the intestines at this time (Smith & Jones, 1963).

Groups of pigs either weaned at 3 weeks (*n* 45), unweaned with commercial solid creep food (*n* 27) or unweaned without creep food (*n* 25) were killed daily from 3 weeks of age. At fixed percentage lengths along the small intestine, measurements were made of mean villus height and crypt depth, lactase (*EC* 3.2.1.23) and sucrase (*EC* 3.2.1.48) activities, and number of coliform bacteria. Stomach and caecal pH were recorded and intestinal contents examined for rotavirus.

Large changes in small intestinal structure and brush-border enzymes occurred in weaned pigs. Villus height decreased dramatically to reach minimum values 5 d after weaning; increased crypt depth followed these changes. Lactase and sucrase activities fell markedly, and coliform numbers increased in some pigs in this critical period. The caecal pH dropped as the size and contents of the large intestine expanded but, although faecal water increased, diarrhoea did not occur. Malabsorption was confirmed by xylose absorption tests on one litter of pigs before and 5 d after weaning.

We consider that the changes described involving loss of small intestinal absorptive area and brush-border enzyme activity are an important cause of post-weaning malabsorption. Pathogenic *E. coli* appear to increase whilst these changes occur, and they may precipitate diarrhoea when they reach sufficient numbers.

This work was supported by the Agricultural Research Council.

Gay, C. C., Barker, I. K. & Moore, P. (1976). *Proceedings of the 4th International Congress on Pig Veterinary Science* VII.
Kenworthy, R. (1976). *Research in Veterinary Science* **21**, 69–75.
Smith, H. W. & Jones, J. E. T. (1963). *Journal of Pathology and Bacteriology* **86**, 387–412.

**Attempts to modify post-weaning changes in the piglet gastrointestinal tract.** By D. J. HAMPSON, D. E. KIDDER and E. M. HAMPSON, *Department of Veterinary Medicine, University of Bristol, Langford House, Langford, Avon BS18 7DU*

Hampson & Kidder (1984) have described post-weaning changes in piglet small intestinal structure and brush-border enzyme activity which were associated with malabsorption.

Endeavours were made to modify these changes, including:

1. Weaning 'abruptly' without prior access to commercial solid creep food.
2. Administering therapeutic levels of oxytetracycline over the weaning period.
3. Weaning to a liquid sow-milk replacer, given every 2 to 3 h.
4. Weaning to a casein-based diet.
5. Weaning to a similar hydrolysed casein diet.

Group 1 contained twenty-eight pigs killed in a time-course after weaning, groups 2–5 each consisted of six pigs killed 5 d after weaning.

Withholding creep food before weaning had no significant effect on piglet growth, mean (with SD) weight at 20 d with creep being 5·19 (SD 1·02) kg ($n$ 90) and without creep 5·02 (SD 1·24) kg ($n$ 82). Abruptly-weaned pigs were not different in intestinal structure and enzyme activity before weaning at 21 d, and went through essentially the same post-weaning changes as those having creep before weaning.

Administering oxytetracycline, or weaning on to milk replacer or casein all resulted in broadly similar changes in structure and enzyme activity by 5 d after weaning, but the oxytetracycline and milk replacer groups had increased faecal water.

The group weaned on to hydrolysed casein had smaller increases in crypt depth and less reduction of brush-border enzyme activity in the distal small intestine. There was a consistent failure of the pH in the caecum to drop after weaning and growth was poor.

There seemed to have been little advantage in offering pigs expensive creep food before weaning at 3 weeks of age. Giving antibiotics or weaning on to different forms of diet had no protective effect on structure, except when a low antigen diet (hydrolysed casein) was used.

The results suggest that consumption of food other than sow's milk causes these important intestinal alterations, and indicate that antigenicity of the diet may be involved in the changes.

This work was supported by the Agricultural Research Council.

Hampson, D. J. & Kidder, D. E. (1984). *Proceedings of the Nutrition Society* **43**, 18A.

**The influence of gastrointestinal surgery on the metabolic status of adult sheep.** By W. H. CLOSE*, F. A. HARRISON and R. P. HEAVENS, *ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

MacRae *et al.* (1982) reported significant increases in the heat production of sheep after re-entrant fistulation of the small intestine. This finding questions the application of results obtained from surgically-prepared sheep to the understanding of the metabolism of unoperated sheep. In a communication to the Physiological Society, Close *et al.* (1982) presented preliminary observations on the effects of rumen and single duodenal re-entrant fistulations on the metabolic status of adult sheep. The results showed no change in the heat loss of two sheep at 6 weeks after duodenal re-entrant surgery. The observations have now been extended to 6 months and include results for four animals after re-entrant fistulation.

Four pairs of Clun Forest ewes, aged 1–5 years, were studied for several 7-d periods in a direct heat-sink calorimeter (Close & Mount, 1975). They were kept at an environmental temperature of 20±1° and were given 500 g lucerne nuts and 600 g chopped hay, once daily. After the initial determination of heat loss, one animal from each pair was prepared with a rumen fistula. Six months later, after shearing, the rumen-fistulated animals were provided with a single re-entrant fistulation of the duodenum just distal to the pylorus (Harrison & Hill, 1962). All surgery was performed under general anaesthesia and, during surgery, the control animals were also clipped and anaesthetized. The results are presented in the Table.

*Body-weight and heat loss of surgically-prepared and control sheep*

| Weeks following | | | | | | | |
|---|---|---|---|---|---|---|---|
| Rumen fistulation | −1·5 | 2·5 | 10·5 | 20·0 | 30·5 | 34·5 | 51·0 |
| Duodenal fistulation | — | — | — | — | 2·5 | 6·5 | 23·0 |
| Body-weight (kg) | | | | | | | |
| Operated | 52·2 | 51·8 | 53·1 | 54·1 | 52·2 | 51·9 | 51·5 |
| Control | 44·7 | 45·0 | 46·7 | 48·2 | 47·9 | 48·1 | 48·7 |
| Heat loss (kJ/kg$W^{0.75}$ per d) | | | | | | | |
| Operated | 409 | 409 | 386 | 363 | 425 | 417 | 410 |
| Control | 422 | 434 | 408 | 421 | 454 | 444 | 410 |

There was no significant difference ($>0.05$) in heat loss between fistulated and control animals, suggesting that in this experiment there was no long-term alteration in the metabolic status of the sheep following the fistulations. The four operated animals maintained appetite, body-weight and condition for over 1 year after re-entrant surgery.

Close, W. H., Harrison, F. A. & Heavens, R. P. (1982). *Journal of Physiology* **332**, 81P–82P.
Close, W. H. & Mount, L. E. (1975). *British Journal of Nutrition* **34**, 279–290.
Harrison, F. A. & Hill, K. J. (1962). *Journal of Physiology* **162**, 225–243.
MacRae, J. C., Smith, J. S. & White, F. (1982). *British Journal of Nutrition* **47**, 637–644.

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**The effect of Avoparcin on rumen fermentation in vitro.** By R. C. MacGregor and D. G. Armstrong, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Avoparcin is an antibiotic exhibiting growth promoting properties when given to ruminants (Mudd & Smith, 1982). In order to assess the action the antibiotic might have on microbial fermentation in the rumen, the effect of six different concentrations of Avoparcin (0, 2, 4, 8, 10 and 20 μg/ml of incubation media) on volatile fatty acids (VFA), methane and hydrogen production was measured in an in vitro rumen incubation system (Allen, 1981). The substrate used in the in vitro fermentation flasks comprised seven parts dried grass and three parts barley, which was also the formulation of the ration given to the sheep from which the total rumen liquor sample was obtained. On each of three experimental runs each of the six treatments was replicated three times. On no occasion was hydrogen gas detected in the fermentation gases. The rate of fermentation in the flasks was measured as μmol VFA produced/h while in order to assess the effect of Avoparcin on the fermentation pattern independently from any effect on over-all fermentation rate, the amount of hexose theoretically fermented was calculated (Marty & Demeyer, 1973) and the production of acetate, propionate, butyrate and methane expressed as μmol produced/mmol hexose theoretically fermented. The relationships between product production ($Y$) and Avoparcin (μg/ml) concentrations ($X$) are shown below.

(Mean values, SEM in parentheses)

| Dependant variable | Equation | $r^2$ | $P$ |
|---|---|---|---|
| Acetate | $Y = 1122 - 7{\cdot}2\,(3{\cdot}6)\,X + 0{\cdot}2\,(0{\cdot}1)\,X^2$ | 0·60 | <0·05 |
| Propionate production | $Y = 313 + 7{\cdot}9\,(2{\cdot}0)\,X - 0{\cdot}26\,(0{\cdot}1)\,X^2$ | 0·59 | <0·001 |
| Methane production | $Y = 496 - 12\,(2{\cdot}9)\,X + 0{\cdot}31\,(0{\cdot}14)\,X^2$ | 0·68 | <0·001 |
| Butyrate production | $Y = 258 + 3{\cdot}8\,(1{\cdot}9)\,X - 0{\cdot}45\,(0{\cdot}09)\,X^2$ | 0·13 | 0·735 |
| Fermentation rate | $Y = 130 - 2{\cdot}71\,(1{\cdot}8)\,X + 0{\cdot}08\,(0{\cdot}08)\,X^2$ | 0·32 | 0·061 |

Increasing Avoparcin concentration enhanced propionate production (312 μmol/mmol hexose at 0 ppm *v.* 361 μmol/mmol hexose at 8 ppm Avoparcin), and reduced that of acetate (1125 *v.* 1086) and methane (502 *v.* 420); there was little change in butyrate production. There was a slight tendancy for the over-all fermentation rate to be reduced as Avoparcin concentration increased (124 μmol/h at 0 ppm *v.* 112 μmol/h at 8 ppm Avoparcin). It would appear from these in vitro studies that the presence of Avoparcin in the media enhanced the yield, per unit hexose theoretically fermented, of products useful to the ruminant animal.

Allen, J. D. (1981). Studies on manipulation of rumen fermentation. PhD Thesis, University of Newcastle upon Tyne.

Marty, R. J. & Demeyer, D. I. (1973). *British Journal of Nutrition* **30**, 369–376.

Mudd, A. J. & Smith, H. (1982). *Animal Production* **34**, 54A.

**In vivo incorporation of $^{14}C$ into plasma fractions of pigs after injection of [U-$^{14}C$]sodium acetate into the caecum.** By EVA A. LATYMER and SUSAN C. WOODLEY, *National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT*

There has recently been increasing interest in the fate of volatile fatty acids produced in the large intestine of monogastric animals, in view of their possible role in nutrition and metabolism.

To assess in vivo the fate of exogenous acetate, $^{14}C$-labelled sodium acetate was injected into the caecum of pigs in a preliminary experiment.

Three Large White × Landrace boars of initial live weight 22, 26 and 28 kg were fitted with a permanent cannula in the caecum. The pigs were fed on a practical diet based on barley and soya. [U-$^{14}C$]sodium acetate was dissolved in 30 ml homogenized caecal contents and injected at a level of *ca.* 9 μCi/kg body-weight through the caecal cannula. Starting 30 min after the injection, samples of portal blood were taken at 15 min intervals for 5 h. The blood plasma samples were separated into total lipid (TL), plasma protein (PP) and water soluble compounds (WS) and counted for radioactivity. The plasma TL classes were separated by thin-layer chromatography (Storry & Tuckley, 1967; Tuckley & Storry, 1974).

At 30 min after the injection the plasma $^{14}C$ level was already maximum in all three pigs and the peak was maintained during the following 5 h of sampling. During this period $^{14}C$ was found in all three fractions of the plasma; the proportions in WS decreased and those in PP increased. Moreover, at a later occasion, 96 h after injection of a similar dose of labelled acetate into two of these pigs, the PP contained 61 and 65% of the total count. The distribution of $^{14}C$ during the 5 h after injection in the individual lipid classes of TL portion was as follows:

| | Percentage of total phospholipid fraction | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Phospho-lipids | | Monoacyl-glycerols | | Non-esterified fatty acids | | Free cholesterol | | Diacyl-glycerols | | Triacyl-glycerols | | Cholesteryl esters | |
| Live weight (kg) | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| 22 | 8 | 2–18 | 3 | 2–5 | 6 | 4–10 | 28 | 20–35 | 3 | 1–7 | 31 | 24–38 | 21 | 9–32 |
| 26 | 14 | 8–21 | 9 | 6–12 | 10 | 3–20 | 15 | 11–19 | 11 | 7–15 | 31 | 20–40 | 11 | 7–18 |
| 28 | 14 | 10–22 | 4 | 3–5 | 4 | 2–6 | 18 | 15–24 | 9 | 7–11 | 35 | 24–49 | 15 | 5–20 |
| Mean | 12 | | 5·3 | | 6·7 | | 20·3 | | 7·7 | | 32·3 | | 15·7 | |

The results show that acetate was rapidly absorbed and metabolized into a number of important compounds, which persisted in the body for a number of days.

Storry, J. E. & Tuckley, B. (1967). *Lipids* **6**, 501–502.
Tuckley, B. & Storry, J. E. (1974). *Lipids* **7**, 443–444.

**Fermentation of poultry excreta, urea and casein with pure carbohydrate sources in an artificial rumen.** By A. AKBAR, J. R. SCAIFE and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Poultry excreta (PE) contains at least 5% nitrogen of which approximately 60% is in the form of uric acid. The slower release of ammonia from uric acid as compared with that from urea may result in a more efficient utilization of dietary carbohydrate.

In vitro fermentations were carried out using the artificial rumen technique of Czerkawski & Breckenridge (1969). The nine nutrient combinations studied were starch or cellulose along with either PE or casein and/or urea. Control incubations were carried out using grass cubes and hay. With the exception of the mixture containing grass cubes, hay and PE, all other combinations were isonitrogenous and had the same dry matter content. Total gas production was measured manometrically and samples were withdrawn at regular intervals for measurement of pH, volatile fatty acids (VFA) and ammonia. In all control vessels, gas production increased rapidly following addition of substrates. Ammonia concentrations remained relatively constant and pH values decreased by 0·3–0·5 units.

*Concentration of VFA (*mM*) and ammonia (*mM*) in different nutrient combinations in vitro*

| Time (min) . . . | 0 | | | 60 | | | 390 | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrients | pH | Ammonia | Total VFA | pH | Ammonia | Total VFA | pH | Ammonia | Total VFA |
| Control | 7·3 | 12·1 | 79·1 | 7·1 | 14·6 | 91·6 | 7·0 | 11·2 | 122·8 |
| Starch + PE | 7·4 | 11·3 | 79·0 | 7·4 | 29·3 | 80·2 | 6·9 | 15·3 | 125·6 |
| Control | 7·4 | 8·8 | 103·7 | 7·1 | 9·4 | 105·1 | 6·9 | 5·0 | 151·0 |
| Cellulose + PE | 7·4 | 7·5 | 90·5 | 7·3 | 21·5 | 91·5 | 7·2 | 31·6 | 108·8 |
| Control | 7·2 | 5·1 | 66·7 | 7·1 | 7·0 | 78·5 | 6·9 | 5·5 | 99·2 |
| Grass cubes + hay + PE | 7·1 | 5·3 | 65·0 | 7·0 | 22·6 | 78·7 | 6·9 | 26·3 | 113·0 |

Incubation of PE with either starch or cellulose resulted in a slow release of ammonia. The fermentation of starch and PE led to a marked decrease in pH and after a lag of between 15 and 135 min gas production was greater than that of the control at 390 min. Acetate, propionate and *n*-butyrate levels rose from 59·6, 13·6 and 5·9 mM respectively at zero time to 87·2, 30·4 and 7·9 mM at 390 min. Replacement of half the grass cubes and hay with PE resulted in a pattern of gas production and changes in pH similar to those of the controls. However, this change resulted in higher ammonia concentrations and total VFA production.

It may be concluded that the fermentation of PE as a nitrogen source results in a slow release of ammonia which may bring about an efficient utilization of carbohydrates as measured by VFA and total gas production.

Czerkawski, J. W. & Breckenridge, G. (1969). *British Journal of Nutrition* **23**, 51–66.

**Effect of choice of calibration curve range on results of *Lactobacillus casei* assay of folate.** By A. J. A. WRIGHT and D. R. PHILLIPS, *ARC Food Research Institute, Colney Lane, Norwich, Norfolk NR4 7UA*

A microbiological assay method using *Lactobacillus casei* is widely used for the measurement of folate in foods and biological fluids. Most of the folate in food exists as polyglutamate forms of 5-formyl-tetrahydrofolic acid and 5-methyl-tetrahydrofolic acid which, after extraction and deconjugation steps, are usually present in low concentrations, whereas serum and whole blood samples contain comparatively high concentrations of folate mainly as 5-methyl-tetrahydrofolic acid.

A recent publication (Phillips & Wright, 1982) gave evidence of a reduced response from *L. casei* to 5-methyl-tetrahydrofolic acid when the assay was performed at pH 6·8 using a 0–1 ng calibration range, the conditions in the method described by Bell (1974) which was used to provide the values for *McCance and Widdowson's, The Composition of Foods* (Paul & Southgate, 1978). We now present evidence to show that in assay systems measuring higher concentrations of folate having a working range 0–10 ng per assay, *L. casei* appears to respond equally to 5-methyl- and 5-formyl-tetrahydrofolic acid and folic acid (pteroylmonoglutamic acid). The Table shows the values for calibration curves in the range 0–8 ng/10 ml assay for 5-methyl-tetrahydrofolic acid and folic acid. When these curves were plotted on semi-logarithmic paper with the folate concentration on the abscissa as the logarithmic plot, the reduced response of *L. casei* to 5-methyl-tetrahydrofolic acid was seen in the 0–2 ng range. This is the reason why 'positive drift' (where serial dilutions fail to give a linear plot) is not seen in assays in the concentration range 2–10 ng of folate.

*Comparison of* L. casei *growth response*

(Initial incubation pH 6·8, 10 ml assay volume, nephelometer reading (% f.s.d.))

| Folate concentration (ng/10 ml assay) . . . | 0·2 | 0·4 | 0·6 | 0·8 | 1·0 | 2·0 | 4·0 | 6·0 | 8·0 |
|---|---|---|---|---|---|---|---|---|---|
| Folic acid | 9 | 21 | 32 | 40 | 45 | 70 | 87 | 94 | 102 |
| 5-Methyl-tetrahydrofolic acid | 0 | 5 | 14 | 24 | 34 | 68 | 88 | 95 | 103 |

The commonly used method for estimation of folate levels in biological fluids is that described by Herbert (1966) which has a calibration range of 0–30 ng and a working range of 0–10 ng/assay. The more sensitive 0–1 ng assay system is mainly used to measure the much lower concentrations of folate present in extracts of foods.

Bell, J. G. (1974). *Laboratory Practice* **23**, 235–242, 252.
Herbert, V. (1966). *Journal of Clinical Pathology* **19**, 12–16.
Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's The Composition of Foods*. London: HM Stationery Office.
Phillips, D. R. & Wright, A. J. A. (1982). *British Journal of Nutrition* **47**, 183–189.

**The use of detergent extraction procedures for estimating fibre carbohydrates in feeds and digesta.** By A. B. McAllan and E. S. Griffith, *National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT*

Neutral detergent and acid detergent extraction techniques (Van Soest, 1963; Van Soest & Wise, 1967) are widely used to identify two main components in fibre carbohydrates. It is frequently assumed that the difference between neutral detergent fibre (NDF) and acid detergent fibre (ADF) represents the hemicellulose content of the sample, and that the difference between ADF and lignin represents the cellulose content. The methods were originally developed for the classification of forage carbohydrates but are of practical value only if digestibilities of the components are also assessed. We have examined the use of these techniques with digesta samples.

Six Friesian steers, each equipped with rumen and abomasal cannulas, were given diets of approximately equal parts of concentrates (flaked maize + tapioca) and either untreated barley straw (BS) or alkali-treated barley straw (BSA). Other similar diets were supplemented with urea (BSU and BSAU) or fishmeal (BSF and BSAF). Abomasal digesta flows were calculated using polyethylene glycol and $^{103}$Ru as markers.

It was found that for cellulose, estimation of flows and mouth to abomasum digestibility by ash-free ADF-lignin gave results which were 1·056 (SE 0·002) of those obtained by direct cellulose analysis (McAllan & Smith, 1974). Values for hemicellulose estimated as (A) NDF-ADF (ash free) or (B) xylose + arabinose were:

| | Intakes (g/d) | | Abomasal flow (g/d) | | Mouth to abomasum digestibility | |
|---|---|---|---|---|---|---|
| Diet | A | B | A | B | A | B |
| BS | 416 | 269 | 271 | 201 | 0·35 | 0·25 |
| BSU | 461 | 250 | 172 | 160 | 0·62 | 0·36 |
| BSF | 473 | 281 | 155 | 161 | 0·67 | 0·43 |
| BSA | 148 | 233 | 105 | 110 | 0·29 | 0·53 |
| BSAU | 150 | 239 | 58 | 79 | 0·61 | 0·67 |
| BSAF | 176 | 270 | 42 | 72 | 0·76 | 0·73 |

Hemicellulose intakes estimated by method A were markedly greater for untreated straw and smaller for alkali-treated straw than those estimated by method B. It appeared that alkali treatment solubilized a substantial part of the straw hemicellulose. Digestibilities were substantially different for the different methods indicating the need for caution in interpreting results.

McAllan, A. B. & Smith, R. H. (1974). *British Journal of Nutrition* **31**, 77–88.
Van Soest, P. J. (1963). *Journal of the Association of Official Analytical Chemists* **46**, 825–829.
Van Soest, P. J. & Wise, R. H. (1967). *Journal of the Association of Official Analytical Chemists* **50**, 50–55.

**The influence of crude fibre on the digestibility, metabolizability and utilization of metabolizable energy in diets for growing pigs.** By A. JUST (Introduced by A. G. LOW), *National Institute of Animal Science, Rolighedvej* 25, 1958 *Copenhagen V, Denmark*

The influence of crude fibre on the utilization of energy in balanced diets was studied in growing Danish Landrace pigs (20–90 kg) using the comparative slaughter technique. The depressive influence of crude fibre on energy utilization depended on the source of fibre. A 1% increase in dietary crude fibre from cereals, barley straw or cellulose (Just, 1970; Just *et al.* 1983) decreased the apparent digestibility of gross energy (GE) by 2·9, 2·1 and 1·1 percentage units, decreased the utilization of metabolizable energy (ME) by 0·4, 0·7 and 0·5 percentage units respectively, and increased the amount of gut fill at slaughter by 0·4–0·5 kg.

Experiments with cannulated pigs using the same diets showed that increasing levels of crude fibre in the diets increased the concentration of volatile fatty acids (VFA) in ileal digesta and faeces except for ileal digesta from the cellulose-supplemented diets.

*Change per 1% increase in dietary crude fibre (dry matter basis)*

| Source of fibre . . . | Cereal* | | Barley straw* | | Cellulose* | |
|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE |
| VFA in ileal digesta (mmol/d) | 38 | 27 | 17 | 3 | −2 | 4 |
| VFA in faeces (mmol/d) | 13 | 3 | 15 | 4 | 15 | 1 |
| % of digestible energy disappearing from caecum–colon | 3 | 2·1 | 1 | 0·9 | 1 | 0·2 |

*Regression coefficients, $b \pm s_b$.

In addition a positive relationship between the level of crude fibre in the diets and the proportion of digested energy disappearing in the caecum–colon was found, which in turn showed a negative relationship to the utilization of ME. When one more percentage unit of the energy digested in the whole tract disappeared in the caecum–colon the utilization of ME was depressed by approximately 0·5%.

The explanation for the large negative influence of crude fibre from cereals on the digestibility of GE is primarily that the crude fibre content of the diet is positively correlated with the content of nitrogen-free extractives which are only digested in the large intestine. The negative relationship between dietary crude fibre and the utilization of ME is partly due to the different chemical composition of the nutrients absorbed from the small intestine (amino acids, fatty acids, hexoses, etc.) and from the hind gut (ammonia, amides, VFA, etc.). In addition, the fermentation processes result in loss of energy as methane, hydrogen, etc. and fermentation heat.

Just, A. (1970). *Beretning fra Forsøgslaboratoriet*, no. 381. 212 pp.
Just, A., Jørgensen, H. & Fernandez, J. A. (1983). *Livestock Production Science* 10, 171–186.

**Energy and protein retention of axenic and holoxenic chickens given the same level of feed.** By G. CHARLET-LERY, M. T. MOREL and M. FISZLEWICZ, *Laboratoire de Physiologie de la Nutrition, INRA-CNRZ, 78350 Jouy-en-Josas, France*

Previous experiments (Szylit & Charlet-Lery, 1981) have shown that the total microflora may have a depressive effect on energy and protein retention in chickens given a low level of protein (180 g/kg dry matter (DM)). However, the male chickens used were chosen at the age of 2 d, pair-housed in cages, fed *ad lib.* and exhibited very different weights at slaughter. We have therefore made three replicates of this experiment with chickens given the same feed level, whatever their microbiological status.

The animals were axenic (germ-free) and half of them were holoxenized (conventional) at the age of 7 d (75 g). They were housed individually, given one of the diets previously described (30 g long-chain dextrins and 180 g protein/kg DM) and slaughtered later (at the live weight of 450 g) than those of the former experiments.

Metabolizable energy (ME) was measured by the total collection procedure during 3 d before slaughter. By comparison with body compositions of chickens killed at the beginning of the experiment (a regression was obtained between body composition and live weight), energy and nitrogen retentions were calculated. Results (mean with standard errors) are given in the Table.

| | | Weight gain (g) | | ME intake (MJ) | | Protein intake (g) | | Deposited energy (MJ) | | Deposited protein (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Axenic | 21 | 361·7 | 8·1 | 12·17 | 0·15 | 134 5 | 1·7 | 3·30 | 0·09 | 69·2 | 1·5 |
| Holoxenic | 15 | 381 3 | 8·9 | 13·16 | 0·18 | 143·1 | 1·7 | 3·56 | 0·14 | 73·6 | 2·0 |

Differences between axenic and holoxenic chickens were small. In spite of a statistically higher intake for holoxenic chicks, weight gains and depositions were not statistically different and the efficiencies of energy utilization and nitrogen retention were the same (energy retention % ME = 27·1 and 27·0, N retention % N intake = 51·5 and 51·4 for axenic and holoxenic chickens respectively).

It may be concluded that, when chickens are in restricted feeding conditions, there is no depressive effect of the total flora. However, in *ad lib.* conditions, the metabolic mechanisms involved in the possible induction of satiety by the microflora in young growing chickens are still unknown.

Szylit, O. & Charlet-Lery, G. (1981). *British Poultry Science* **22**, 305–315.

**Effect of guar gum on gastric emptying of liquid test meals and high-solid meals in growing pigs.** By ANNA L. RAINBIRD, A. G. LOW and I. E. SAMBROOK, *National Institute for Research in Dairying, Shinfield, Reading, Berkshire RG2 9AT*

It is widely accepted that addition of guar gum to foods has a beneficial effect on glucose tolerance in normal and diabetic man. Although its mode of action remains unclear it has been suggested that it reduces the rate of gastric emptying. We have recently found that guar gum had little effect on the gastric emptying rate of dry matter from high-solid meals in pigs (Rainbird *et al.* 1983). This contrasts with the reductions caused by guar gum in emptying rate of a glucose solution given by orogastric intubation in rats (Leeds *et al.* 1979) and of a milky drink consumed by obese humans (Wilmshurst & Crawley, 1980). Leeds *et al.* (1979) measured the residual gastric contents after killing the rats, while Wilmshurst & Crawley (1980) measured the appearance of $^{24}Na$ from the meal in the blood reaching the head.

We have now compared the effects of guar gum on gastric emptying of the three types of meal referred to above in six 30–60 kg pigs. Throughout the trial the pigs received a semi-purified diet given at 30 g/kg body-weight per d in two equal meals, except on five mornings of each week when they received either this diet and water or 1 l of glucose solution (Leeds *et al.* 1979) or 1 l of low-energy milky drink (Wilmshurst & Crawley, 1980), in each case with or without 60, 20 or 10 g guar gum (Meyprogat 150; Meyhall Chemical Co) /kg diet respectively. Each of the six treatments was given to each pig in the morning for 5 d, on which the gastric contents were measured immediately before or 0·5, 1, 2 or 4 h after the meal (Low & Rainbird, 1983).

There was little evidence that guar gum delayed gastric emptying of any of the meals; the only significant effect on the emptying of dry matter was seen for the glucose solution 0·5 h after the meal ($P<0·05$). Guar gum also significantly delayed the emptying of glucose from the glucose solution 0·5, 1 and 2 h after the meal ($P<0·05$). The volume of digesta in the stomach following the semi-purified diet meal was significantly increased ($P<0·01$) by guar gum after 1 h (23%), 2 h (38%) and 4 h (74%), while it was also significantly increased ($P<0·01$) by 47% by guar gum 1 h after consumption of the milky drink.

We suggest that further evaluation of the methods of measuring gastric emptying is necessary before the discrepancies between the results of the present study and those of Wilmshurst & Crawley (1980) can be accounted for.

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Leeds, A. R., Bolster, N. R., Andrews, R. & Truswell, A. S. (1979). *Proceedings of the Nutrition Society* **38**, 44A.
Low, A. G. & Rainbird, A. L. (1983). *Proceedings of the Nutrition Society* **42**, 24A.
Rainbird, A. L., Low, A. G. & Sambrook, I. E. (1983). *Proceedings of the Nutrition Society* **42**, 25A.
Wilmshurst, P. & Crawley, J. C. W. (1980). *British Journal of Nutrition* **44**, 1–6.

**Anti-hypertensive drugs for the treatment of leanness.** By A. G. DULLOO and D. S. MILLER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Energy balance studies were conducted on lean CFLP mice treated with drugs capable of inhibiting the sympathetic nervous system at different loci. The drugs were incorporated in a stock diet (CRM, Christopher Hill group, London) and given to groups of four adult (3–4 months old) female mice for a period of 7 weeks. Body-weight and metabolizable energy intake were monitored throughout. At the end of the experiment, the animals were killed and their carcasses analysed for energy, fat and protein. Total heat production over the whole experimental period was estimated by the comparative carcass technique (Miller & Stock, 1969). The results are presented in the Table.

| | | Body-weight (g) | | Body fat (g) | | Body protein (g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Drug | Dose (g/kg diet) | Mean | SE | Mean | SE | Mean | SE | Food intake† (kJ/mouse) | Heat production† (kJ/mouse) | Balance† (kJ/mouse) |
| Propranolol | 2·0 | 30·8 | 0·6 | 2·88 | 0·05 | 5·89 | 0·21 | 2643 | 2629 | +14 |
| α-MPT | 1·0 | 32·4 | 0·8 | 4·06** | 0·46 | 5·71 | 0·14 | 2332 | 2277 | +55 |
| Reserpine | 0·005 | 30·4 | 1·4 | 3·67** | 0·20 | 5·53 | 0·30 | 2156 | 2119 | +37 |
| Bethanidine | 2·0 | 32·5* | 0·5 | 4·33*** | 0·74 | 6·13 | 0·62 | 2450 | 2373 | +77 |
| No-drug control | | 31·2 | 0·3 | 2·95 | 0·10 | 5·68 | 0·19 | 2705 | 2693 | +12 |
| Initial control | — | 31·2 | 0·4 | 2·59 | 0·05 | 5·79 | 0·06 | — | — | — |

Significant difference between treated group and no-drug control group: *$P<0.05$, **$P<0.01$, ***$P<0.001$.
†Values represent grouped results.

Treatment with α-methyl-*p*-tyrosine (α-MPT), reserpine and bethanidine resulted in significantly higher carcass fat without a change in body protein. This was achieved by a reduction in metabolic rate despite an accompanying decrease in food intake. In contrast, the β-blocker propranolol had no effect on body composition and little influence on energy balance.

The results indicate that α-MPT, bethanidine and reserpine (which are used as anti-hypertensive agents), by virtue of their ability to reduce the level of noradrenaline at the sympathetic nerve synaptic junction, caused reduction in heat production and elevation of the energetic efficiency of lean animals. These drugs might have some practical value in promoting weight gain in people who are underweight but without anorexia.

Miller, D. S. & Stock, M. J. (1969). *Proceedings of the Nutrition Society* **28**, 70A.

**Antidepressants as thermogenic drugs for the treatment of obesity.** By A. G. DULLOO and D. S. MILLER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Drugs known to stimulate the sympathetic nervous system at different sites and with different modes of action were screened for their ability to increase metabolic rate. These drugs were tested on animals made obese by lesions in the hypothalamus using monosodium glutamate (Djazayery *et al.* 1979). Energy balance studies were conducted in two separate trials on nine of these drugs which were incorporated into the diet and given to groups of four adult obese mice for either 7 or 10 weeks. Food intake and body-weight were monitored throughout. The daily oxygen consumption measurements (Boroumand & Miller, 1976) were made at least once during the experimental period. Total heat production was estimated by the comparative carcass slaughter technique (Miller & Stock, 1969). The effects of each drug have been expressed in ratios relative to an appropriate no-drug control group and such ratios are presented in the Table.

| Drug | Dose (g/kg diet) | Body-weight | Carcass fat | Carcass protein | Metabolizable energy intake | Total heat production | 24 h oxygen consumption |
|---|---|---|---|---|---|---|---|
| Tranylcypromine | 0·5 | 0·66 | 0·36 | 0·80 | 1·05 | 1·33 | 1·38 |
| Maprotiline | 0·5 | 0·82 | 0·66 | 1·02 | 1·00 | 1·16 | 1·16 |
| Protriptyline | 0·5 | 0·64 | 0·42 | 0·86 | 0·90 | 1·18 | 1·40 |
| Butriptyline | 1·0 | 0·58 | 0·24 | 1·08 | 1·04 | 1·42 | 1·54 |
| Amitriptyline | 0·5 | 0·71 | 0·46 | 0·81 | 0·89 | 1·12 | 1·30 |
| Iprindole | 1·0 | 0·79 | 0·58 | 0·93 | 0·92 | 1·07 | 1·07 |
| Ephedrine | 1·0 | 0·80 | 0·58 | 0·91 | 0·96 | 1·12 | 1·20 |
| Methoxyphenamine | 0·7 | 0·64 | 0·24 | 0·83 | 1·06 | 1·38 | 1·29 |
| Yohimbine | 0·5 | 0·66 | 0·33 | 0·80 | 0·90 | 1·17 | 1·22 |

All nine drugs (six of which are used as antidepressants) caused much loss of body-weight and body fat, with little change in body protein and food intake: they acted mainly by increasing metabolic rate.

The results indicate that the bulk of these drugs, by virtue of their ability to elevate the levels of noradrenaline at the sympathetic nerve junctions, are thermogenic and could therefore be useful in the treatment of obesity.

Boroumand, N. & Miller, D. S. (1976). *Proceedings of the Nutrition Society* **36**, 12A.
Djazayery, A., Miller, D. S. & Stock, M. J. (1979). *Nutrition and Metabolism* **23**, 357–367.
Miller, D. S. & Stock, M. J. (1969). *Proceedings of the Nutrition Society* **28**, 70A.

**Effect of treatment of obese Wistar rats with a new anti-obesity agent Org 6837.** By D. M. ANDERSON, P. MCKEOWN and K. PARKINSON, *Department of Pharmacology, Scientific Development Group, Organon Laboratories Ltd, Newhouse, Motherwell*

Experiments using anti-obesity agents are usually carried out in young, non-obese laboratory rats. In order to produce a more appropriate model, normal male Wistar rats were made obese. This was achieved by giving the rats a choice of either a normal rat diet (210 g crude protein (N × 6·25; CP)/kg diet, 16·7 MJ/kg) or a diet containing (g/kg) 550 chocolate, 145 digestive biscuits, 110 casein, 55 maize flakes, 36 sucrose, 102 Arachis oil plus vitamins and minerals. The mixture was blended into a solid block. The CP of this mixture was calculated as 155 g/kg diet and the gross energy as 20·9 MJ/kg. The rats were introduced to this feeding regimen when their body-weight was approximately 350 g. In addition, the rats were kept at 26–28° for 2 weeks, then at 15–17° for 2 weeks and returned to 26–28° thereafter. With this treatment male Wistar rats continued to put on weight in excess of the normal plateau, which is about 550 g, and individual weights of up to 1·2 kg have been achieved.

In a previous communication a novel anti-obesity agent Org 6837 has been described which both reduced food intake and caused an additional loss of weight (Anderson, 1981). Three groups of obese rats (body-weight initially 764 (SE 39) g, 769 (SE 34) g and 731 (SE 33) g) were assigned to one of three treatments: (a) Org 6837, (b) pair-fed with the drug-treated group and (c) a normal control group. Treatment was carried out for 56 d and the rats were then allowed to recover for 27 d. For the first 5 d of treatment the rats were given 20 mg Org 6837/kg and for the next 4 d 10 mg Org 6837/kg. A dose of 15 mg Org 6837/kg was used throughout the remaining treatment period.

Org 6837 induced such a severe weight loss in three rats (the most obese) that they had to be removed from the experiment and the results are therefore calculated on five rats/group with initial weights of 706 (SE 27) g, 723 (SE 29) g and 681 (SE 25) g respectively.

The rats receiving Org 6837 (15 mg/kg per d) lost 204 (SE 36) g which was significantly greater than the loss shown by the pair-fed rats (129 (SE 20) g; $P = 0{\cdot}01$). The control rats gained 32 (SE 6) g in the same period. During treatment the loss of weight in the drug-treated rats was continuous, i.e. 'tolerance' did not develop. The pair-fed group gained slightly more weight during the 'recovery period' (144 (SE 13) g) than the Org 6837 group (107 (SE 12) g) although this difference was not statistically significant. Both groups gained significantly more than the controls which gained 39·8 (SE 6) g.

The compound Org 6837 is therefore an extremely effective anti-obesity agent in obese rats. The animals do not develop tolerance and recovery of body-weight is no faster than in rats which have undergone reduction in weight by dietary means alone.

Anderson, D. M. (1981). *Proceedings of the Nutrition Society* **40**, 110A.