The Two Hundred and Eighty-ninth Scientific Meeting of the Nutrition Society (One Hundred and Fifteenth of the Scottish Group) was held in Lecture Theatre No. 2, Ninewells Hospital and Medical School, Dundee, on Saturday, 7 February 1976, at 10.30 hours, when the following papers were read:

Effects of various salts of lignosulphonate on the colon of guinea-pigs.

By J. WATT and S. N. MARCUS, *Department of Pathology, University of Liverpool, Liverpool L69 3BX* (introduced by G. A. J. PITT)

The sodium salt of lignosulphonate supplied in the drinking fluid to guinea-pigs is known to interfere with weight gain and produce ulceration in the large bowel (Watt & Marcus, 1974). In a comparative study, we have investigated the effects of two other salts of lignosulphonate, calcium and magnesium, on the colon of the guinea-pig.

Adult male guinea-pigs of mixed colours (mean body-weight 582 g) were fed on a lignosulphonate-free standard cube diet (SG 1; Nutrients Ltd, Liverpool 1), supplemented with fresh cabbage and hay. They were randomized into four groups of eight animals each. Three groups were given aqueous solutions (40 g/l) of sodium, calcium and magnesium lignosulphonate respectively as drinking fluid over a period of 8 weeks; allowing for spillage from drinking bottles, the average daily lignosulphonate consumption per animal in the three experimental groups was less than 4·5, 4·7 and 5·8 mg/g body-weight, respectively. A control group received water without added lignosulphonate.

At the end of 8 weeks, the average weight gain (±SE) of the guinea-pigs in the group receiving sodium lignosulphonate was 117±55 g, in the group receiving calcium lignosulphonate 144±30 g, in the group receiving magnesium lignosulphonate 210±29 g and in the control group 253±14 g.

All animals were killed by diethyl ether anaesthesia, and the large intestine was emptied of faeces and carefully examined by transmitted light. Multiple focal ulcers in the caecum were found in three out of eight animals receiving sodium lignosulphonate and in three out of eight animals receiving calcium lignosulphonate. No ulcers were found in the large bowel in any of the animals receiving magnesium lignosulphonate, or in any of the control animals which received water only as a drinking fluid.

These results indicate that of the three salts of lignosulphonate investigated, magnesium lignosulphonate interfered least with weight gain and was not associated with ulceration of the colon. For use as a food-binder in the formulation of cubes or pellets supplied to guinea-pigs, magnesium lignosulphonate would appear to be preferable to the sodium or calcium salts.

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Sex differences in the fatty acid composition of total carcass lipids of obese and lean Zucker rats. By K. W. J. WAHLE and J. D. RADCLIFFE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Sex differences in the fatty acid composition of lipids from various tissues have been observed in normal rats fed on essential fatty acid (EFA)-deficient diets. These differences relate in part to the greater capacity of female rats to maintain higher proportions and concentrations of linoleic and arachidonic acid in their tissue lipids than male rats during progressive EFA deficiency (Aftergood & Alfin-Slater, 1965; Ostwald, Bouchard, Miljanich & Lyman, 1965; Lyman, Ostwald, Bouchard & Shannon, 1966). Male rats had a greater EFA requirement than females and this was attributed to their greater growth rate and larger body mass (*cf.* Alfin-Slater & Aftergood, 1968).

The fatty acid composition of tissue lipids of male, genetically obese rats differs greatly from that of their lean litter-mates of the same sex with respect to the proportions of monounsaturated fatty acids and of linoleic acid (Wahle, 1974). The present studies were undertaken to determine whether these differences in the composition of tissue lipids are also reflected in total carcass lipids of obese and lean male and female rats.

Obese and lean rats (four males and four females/group) were fed to appetite on a standard Oxoid diet (Herbert C. Styles (Bewdley) Ltd, Bewdley, Worcs.) for 10 d and killed at 34 d of age. The entire carcasses were minced and freeze-dried and samples were taken for analysis. Extraction of total carcass lipids and the preparation and determination of their component fatty acids (as methyl esters) by gas–liquid chromatography were done using the methods of Duncan & Garton (1967).

In lean male rats the total carcasses had greater fresh and dry weights than those of the corresponding females. Total fresh and dry weights were greater in obese than in lean rats of both sexes, but no differences were found between obese female rats and obese males. The total lipid content of the carcass did not differ between sexes, although in obese rats it was four times greater than in the corresponding lean animals.

Obese female rats had similar ratios of 16:1/16:0 and 18:1/18:0 fatty acids in total carcass lipids to those observed in obese males. These were greater than those found in the corresponding lean rats and probably reflect enhanced $\Delta 9$-desaturase activity in obese animals (*cf.* Wahle, 1974). The proportions of 18:2 and 20:4 fatty acids were greater in lean male rats than in females, but were markedly reduced in the carcass lipids of obese males compared with their lean litter-mates. This was probably due to the increase in endogenous production of fatty acids in obese rats which is largely responsible for the fourfold increase in carcass lipid. No reduction in the proportion of 18:2 and 20:4 fatty acids was, however, observed in obese females compared with their lean counterparts. Female obese rats are therefore

able to maintain the same relative proportions of EFA in their carcass lipids as the lean litter-mates despite a fourfold increase in total carcass lipid which is largely of endogenous origin. These observations indicate a difference in EFA metabolism between male and female rats and could be related to the greater ability of female rats to maintain the proportions of tissue EFA during progressive EFA deficiency (*cf.* Aftergood & Alfin-Slater, 1965). This sex difference needs to be taken into account when EFA metabolism of rats (and presumably other species) is being investigated.

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The effect of bran on the excretion of faecal cations. By M. A. EASTWOOD and W. D. MITCHELL, *Wolfson Gastrointestinal Laboratories, Western General Hospital, Edinburgh,* and J. L. PRITCHARD, *Department of Clinical Chemistry, Western General Hospital, Edinburgh*

The role of dietary fibre in human nutrition is of considerable interest (Cummings, 1973). Cereal fibre has been postulated to lower serum triglycerides (Heaton & Pomare, 1974) and to alter stool weight, transit time and faecal steroid concentration (Eastwood, Kirkpatrick, Mitchell, Bone & Hamilton, 1973). Dietary fibre may also interact with dietary cations (McConnell, Eastwood & Mitchell, 1974), e.g. calcium (Heaton & Pomare, 1974) and zinc (Reinhold, Nasr, Lahimgarzadeh & Hedayati, 1973).

We have studied the effect of a diet with 16 g bran/d for 3 weeks on the faecal excretion of sodium, potassium, Ca and magnesium in eight healthy subjects. Faeces were collected for 5 d on the normal diet and after 3 weeks on bran. Faeces were weighed, freeze-dried and the Na, K, Ca and Mg contents were measured after digestion with concentrated nitric acid. Table 1 shows the mean change in the faecal cations (mmol/d). The change in faecal water content was from $83{\cdot}4 \pm 38{\cdot}4$ (SD) to $143{\cdot}4 \pm 33{\cdot}8$ g/d ($P<0{\cdot}005$), and that in dry weight was from $26{\cdot}1 \pm 8{\cdot}8$ to $42{\cdot}4 \pm 7{\cdot}7$ g/d ($P<0{\cdot}005$).

The daily excretion of all the cations measured except Ca increased significantly during the bran regimen, as did the faecal water content and dry weight. Regression analysis showed significant correlation between change in daily faecal dry weight and change in daily faecal K (r 0·9603; $P<0{\cdot}001$) and change in daily faecal Ca (r 0·8019; $P<0{\cdot}01$).

The water-holding capacity of the bran is a factor in increasing the water content of the stool. Increased intake of bran significantly increased the daily faecal

Table 1. *The effect of a diet with 16 g bran/d for 3 weeks following a 5 d control period on faecal cation excretion in normal subjects*

(Mean values and standard deviations for eight subjects)

	Faecal cations (mmol/d)							
	Na^+		K^+		Ca^{2+}		Mg^{2+}	
Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	2·02	2·57	10·70	4·37	76·11	18·15	35·75	8·65
Bran	3·62	3·70	18·66	5·79	90·53	35·90	49·84	20·48
Significance (paired *t* test)	$P<0·025$		$P<0·01$		NS		$P<0·05$	

NS, not significant.

excretion of Na, K and Mg and to a lesser extent Ca. This increase is probably due to bran acting as a polyfunctional cation-exchanger.

These results suggest that unabsorbable cereal fibre may alter the metabolism of cations.

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A study of infant feeding practices in Edinburgh. By T. R. KIRK, *Department of Science and Dietetics, Queen Margaret College, Edinburgh*

This study was a pilot project, designed to identify infant feeding practices requiring further investigation and to provide practical, educational experience for student dietitians.

Twenty-two student dietitians interviewed ninety-eight mothers of infants up to 14 months old in four Edinburgh postnatal clinics. Ninety-four questionnaires were successfully completed. Some of the findings are summarized here. Table 1 shows the incidence and duration of breast-feeding (total breast-feeding). Data for partial breast-feeding are also available. The frequency and duration of breast-feeding was found to increase with increasing social class.

Table 1. *Duration of breast-feeding by Edinburgh mothers (%)*

At birth	42·5
2 weeks	29·8
1 month	27·2
4 months	10·2

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The frequencies with which the different forms of artificial milk available were used as the first milk feed and as the first and second change of milk feed were ascertained. National Dried Milk (Welfare Foods) was the most popular artificial milk used (42·4%). It was the most frequently changed to and least frequently changed from artificial milk.

From data concerning the types of milk used during the first 14 d of life the extent of use of high-solute milks during this period was determined. This information is summarized in Table 2.

The duration of use of the various forms of artificial milks introduced in hospital was examined. Of those infants having artificial milk in hospital, 54·3% were changed to another type immediately on discharge and a further 13·0% were changed within 2 weeks.

Table 2. *Frequency of use of high- and low-solute infant milks in the first 14 d of life by Edinburgh mothers (%)*

Human milk only	29·8
Human followed by low-solute	4·3
Human followed by high-solute	10·6
Low-solute only	6·4
Low-solute followed by high-solute	4·3
High-solute followed by low-solute	4·3
High-solute only	40·4
	100·0
Human or low-solute or both	40·4
At least some high-solute	59·6

The incidence of addition of gluten-containing cereal to the bottle-feed was found to be 0 at 2 weeks, 4·4% at 1 month, 14·6% at 2 months and 20·8% at 4 months. The incidence of introduction of such cereals as solid food was 2·1% at 2 weeks, 6·4% at 1 month, 19·4% at 2 months and 51·4% at 4 months.

The findings were discussed with the students in the light of the recommendations of the Oppé Report (Department of Health and Social Security, 1974) and were compared with those of Arneil (1967).

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Advice received by ninety-four mothers on infant feeding in Edinburgh.

By T. R. KIRK, *Department of Science and Dietetics, Queen Margaret College, Edinburgh*

As part of a survey carried out by student dietitians on the feeding practices of ninety-four Edinburgh infants, information was obtained concerning advice received by mothers on the milk feeding of their infant. All mothers had had their youngest child in hospital.

The first column of Table 1 shows the sources of advice and the frequency with which each was used. The large variety of sources used was striking. The small proportion of mothers receiving advice from dietitians was of particular interest to the students.

Table 1. *Advice on infant feeding given to Edinburgh mothers*

Source of advice	Mothers receiving advice (%)	Method of feeding advocated (%)		
		Breast-feeding	Bottle-feeding	Not specified
Hospital clinic	53·1	54·0	0·0	46·0
Doctor's welfare clinic	18·1	35·3	0·0	64·7
Health visitors' welfare clinic	27·7	46·2	11·5	42·3
General practitioner	10·6	60·0	10·0	30·0
Ward sister and staff	57·4	46·3	9·3	44·4
Health visitor at home	42·5	40·0	10·0	50·0
District nurse	19·1	44·4	5·6	50·0
Midwife	7·4	57·1	14·3	28·6
Dietitian	5·3	60·0	0·0	40·0
Own mother	45·7	46·5	27·9	25·6
Husband	29·7	16·6	36·6	46·6
Magazines and newspapers	32·9	64·5	19·3	16·1
Radio and television	10·6	90·0	0·0	10·0
Books	31·9	73·3	3·3	23·3

For each source of advice it was asked if breast-feeding was encouraged, if bottle-feeding was encouraged or if neither in particular was encouraged. The results are shown in the last three columns of Table 1. It is clear that advice given was inconsistent. Although no professional source consistently encouraged bottle-feeding, in a high proportion of instances breast-feeding was not encouraged. Grandmothers and husbands frequently encouraged bottle-feeding.

As it is believed that breast-feeding is desirable, particularly in the first 2 weeks of life (Department of Health and Social Security, 1974), breast-feeding should be actively encouraged by all professional persons advising mothers on infant nutrition.

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The effect of variations in dietary protein levels on the collagen of rat skin. By R. DAWSON and G. MILNE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Several authors have reported appreciable losses of collagen, particularly from the skin of rats and mice, under conditions of protein deprivation (Harkness, Harkness & James 1958; Cabak, Dickerson & Widdowson, 1963; Waterlow & Stephen, 1966; Anasuya & Narasinga Rao, 1970). However, in a study of malnourished children, Picou, Halliday & Garrow (1966) found that the amount of

collagen continued to increase in spite of severe protein deficiency. Such conflicting results have given rise to the belief that species differences exist with regard to the lability of skin collagen (ARC/MRC Committee, 1974).

We have investigated the effect of varying dietary nitrogen level on the collagen content of rat skin. Male rats of approx 120 g body-weight were used. One group was given a high-protein (HP) diet and another group a diet with 40 g casein/kg (LP). After 6 weeks half of each group were transferred to a non-protein (NP) diet, giving two additional groups (HP-NP and LP-NP). During the experimental period weekly determinations were made of the urinary excretion of N, hydroxyproline and creatinine by one animal from each group. Animals from each group were killed after 4 d, 3 weeks and 6 weeks of the NP feeding, the skins were removed and analysed for N, hydroxyproline and dry matter contents and the results compared with those of an initial control group killed at the beginning of the experiment. The following results were obtained:

Group	Mean weight of skin per animal (g) (with mean body-wt (g) in parentheses)		Mean total N content per skin (mg)		Mean total hydroxyproline content per skin (mg)	
Initial control . . .	5·02 (122)		191		71	
Time on NP diet (weeks) . . .	3	6	3	6	3	6
HP	17·88 (420)	18·08 (450)	1017	1050	578	625
HP-NP	11·51 (308)	7·99 (278)	659	536	402	335
LP	4·33 (131)	4·60 (135)	209	248	119	141
LP-NP	2·83 (110)	2·52 (96)	148	166	86	101

It appears that the response of rat skin collagen to dietary protein deprivation depends on previous dietary history. Thus in the HP-NP group 16% of the skin collagen is lost during the 3rd–6th weeks of NP feeding. This is similar to the loss reported by Harkness *et al.* (1958). However, in the animals on the LP diet, skin collagen increased during the same period and at 6 weeks was twice that of the initial controls which were of similar weight. On the LP-NP diet skin collagen again increased and after 6 weeks NP feeding collagen content was about 50% higher than in the initial controls, in agreement with the results of Picou *et al.* (1966).

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Arsanilic acid and its relationship to selenium and vitamin E in the nutrition of the pig. By S. J. PARKER and H. F. WALKER, *Division of Agricultural Chemistry and Biochemistry, School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

A 10-week trial was conducted with twenty young pigs to determine the effects of the addition of initially 500 mg arsanilic acid (ASA)/kg for 14 d and later 350 mg ASA/kg to a diet of low selenium and vitamin E content. The effects of adding supplementary Se and vitamin E to such a diet were also studied.

The diet was based on Se-deficient barley (742 g/kg) and dried skim-milk (247 g/kg). The vitamin E content of the barley was reduced by heat treatment (Swahn & Thafvelin, 1962). The basal diet contained 0·075 mg Se/kg. Experimental diets were as follows: basal (B); B+ASA (BA); B+ASA+20 mg DL-α-tocopheryl acetate/kg (BAE); B+ASA+1 mg Se as sodium selenite/kg (BAS); B+ASA+1 mg Se+20 mg DL-α-tocopheryl acetate/kg (BASE).

Three pigs, one in BA group and two in BAS group, developed moderate symptoms of organo-arsenical toxicity.

Blood glutathione peroxidase (*EC* 1.11.1.9) was assayed by the principle of the method of Paglia & Valentine (1967). The results (Fig. 1) show that

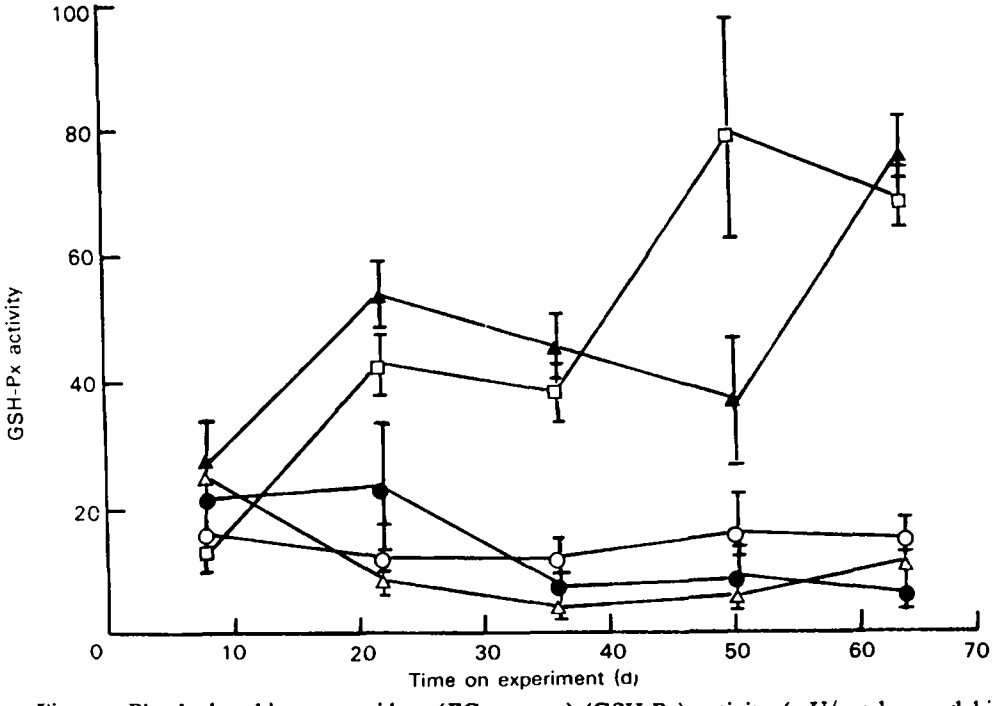

Fig. 1. Blood glutathione peroxidase (*EC* 1.11.1.9) (GSH-Px) activity (mU/mg haemoglobin) in pigs given: ○, basal diet (B) (see text for details); ●, B+500 mg arsanilic acid (ASA)/kg; △, B+500 mg ASA+20 mg DL-α-tocopheryl acetate/kg; ▲, B+500 mg ASA+1 mg selenium/kg; □, B+500 mg ASA+20 mg DL-α-tocopheryl acetate+1 mg Se/kg. The vertical bars represent the standard errors.

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the activity is dependent on dietary Se level but not on that of vitamin E. The effect of arsenic was indecisive.

Blood As was assayed by the method of Kingsley & Schaffert (1951). Table 1 shows that either supplementary Se or vitamin E can increase blood As levels, but levels are lowered by the addition of both nutrients simultaneously.

Table 1. *Blood arsenic levels (mg/l) after 65 d for pigs consuming diets differing in vitamin E, selenium and As content*

(Mean values and standard deviations for four pigs/diet)

	Blood As	
Diet*	Mean	SD
B	0·26	0·08
BA	0·57[a]	0·21
BAE	1·18[b]	0·22
BAS	1·26[c]	0·33
BASE	0·48[d]	0·33

Superscript letters denote: b>a ($P<0·001$); c>a ($P<0·01$); d, interaction significant ($P<0·001$).
*For details of diets, see text.

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Nutritional effects of field-bean (*Vicia faba* L.) protease inhibitors and field beans fed to rats. By B. W. ABBEY, R. J. NEALE and G. NORTON, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

Raw field beans (*Vicia faba* L.) have been shown to produce adverse effects on growth when consumed by rats (Nitsan, 1971) and chicks (Wilson & McNab, 1972). Such effects have been attributed to protease inhibitors (PI) present in the raw beans. In this study partially purified PI were included at various levels in a basal casein diet (120 g crude protein (nitrogen×6·25)/kg) supplied to rats and the effects on growth, apparent digestibility of N and the physiology of the pancreas and small intestine investigated. All observations were compared with rats supplied with the respective control diets containing autoclaved PI. Rats were also given diets containing raw or autoclaved bean meal (FBD) supplemented with DL-methionine (2·5 g/kg). Time-related changes were followed over a 21 d period.

Table 1. *Growth rate, nitrogen digestibility, pancreatic and intestinal trypsin* (EC 3.4.4.4) *and chymotrypsin activities of rats fed on field-bean protease inhibitor-supplemented diets at levels of* 1·25, 2·5, 5 *and* 10 *g/kg and raw and autoclaved field-bean meal diets (FBD) providing* 120 *g crude protein* ($N \times 6{\cdot}25$)*/kg over* 7 *d*

Protease inhibitor level (g/kg diet)	Growth rate (g/d)	Apparent N digestibility	Pancreas weight (g/kg body-weight)	Pancreatic trypsin activity (TU/g dry chyme)	Pancreatic chymotrypsin activity (CU/g dry chyme)	Intestinal trypsin activity (TU/g dry chyme)	Intestinal chymotrypsin activity (CU/g dry chyme)
1·25	5·64[a]	0·916[a]	6·43[a]	229·4[a]	163·7[a]	26·4[a]	15·7[a]
1·25 API	5·66[a]	0·916[a]	6·60[a]	258·2[a]	169·3[a]	17·2[a]	14·3[a]
2·5	5·76[a]	0·916[a]	6·94[a]	203·9[a]	155·8[a]	36·8[a]	21·9[a]
2·5 API	7·32[b]	0·931[a]	6·72[a]	263·0[b]	175·0[a]	18·8[b]	17·2[a]
5	5·40[a]	0·925[a]	8·35[a]	169·2[a]	130·4[a]	39·5[a]	27·7[a]
5 API	7·53[b]	0·932[a]	6·47[b]	241·0[b]	171·8[b]	20·6[b]	16·6[b]
10	5·13[a]	0·891[a]	8·17[a]	128·9[a]	125·2[a]	35·9[a]	22·2[a]
10 API	7·84[b]	0·900[a]	6·26[b]	222·4[b]	183·9[b]	15·7[b]	12·5[b]
Raw FBD	1·94[a]	0·839[a]	6·85[a]	134·6[a]	57·2[a]	13·8[a]	14·3[a]
Autoclaved FBD	4·52[b]	0·831[a]	5·82[b]	152·0[b]	71·7[a]	11·6[b]	12·9[b]

TU, trypsin units; CU, chymotrypsin units; API, autoclaved protease inhibitor.

[a,b] Values in the same column and the same treatment with different superscript letters are significantly different ($P<0{\cdot}001$).

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After 7 d the growth rate was significantly reduced ($P<0.001$), while pancreas weights increased in rats given diets containing 1·25 g PI/kg or higher (Table 1). The weight gain of rats given raw FBD was depressed while pancreas weight increased. Since pancreas weight reached a maximum in rats given a diet supplemented at the 5 g PI/kg level, it was concluded that there was adaptation to high levels of dietary PI. As the dietary level of PI increased, pancreatic levels of trypsin (*EC* 3.4.4.4) and chymotrypsin activity fell whilst intestinal levels increased. It was concluded that the PI level in FBD (<1·25 g/kg) was insufficient to produce the adverse effects observed in rats when fed solely on raw FBD.

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Copper and zinc deposition in the foetal lamb. By R. B. WILLIAMS and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The ability of the foetal liver to accumulate copper is well established (Underwood, 1971). In the instance of the lamb, however, there appears to be some disagreement on the relation between foetal age and liver Cu concentrations (McDougall, 1947; Pryor, 1964). Less is known of the deposition of zinc in this tissue. In the human foetus, however, Widdowson, Dauncey & Shaw (1974) showed that about one-quarter of the total Zn present at term occurs in the liver.

In this experiment fifty-six foetal lambs were obtained from twenty multiparous ewes maintained on the diet described by Wainman, Blaxter & Pullar (1970) and slaughtered at stages of pregnancy ranging from 80 to 140 d. Cu and Zn concentrations were measured on samples of fresh liver and on freeze-dried samples of whole foetuses (Table 1).

Liver Cu concentration was found to increase towards the end of gestation in agreement with the findings of McDougall (1947). Liver Zn concentration, initially very high, declined rapidly as pregnancy proceeded. While no definite trend in whole-body Zn concentration was observed, there was some indication that minimum values occurred between 105 and 128 d. Whole-body Cu concentration rose steadily from 80 to 128 d.

Approximately 80% of the Cu and Zn in the 80 d foetus was calculated to be in the liver. Thereafter, the liver Zn contribution declined rapidly, while not less than half the total body Cu occurred in the liver at any time; this latter observation is in agreement with that of Pryor (1964).

Total Cu and Zn deposition in the whole foetal burden of the ewe at 140 d was calculated to be approximately 30 and 180 mg respectively. Over the period of pregnancy examined this corresponded to mean daily accretion rates of less than 0·5 and 3 mg, respectively, for each element.

35 (2) 10

Table 1. *Concentrations of copper and zinc in whole body and liver of foetal lambs*

(Mean values with their standard errors)

Foetal age (d)	No. of Ewes	No. of Foetuses	Foetal weight (kg) Mean	SE	Concentration in whole body (mg/kg)* Cu Mean	SE	Zn Mean	SE	Concentration in liver (mg/kg)* Cu Mean	SE	Zn Mean	SE	Proportion of total body metal in liver Cu Mean	SE	Zn Mean	SE
80–81	2	5	0·35	0·01	1·55	0·05	20·1	1·1	17·1†		201·4†		0·96	0·08	0·87	0·05
91–92	6	16	0·69	0·02	1·78	0·08	17·7	0·7	14·1	1·2	117·0	9·5	0·61	0·04	0·50	0·03
105–106	2	5	1·51	0·11	2·01	0·09	14·8	0·6	18·8	2·5	74·8	11·7	0·63	0·07	0·34	0·04
113	3	9	1·66	0·06	2·32	0·11	14·5	0·3	26·3	2·0	59·5	4·2	0·70	0·08	0·25	0·02
121	1	4	2·16	0·07	2·36	0·05	12·7	0·4	21·0	1·9	26·9	0·9	0·51	0·05	0·12	0·01
128	2	5	3·03	0·08	2·81	0·31	15·8	1·1	47·5	9·9	65·3	8·1	0·89	0·18	0·21	0·01
135–136	2	5	3·71	0·26	1·88	0·14	16·0	1·1	25·5	2·0	36·4	4·8	0·64	0·01	0·11	0·02
140	2	7	2·92	0·35	2·60	0·11	16·2	0·4	54·1	5·8	32·3	2·6	0·78	0·10	0·09	0·01

*Metal concentrations are expressed on a fresh tissue basis.

†Pooled samples.

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Determination of available lysine in barley using *Tetrahymena pyriformis*.

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The protein quality of barley is determined principally by the amount and availability of lysine. The microbiological method for the measurement of available lysine, devised by Stott & Smith (1966) and modified by Shorrock & Ford (1973), has been adapted to compare different barleys and the results compared with those obtained from a protein efficiency ratio (PER) test (Table 1).

Samples of barley were finely ground in a ball mill, predigested with papain (*EC* 3.4.4.10) according to the method of Ford (1964) and assayed at five graded levels of total nitrogen. Lysine hydrochloride was used as a standard. Each assay flask, after sterilization, was inoculated with a 3 d broth culture of *Tetrahymena pyriformis* and incubated at 25° for 96 h in a shaker–incubator. The growth response was measured by counting the number of protozoa per unit volume in a haemocytometer.

PER values were obtained by mixing the barley with groundnut meal so that each constituent provided half of the protein in a diet containing 100 g protein/kg and feeding this diet to weanling rats for 4 weeks. Total lysine content was determined with an amino acid analyser.

Table 1. *Available lysine content of barley, as measured with* Tetrahymena pyriformis, *compared with total lysine content, protein efficiency ratio* (PER) *value and the corresponding values for casein*

	Total lysine (g/kg CP)	Available lysine (g/kg CP)	Availability (%)	PER value
Casein	81·6	76	93·1	2·97
Barley Nackta (5)	27·5	20	72·7	2·27
Barley Nackta (15)	39·4	28	71·1	2·51
Barley before heating	34·1	21	61·6	nd
Barley after heating at 100° for 21 h	31·7	17	53·6	nd
Barley before micronization	35·4	23	65·0	2·31
Barley after micronization	29·6	15	50·7	2·09

CP, crude protein (nitrogen×6·25); nd, not determined.

Available and total lysine contents of the samples were closely related, but processing two of the barley samples appeared to reduce the availability of lysine. The microbiological assay gave results which were consistent with the different

growth responses of rats, but the assay was found to be too tedious and time-consuming for adoption as a standard procedure.

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Fatty acid composition of triglycerides of goats fed on a barley-rich diet.

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Previous studies have shown that, when sheep were fed on cereal-based diets (barley, maize or wheat), they produced depot triglycerides containing abnormally high proportions of odd-numbered *n*-fatty acids and of methyl-branched fatty acids (Duncan, Ørskov & Garton, 1972, 1974; Garton, Hovell & Duncan, 1972; Duncan, Lough, Garton & Brooks, 1974). The biosynthesis of these acids is associated with enhanced production of rumen propionate (Ørskov, Fraser & Gordon, 1974) which serves directly as precursor of odd-numbered acids and indirectly (via its metabolite, methylmalonate) as source of the methyl groups of the branched-chain acids (Scaife & Garton, 1975). It thus became of some interest to see if another ruminant responded in a similar manner to a starch-rich (cereal) diet by producing depot triglycerides of unusual fatty acid composition.

Accordingly, six male early-weaned goats were fed to appetite for about 5 months on a diet which contained whole barley (900 g/kg) and a pelleted mixture of white fish meal, minerals and vitamins (100 g/kg). They were slaughtered when each weighed 40 kg and pieces of adipose tissue (subcutaneous and perinephric) taken for analysis by methods previously described (Garton *et al.* 1972). Similar

Table 1. *Component fatty acids (mg/g total fatty acids) of triglycerides of subcutaneous adipose tissue of feral goats and goats fed on a barley-rich diet*

(Mean values with no. of animals in parentheses)

	Fatty acid							
	14:0	16:0	16:1	18:0	18:1	18:2	*n*-Acids with odd number of C atoms*	Branched-chain acids
Feral goats (3)	40	240	30	250	370	10	30	30†
Barley-fed goats (6)	30	180	40	40	450	20	110	110‡

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

†Mostly iso- and anteiso-acids.

‡Iso- and anteiso-acids, together with acids having one or two substituent methyl groups nearer to the carboxyl group.

analyses were made on corresponding samples of adipose tissue from three feral adult goats (two males and one female) killed in the south-west of Scotland.

These analyses showed (Table 1) that, as in sheep, the subcutaneous fatty acids had an abnormally high content of odd-numbered and branched-chain fatty acids compared with normal values for this species. Whereas in the feral goats the branched acids were mostly of the iso- and anteiso-series, the barley-fed animals produced considerable amounts of acids of unusual structure with methyl branches in various positions nearer to the carboxyl group, similar to those previously found in cereal-fed sheep. Again, as in sheep, perinephric fatty acids showed much less marked changes in composition.

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Urinary excretion of methylmalonic and ethylmalonic acids by sheep fed on a barley-rich diet. By A. K. LOUGH and A. G. CALDER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The occurrence of a variety of mono-, di- and trimethyl-branched fatty acids in the adipose tissue triglycerides of sheep and goats fed on barley-rich diets (Duncan, Lough, Garton & Brooks, 1974; Duncan, Ørskov & Garton, 1976) is attributable to the incorporation of methylmalonate, in place of malonate, into endogenously-synthesized fatty acids (Scaife & Garton, 1975). Methylmalonate is derived by carboxylation of propionate which is produced in unusually large amounts as a result of bacterial fermentation of barley carbohydrates in the rumen (Ørskov, Fraser & Gordon, 1974). The possibility that methylmalonate might also be excreted in enhanced amounts in the urine of barley-fed sheep was therefore investigated. Preliminary investigations indicated that, in addition to methylmalonate, ethylmalonate was also excreted by such sheep and so an analytical method was devised by which both compounds could be determined.

Urine (3 ml), acidified to pH 1·0, is concentrated and subjected to thin-layer chromatography on grooved plates with silica gel G as adsorbent and diethyl ether–formic acid (99:1, v/v) as developing solvent. Methylmalonic and ethylmalonic acids are among the compounds which appear at the top (solvent front) of the chromatogram. These compounds are converted to their methyl esters which are then separated by gas–liquid chromatography on a column of Celite containing, as liquid phase, a mixture of Apiezon L grease and polymerized ethylene glycol adipate. Full details of the method will be published elsewhere.

It was found that, whereas conventionally-fed sheep usually excreted <10 mg methylmalonic acid/l urine, sheep given a diet containing rolled barley (900 g/kg) excreted about twice as much of the acid (10–20 mg/l). The corresponding values

for ethylmalonic acid were <20 mg/l and, surprisingly, 150–550 mg/l. The origin of such considerable amounts of ethylmalonic acid is obscure, though they do not apparently arise from butyrate by carboxylation since, in barley-fed sheep, the proportion of butyrate in rumen contents is somewhat lower than in sheep given conventional diets (Ørskov *et al.* 1974). It is equally puzzling that no ethyl-branched fatty acids have so far been detected in the adipose tissue triglycerides of barley-fed sheep, since it is not unreasonable to suppose that ethylmalonate could, like methylmalonate, be utilized for fatty acid synthesis.

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Utilization of fish-protein hydrolysate for artificial rearing of lambs. By H. S. SOLIMAN and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB,* I. M. MACKIE, *Torry Research Station, Aberdeen,* and T. L. DODSWORTH, *North of Scotland College of Agriculture, Aberdeen.*

Fish-protein concentrate (FPC) has been satisfactorily used (Huber, 1975) to replace 35% of milk protein in calf milk-substitutes. Complete replacement of milk protein with protein from FPC gave poor performance and high mortality (Huber & Slade, 1967). In a recent experiment Smits, Boeve & Nieboer (1974) found that the digestibility of protein was decreased when protein from FPC replaced 50–75% of the milk protein but not when the same concentrations of fish-protein hydrolysates (FPH) was used.

In our experiment (see Table 1) we have compared the effect of replacing all milk protein with protein from undried FPH (Mackie, 1974) made from cod fillets and the effect of replacing all milk fat with lard–coconut oil (9:1, w/w). The diets contained (g/kg dry matter (DM)) 280 protein, 300 fat and 370 lactose. Sixteen crossbred lambs (Suffolk×(Finnish Landrace×Dorset Horn)) were taken from their mothers at 1–2 d of age. They were automatically fed every 3 h, the level of feeding being 1·045 MJ/kg$^{0.75}$ per d. There were two experimental periods, of 15 and 18 d. The apparent digestibility of DM, nitrogen and diethyl ether extract was estimated at 6 weeks of age.

All the animals remained healthy during the experiment. The live-weight gains of lambs given the FPH diets were 71% and 92% of those of lambs fed on dried skim-milk in the first and second periods respectively (Table 1).

Digestibility of DM, N and diethyl ether extract in diets containing milk and fish protein was not significantly different at 6 weeks, but there were small, significant differences in the digestibility due to fat source. The experiment showed that lambs could be artificially reared successfully without milk protein.

Table 1. *The effect of using fish-protein hydrolysate (FPH) and lard as complete replacements for milk protein and fat in diets for lambs*

Protein and carbohydrate source	Fat source	Body-weight gain (g/d) for days: 0–15	16–33	Over-all food conversion ratio*	Digestibility: DM	Nitrogen	Diethyl ether extract
Dried skim-milk	Butter oil	127	185	1·03	0·965	0·959	0·987
Dried skim-milk	Lard and coconut oil	108	188	0·99	0·945	0·934	0·965
FPH and lactose	Butter oil	88	183	1·05	0·964	0·955	0·989
FPH and lactose	Lard and coconut oil	80	158	1·12	0·938	0·928	0·962
SE of means		21·4	13·9	0·07	0·0076	0·0067	0·0083

DM, dry matter.

*kg Food DM intake/kg body-weight gain.

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