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# PROCEEDINGS OF THE NUTRITION SOCIETY

## ABSTRACTS OF COMMUNICATIONS

*The Three Hundred and Ninety-fifth Meeting of the Nutrition Society (One Hundred and Fifty-sixth of the Scottish Group) was held in Lecture Theatre 4, Chemistry Building, Meston Walk, Old Aberdeen on Wednesday and Thursday, 4/5 April 1984, when the following papers were read:*

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**The composition and nutritive value of *Chlorella vulgaris* grown in laboratory scale and pilot plant culture.** By J. J. STRAIN*, H. J. FALLOWFIELD and M. K. GARRETT, *Department of Agricultural and Food Chemistry, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*

A major limitation on the utilization of green algae as novel sources of protein for non-ruminants is the low digestibility of most species of green algae studied (Waslien, 1975). This study considers the nutritional value of a strain of *Chlorella vulgaris* (CCAP no. 211–1e) lacking the resistant cell wall component sporopollenin (Garrett *et al.* 1976). Algal biomass produced axenically in defined medium in laboratory chemostats was compared with the biomass produced in an outdoor pilot plant for the treatment of pig slurry. The inoculated algae were the major component of the harvested biomass from the latter system (Fallowfield & Garrett, 1983).

The centrifuged and lyophilized laboratory and pilot plant products contained (g/kg) respectively: true protein 420, 395–499; lipid 234, 79–141. The fatty acids C18:2 and C18:3 comprised 39·0% of the total fatty acid content in the laboratory product, significantly more than in the pilot plant material. Calculations for the pilot plant based upon a 153 d growing season suggest a possible yield of some 11 tonnes of algal protein/ha in Northern Ireland (H. J. Fallowfield, unpublished observations).

In the laboratory product the sulphur amino acids were limiting (1·56 g/16 gN) but the protein was relatively rich in lysine, phenylalanine and tryptophan (6·01, 8·09 and 1·97 g/16 gN respectively). This product had an essential amino acid content of 45·50 g/16 gN. The pilot plant product was more variable. The sulphur amino acids were still limiting (0·73–3·00 g/16 gN) but the lysine content was relatively low (2·24 g/16 gN).

In rat-feeding experiments the laboratory product had a digestibility of 81·7, a net protein utilization of 48·2 and a biological value of 59·0. It is proposed that the absence of sporopollenin from the cell walls of this strain of *C. vulgaris* may be responsible for the relatively high N digestibility and, further, that elaborate and expensive processing, e.g. methanol extraction, heat treatment or homogenization, should not be necessary to enhance the digestibility of this product.

Fallowfield, H. J. & Garrett, M. K. (1983). *British Phycology Journal* **18**, 203.
Garrett, M. K., Strain, J. J. & Allen, M. D. B. (1976). *Journal of the Science of Food and Agriculture* **27**, 603–611.
Waslien, C. I. (1975). *Critical Reviews in Food Science and Nutrition* **6**, 77–151.

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**Production of single cell protein on potato-processing-waste media.** By M. M. ABOUZIED and M. M. MOSTAFA, *Food Science Department, Faculty of Agriculture, Minufiya University, Shebin El-kom, Egypt*

During potato processing about half the potato is lost in various forms of waste such as peel, trim, filterable particulates, processing water and blanching water (Lemmel *et al.* 1980). Fermentation would remove part of the organic load of the waste water and produce a utilizable single cell protein (SCP). Kostina *et al.* (1975) reported that fungi, in general, were good sources of high quality protein. Szabo *et al.* (1975) found that the protein content of Aspergilli ranged from 310 to 340 g/kg. The present study was designed to study the possibility of growing amylolytic micro-organisms such as *Aspergillus niger, A. foetidus, A. awamori* and *Saccaromyces fibuligera* on potato-processing waste (PPW).

The total crude protein (TCP, nitrogen × 6·25; g/l) values were found to be highest with *A. niger* (5·18), lowest with *A. foetidus* (2·82) and intermediate with *A. awamori* and *S. fibuligera* (4·90 and 4·97 respectively). Comparing different media for the production of *A. niger* biomass, it was found that TCP reached a maximum on Czapek medium (Difco, 1974) of 5·99 g/l at 40 g PPW/l whereas TCP was 6·42 g/l at 20 g PPW/l on the basal medium suggested by Abouzied & Reddy (1982).

Gelatinized starch caused an increase in the protein and a decrease in the biomass of all micro-organisms except *A. niger*. However, *A. niger* gave high values of TCP in both gelatinized and non-gelatinized starch.

A mixed culture of *S. cerevisiae, A. foetidus* and *A. awamori* was favourable for producing a high percentage of cell biomass protein but a pure culture of *A. niger* and *S. fibuligera* was more practical.

Abouzied, M. M. & Reddy, C. A. (1982). *82nd Annual Meeting of the American Society of Microbiology*, Atlanta, Georgia.

Difco (1974). *Difco Manual* 9th ed. (reprinted), Detroit, Michigan: Difco Laboratories.

Kostina, A. M., Lobanko, A. G., Babitskaya, V. G. & Ovrutskaya, I. Y. (1975). *Chemical Abstracts* **83**, 19137e.

Lemmel, S. A., Heimsch, R. C. & Korus, R. A. (1980). *Applied Environmental Microbiology* 387–393.

Szabo, M., Scarpino, P. V. & Rogers, C. (1975). *Journal of Agricultural and Food Chemistry* **23**, 212–216.

**Activities of glutathione peroxidase (*EC* 1.11.1.9), glutathione transferase (*EC* 2.5.1.18) and pyruvate kinase (*EC* 2.7.1.40) in tissues of rainbow trout (*Salmo gairdneri*) deprived of selenium and vitamin E.** By J. G. BELL and C. B. COWEY, *NERC Institute of Marine Biochemistry, St Fittick's Road, Aberdeen AB1 3RA*

Deficiency symptoms could not be induced in rainbow trout given diets low in selenium (Hilton *et al.* 1980) although in a related salmonid, Atlantic salmon (*Salmo salar*), both vitamin E and Se were required to prevent muscular dystrophy (Poston *et al.* 1976). This may possibly be due to a Se-independent glutathione (GSH) peroxidase activity, the presence of which has recently been reported in another fish, black bullhead (species unspecified), by Heisinger & Dawson (1983). In mammals the Se-independent GSH peroxidase has been identified as a GSH transferase (Lawrence *et al.* 1978).

GSH transferase was purified from trout liver and shown to be without any GSH peroxidase activity with hydrogen peroxide and cumene hydroperoxide as substrates. GSH peroxidase purified 600-fold from trout liver did not prevent or reduce malondialdehyde formation in the NADPH-dependent microsomal system (Tien & Aust, 1982) in vitro but GSH transferase very greatly reduced its formation. The possibility that an increase in the GSH transferase activity might in some measure be responsible for prevention of Se-deficiency symptoms was examined by giving groups of trout diets deficient in either vitamin E, Se or both nutrients for 40 weeks.

Plasma pyruvate kinase activity was significantly elevated in the dually deficient fish suggesting leakage of the enzyme from the muscle but histologically no muscle damage could be detected. Liver and plasma GSH peroxidase activities were significantly reduced in the Se-deficient fish but the ratio of GSH peroxidase activity assayed with $H_2O_2$ and with cumene hydroperoxide as substrates was constant in all treatments, confirming the absence of any Se independent GSH peroxidase activity. Hepatic GSH transferase activity did not alter in any of the dietary treatments used; there was no compensatory increase in GSH transferase activity in Se deficiency in rainbow trout.

Heisinger, J. F. & Dawson, S. M. (1983). *Journal of Experimental Zoology* **225**, 325–327.
Hilton, J. W., Hodson, P. V. & Slinger, S. J. (1980). *Journal of Nutrition* **110**, 2527–2535.
Lawrence, R. A., Parkhill, L. K. & Burk, R. F. (1978). *Journal of Nutrition* **108**, 981–987.
Poston, H. A., Combs, G. F. & Leibovitz, L. (1976). *Journal of Nutrition* **106**, 892–904.
Tien, M. & Aust, S. D. (1982). *Biochimica et Biophysica Acta* **712**, 1–9.

**Mineral elements affecting rumen microbial synthesis in an in vitro system.** By G. N. MATHUR, J. R. SCAIFE and J. H. TOPPS, *Division of Agricultural Chemistry and Biochemistry, School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

An artificial rumen which was designed by Czerkawski & Breckenridge (1969) and is a closed system, has been used to study the effect of major and trace elements upon microbial synthesis as measured by the simultaneous incorporation of $^{32}P$ and $^{35}S$.

Strained rumen liquor from a sheep fed on ammonia-treated straw and cassava (*Manihot esculenta* Crantz) but with no mineral supplement, was incubated for 2 h in an artificial rumen. The results for incorporation of P and S into microbial material in the presence of different combinations of essential mineral elements are presented in the table. Each treatment was examined in triplicate.

*Incorporation of either P or S into a microbial fraction (mg/g)*

| | P incorporation | | S incorporation | |
|---|---|---|---|---|
| Treatment | Mean | SD | Mean | SD |
| All minerals added* | 7·3 | 3·14 | 0·056 | 0·020 |
| No mineral added | 1·4† | — | 0·244 | 0·090 |
| All minerals added except: | | | | |
| Mo, Cu | 3·1† | — | 0·366 | 0·231 |
| Mo | 3·1† | — | 1·17 | 0·668 |
| Cu | 2·4† | — | 0·187 | 0·042 |
| Mg, Mn, Co, Zn | 3·0 | 0·65 | 0·063 | 0·029 |
| Zn, Co | 3·2 | 0·44 | 0·059 | 0·031 |
| Fe | 1·2 | 0·14 | 0·036 | 0·010 |
| Zn | 2·8 | 0·45 | 0·074 | 0·022 |
| Co | 3·1 | 0·04 | 0·068 | 0·028 |

*Minerals added (mg/l) to incubation medium: Ca 40·0, P 100·0, Mg 25·0, K 1000·0, S 20·0, Fe 5·0, Cu 0·2, Zn 0·5, Mn 5·0, Co 0·3, Mo 5·0.

†Two replicates only.

The results have been expressed as incorporation of either P or S and not calculated to give synthesis of microbial protein since for both isotopes and for all treatments the extent of synthesis was small which may be related to the short period of incubation. The removal of the high concentration of molybdenum from the mixture of minerals gave a pronounced increase in uptake of $^{35}S$ but not $^{32}P$. The likely explanation for such an effect is that without Mo more of the S in the incubation medium was available to the micro-organisms but not fully incorporated into phosphoproteins. The largest reduction in uptake of both isotopes was found when iron was omitted from the mineral mixture. This surprising result is difficult to explain but it may be related to depletion of the sheep's rumen reserves of minerals or to a low availability of the mineral.

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

**In vitro responses of subcutaneous fat obtained from two sites in unanaesthetized women during pregnancy and postnatally.** By M. A. RADCLIFFE, C. ASHALL and D. M. CAMPBELL, *Department of Physiology, University of Aberdeen, Foresterhill, Aberdeen.*

It is held that basal and catecholamine-stimulated lipolysis are elevated in human white fat during late pregnancy and that this may in part explain the concomitant rise in maternal plasma non-esterified fatty acid levels (Elliott, 1975; Coltart & Williams, 1976). In each of these studies the subjects received a variety of medication prior to abdominal surgery and tissue sampling. The questions arise whether the changes occur in normal untreated subjects, and whether there might be differences in the behaviour of subcutaneous fat obtained from different sites, as suggested by differential change in skinfold thickness (Taggart *et al.* 1967)

Percutaneous needle biopsies were taken from two subcutaneous fat sites in twenty-six normal healthy primigravidae at different stages of gestation and postnatally. Small pieces of white fat tissue were incubated in duplicate or triplicate at 37° in Krebs bicarbonate saline containing 4% bovine albumin and isoprenaline bitartrate at the concentrations shown in the table. Lipolysis was measured by estimating glycerol release into the medium.

Our results suggest that basal and catecholamine-stimulated lipolysis was indeed elevated in late pregnancy in subcutaneous fat from buttock and thigh and that there were no differences in the behaviour of these two sites. However, the results from the buttock–arm comparison are consistent with the hypothesis that differential deposition of subcutaneous fat may be explained by the different lipolytic activities in tissue from these sites.

*Basal and isoprenaline-stimulated lipolysis in fat-tissue pieces from different subcutaneous sites (nmol glycerol/mg tissue in 90 min)*

| | | Gestation | | | | | | | | Postnatal | | | Gestation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week of sampling . . . | | 12 | | 20 | | 30 | | 38 | | 6 | | | 30–38 | |
| *n* . . . | | 5 | | 5 | | 3 | | 3 | | 5 | | | 4 or 5 | |
| Isoprenaline | Site* | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Site* | Mean | SD |
| 0 (basal) | B | 0·26 | 0·26 | 0·23 | 0·12 | 0·23 | 0·18 | 0·30 | 0·18 | 0·12 | 0·08 | B | 0·16 | 0·04 |
| | T | 0·19 | 0·03 | 0·23 | 0·10 | 0·23 | 0·09 | 0·26 | 0·09 | 0·10 | 0·09 | A | 0·25 | 0·14 |
| $10^{-6}$M | B | 0·43 | 0·26 | 0·76 | 0·68 | 0·84 | 0·51 | 0·87 | 0·59 | 0·21 | 0·16 | B | 0·26 | 0·16 |
| | T | 0·30 | 0·04 | 0·67 | 0·78 | 0·60 | 0·28 | 0·68 | 0·18 | 0·32 | 0·10 | A | 0·82 | 1·02 |
| $10^{-5}$M | B | 1·54 | 0·11 | 1·67 | 0·80 | 1·96 | 0·32 | 2·25 | 0·71 | 1·03 | 0·53 | B | 1·20 | 0·70 |
| | T | 1·65 | 0·25 | 1·49 | 0·50 | 1·88 | 0·46 | 2·12 | 0·86 | 1·37 | 0·18 | A | 2·03 | 1·16 |

*B, buttock; T, thigh; A, arm.

This study was supported by a grant from the Scottish Hospital Endowments Research Trust.

Coltart, T. M. & Williams, C. (1976) *British Journal of Obstetrics and Gynaecology* **83**, 241–244.

Elliott, J. A. (1975). *European Journal of Clinical Investigation* **5**, 159–163.

Taggart, N. R. Holliday, R. M., Billewicz, W. Z., Hytten, F. E. & Thomsom, A. M. (1967). *British Journal of Nutrition* **21**, 439–451.

**Cardiac phospholipid fatty acid composition in obese and lean Zucker rats.** By K. W. J. WAHLE, ALISON M. DUNCAN and LESLEY COUTTS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In tissue lipids, long-chain polyunsaturated fatty acids (PUFA) are mainly found as acyl groups of membrane phospholipids (Wahle, 1983). Changes in PUFA composition of the membrane phospholipid bilayer influences membrane integrity and function and may affect eicosanoid synthesis through the availability of precursor (Wahle, 1983). Tissue lipids from obese rats have reduced proportions of PUFA compared with lean animals and the values of 18:2ω6/20:4ω6 indicate an impaired 18:2ω6 metabolism in obese rats (Wahle & Radcliffe, 1977).

Cardiac phospholipids were obtained from obese and lean Zucker rats fed on standard Oxoid diets, on high (HSO; 200 g/kg) and low (LSO; 50 g/kg) sunflower oil diets or on high (HTO) and low (LTO) triolein diets of similar composition. Fatty acid composition was determined by capillary gas chromatography of the prepared methyl esters. Results for 18:2ω6, 20:4ω6 and 22:6ω3 are given in the table. Obese rats fed on the Oxoid diet had lower proportions of 20:4ω6 and 22:6ω3 in phospholipids than lean animals and the values of 18:2ω6/20:4ω6 again indicated impaired 18:2ω6 metabolism. Δ6-Desaturase activity, the rate-limiting step in 18:2ω6 metabolism, did not differ between phenotypes, at least in hepatic microsomes, being 11·6 (SD 2·4) and 11·3 (SD 1·2) nmol 18:3 formed/5 mg protein per 30 min (*n* 6) for obese and lean rats respectively.

*Cardiac phospholipid fatty acid composition (wt%) of obese and lean rats fed on different diets*

| | | 18:2ω6 | | 18:2ω6/ 20:4ω6 | 20:4ω6 | | 22:6ω3 | |
|---|---|---|---|---|---|---|---|---|
| | Diet | Mean | SD | | Mean | SD | Mean | SD |
| Obese | HSO | 26·7 | 2·5 | 1·79 | 14·9 | 0·5 | 3·8 | 0·3 |
| | LSO | 22·4 | 0·9 | 1·46 | 15·3 | 0·5 | 5·2 | 0·8 |
| | Oxoid | 25·8 | 1·8 | 1·96 | 13·2 | 1·5 | 6·4 | 1·2 |
| | LTO | 14·3 | 1·1 | 0·91 | 15·8 | 0·8 | 7·4 | 1·5 |
| | HTO | 12·4 | 1·6 | 0·66 | 18·7 | 1·1 | 10·6 | 0·9 |
| Lean | HSO | 20·8 | 1·6 | 1·10 | 19·3 | 0·3 | 4·0 | 1·6 |
| | LSO | 15·9 | 1·6 | 0·76 | 20·9 | 1·4 | 7·1 | 0·9 |
| | Oxoid | 16·1 | 0·4 | 0·89 | 18·1 | 1·3 | 11·8 | 1·2 |
| | LTO | 12·0 | 1·3 | 0·58 | 20·6 | 1·3 | 6·7 | 1·4 |
| | HTO | 12·9 | 2·5 | 0·56 | 22·9 | 1·5 | 9·0 | 1·9 |

Dietary triolein increased the proportion of 20:4ω6 to a greater extent than sunflower oil and reduced the value of 18:2ω6/20:4ω6 in both phenotypes. These observations show that dietary triolein can alter the proportion of 18:2ω6 and 20:4ω6 in cardiac phospholipids of obese rats to resemble those in lean rats and that it has a specific effect on 18:2ω6 metabolism, possibly at the level of Δ5-desaturase activity.

Wahle, K. W. J. (1983). *Proceedings of the Nutrition Society* **42**, 273–287.
Wahle, K. W. J. & Radcliffe, J. R. (1977). *Lipids* **12**, 135–139.

**Relationship between drinking-water nitrate and total nitrate intake.** By CHRISTINE CAYGILL, BARBARA BARTHOLOMEW, and M. J. HILL, *PHLS/ Centre for Applied Microbiology and Research, Porton Down, Wiltshire SP4 0JG*

Numerous reports, but none unambiguous, suggest an association between nitrate exposure and gastric cancer possibly via the production of locally acting N-nitroso compounds (Correa *et al.* 1975). A major shortcoming is lack of firm evidence regarding nitrate intakes in populations. Nitrate balance studies (Bartholomew *et al.* 1979) show that 24 h urinary nitrate excretion can be used to assess total nitrate intake (66% of intake being excreted in 24 h). Adults (544) drinking well/bore water of varying nitrate concentrations were asked to supply a 24 h urine specimen and a sample of their drinking water on two occasions, 14 d apart. Urinary nitrate, nitrite, creatinine and the drinking water nitrate were measured (Bartholomew, 1984). The nitrate concentrations of the water were similar on the two separate occasions ($r$ 0·97) and did not affect the volume consumed ($r$ 0·04). The total nitrate intake correlated with the drinking water nitrate concentration ($r$ 0·51, $n$ 423 $P<0·001$). The table shows the mean percentage contribution by the water to the total nitrate intake at various water nitrate concentrations.

| Drinking water nitrate conc. (mg/l) | No. of 24 h urine samples | Percentage nitrate intake from drinking water | |
|---|---|---|---|
| | | Mean | Range |
| 0 | 169 | 0 | — |
| 1–50 | 115 | 21 | 1–110 |
| 51–150 | 121 | 47 | 13–160 |
| More than 150 | 18 | 66 | 35–116 |

Thus, drinking water was a significant but minor contributor to total nitrate intake at nitrate concentrations $\leqslant$50 mg/l, but a major contributor at concentrations $>$50 mg/l in one-third of the adults tested.

These studies were supported by the Department of the Environment and the Cancer Research Campaign.

Bartholomew, B. (1984). *Journal of Food Chemistry and Toxicology* (In the Press).
Bartholomew, B., Caygill, C. Darbar, R. & Hill, M. J. (1979). *Proceedings of the Nutrition Society* **38**, 124A.
Correa, P., Haenszel, W., Cuello, C., Tannenbaum, S. & Archer, M. (1975). *Lancet* ii, 58–60.

**The iron status and dietary Fe intake of non-supplemented pregnant women.** By SHEENA TUTTLE, P. J. AGGETT, DORIS M. CAMPBELL and I. MACGILLIVRAY, *Department of Obstetrics and Gynaecology, University of Aberdeen, Foresterhill, Aberdeen*

The purpose of this study was to determine the effect of pregnancy on various haemotological indices in normal healthy primigravidae (group A) and to compare them with those of primigravidae at risk of delivering a growth-retarded baby (group B). It has been suggested that plasma iron is significantly lower in women in the latter group (Bogden *et al.* 1978). The twenty-nine women in group A were followed at 5-weekly intervals from 14 weeks gestation until 6 weeks postpartum, the thirty women in group B from 30 weeks gestation. At each visit, a fasting blood specimen was taken for estimation of haemoglobin (Hb), packed cell volume (PCV), plasma Fe, plasma transferrin and serum ferritin concentrations. Plasma volume was measured antenatally. Nutritional intake was assessed by 7-d weighed dietary surveys at 30 and 35 weeks gestation.

The haematological results are presented in the table. In group A, Hb, PCV, plasma Fe and serum ferritin concentrations fell progressively from 14 to 35 weeks. Plasma transferrin concentration and plasma volume increased. The fall in plasma Fe concentration was not due solely to haemodilution as there was a significant fall in the mean intravascular mass of Fe from 59·4 (SD 25·1) μmol at 14 weeks to 33·6 (SD 17·15) μmol at 35 weeks ($P<0.001$). There was no significant difference in any of the concentrations between groups A and B at 30, 35 weeks or postpartum. Plasma volume expansion was approximately 600 ml less in group B but the mean intravascular mass of Fe was 32·6 (SD 6·03) μmol at 35 weeks. In both groups, Hb and PCV had risen by 6 weeks postpartum but in group A, plasma Fe, transferrin and serum ferritin had not reached early pregnancy levels.

| | Gestation | | | | 6 weeks Postpartum | |
|---|---|---|---|---|---|---|
| | 14 weeks | | 30 weeks | | | |
| | Mean | SD | Mean | SD | Mean | SD |
| Hb (g/l) | 119·5 | 10·5 | 107 | 10·6 | 127 | 8·7 |
| PCV (l/l) | 0·35 | 0·026 | 0·32 | 0·027 | 0·39 | 0·026 |
| Plasma Fe (μmol/l) | 20·1 | 8·28 | 9·8 | 4·60 | 13·6 | 4·57 |
| Plasma transferrin (g/l) | 2·96 | 0·65 | 4·8 | 0·95 | 3·3 | 0·70 |
| Serum ferritin (ng/ml) | 40·8 | 28·40 | 9·9 | 7·38 | 19·9 | 10·79 |

Mean daily energy, protein and Fe intakes were comparable in the two groups at both gestations. At 30 weeks, mean Fe intake was 10·4 (SD 2·13) mg in group A and 10·3 (SD 2·75) mg in group B. These are less than the recommended daily amounts. However, no relation was found between maternal Fe status or Fe intake and the subsequent delivery of a growth-retarded baby.

Bogden J. D., Thind, I. S., Kemp, F. W. & Caterini, H. (1978). *Journal of Laboratory and Clinical Medicine* **92**, 455–462.

**Factors influencing Cu:Zn superoxide dismutase (*EC* 1.15.1.1) activity in ovine blood and its use in the diagnosis of hypocuprosis.** By D. W. PETER* and N. F. SUTTLE, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

Suttle & McMurray (1983) suggested that the rapid increase in activity of the copper-containing enzyme in erythrocytes, superoxide dismutase (SOD; *EC* 1.15.1.1), observed when Cu-depleted ewes were acutely repleted, may have resulted partly from reconstitution of an apo-enzyme in circulating erythrocytes. This suggestion was examined more closely by comparing the SOD activity in young and old cells during the early stages of repletion.

Four out of eight hypocupraemic ewes (mean plasma Cu 1·4 (SE 0·12) μmol/l), all maintained on a Cu-deficient diet, were given 0·19 mmol Cu, subcutaneously, as a water soluble, Cu-chelate. Whole blood samples were collected from all sheep before treatment and 1 and 7 d later. Erythrocytes were separated into age groups by the division of the centrifuged cells into an upper (younger) and a lower (older) cell fraction: this procedure was repeated three times with resuspension of separated fractions in isotonic buffer. SOD activities were then assayed in haemolysates (1:250, v/v) of whole blood and both the 'oldest' and 'youngest' cells of the fourteen fractions by a modification of the method of Jones & Suttle (1981).

There were no significant differences between the cell fractions (see Table).

| Day . . . | | SOD activity (IU/g haemoglobin) 0 | | SOD activity (% of value at day 0) 1 | | 7 | |
|---|---|---|---|---|---|---|---|
| | Treatment | Mean | SE | Mean | SE | Mean | SE |
| Whole blood | Nil | 482 | 81 | 78 | 6 | 88 | 7 |
| | + Cu | 535 | 56 | 97 | 4 | 106 | 4 |
| 'Young' washed cells | Nil | 754 | 102 | 111 | 6 | 107 | 10 |
| | + Cu | 787 | 93 | 135 | 7 | 142 | 14 |
| 'Old' washed cells | Nil | 809 | 128 | 100 | 12 | 91 | 7 |
| | + Cu | 964 | 181 | 128 | 6 | 114 | 7 |

One day after injecting Cu, mean SOD activities in whole blood and washed cells, expressed as a percentage of pre-treatment values, were significantly higher ($P<0·05$ and $P<0·01$ respectively) in treated animals compared with controls: plasma Cu had increased by almost threefold after 24 h. Because increases were similar in both 'old' and 'young' cells, they were not due to enhanced SOD synthesis: rapid reconstitution of an apo-enzyme may have occurred. The SOD activity of washed cells was between 47 and 129% (mean 92%) higher than that measured in whole blood due to an interference by plasma in the SOD assay. The SOD activity of washed cells and their response to in vitro repletion might be used for diagnostic purposes.

Jones, D. G. & Suttle, N. F. (1981). *Research in Veterinary Science* **31**, 151–6.
Suttle, N. F. & McMurray, C. H. (1983). *Research in Veterinary Science* **35**, 47–52.

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**Plasma metallothionein-I assays in the diagnosis of zinc deficiency in rats.** By M. SATO, R. K. MEHRA and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Unequivocal diagnosis of zinc deficiency on the basis of a reduction in plasma Zn concentrations is not possible, since these are also reduced after stress. There is a need for a test which will differentiate between different causes of hypozincaemia. Since stress and Zn deficiency have opposite effects on liver metallothionein (MT) concentrations, and plasma concentrations of MT-I are partly related to hepatic concentrations of the protein (Mehra & Bremner, 1983, 1984), a study has been made of the effects of endotoxin administration and of Zn deficiency on plasma MT-I levels in rats.

Groups of five male Hooded Lister rats, aged 3–6 weeks, were treated as follows: (a) injected intraperitoneally with endotoxin suspended in saline solution (1 mg/kg body-weight) 10 h before slaughter, (b) given a Zn-deficient diet (<1 mg Zn/kg) for 2 weeks, (c) given the same diet but with 40 mg Zn/kg for 2 weeks, either *ad lib.* or pair-fed against Zn-deficient animals, (d) given the Zn-deficient diet for 2 weeks and injected with endotoxin as in (a), (e) given a diet with 3 mg Zn/kg for 3 weeks and injected with endotoxin. Liver and heparinized plasma were collected and analysed for MT-I by radioimmunoassay (Mehra & Bremner, 1983).

| | Treatment | Plasma Zn (μg/ml) | | Liver MT-I (μg/g wet wt) | | Plasma MT-I (ng/ml) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SEM | Mean | SEM | Mean | SEM |
| (a) | Pre-injection | 1·42 | 0·06 | 24·9 | 1·8 | 5·6 | 1·1 |
| | Endotoxin | 0·76 | 0·10 | 68·4 | 5·8 | 28·9 | 4·8 |
| (b) | Zn-deficient | 0·43 | 0·02 | nd | — | nd | — |
| (c) | +Zn, *ad lib.* | 1·67 | 0·03 | 1·1 | 0·3 | 2·9 | 0·7 |
| | +Zn, pair-fed | 1·80 | 0·10 | 29·2 | 7·0 | 9·4 | 1·5 |
| (d) | Zn-deficient, control | 0·45 | 0·03 | nd | — | nd | — |
| | Zn-deficient, endotoxin | 0·24 | 0·02 | 13·8 | 0·5 | 8·2 | 2·0 |
| (e) | 3 mg Zn/kg, control | 0·79 | 0·10 | nd | — | nd | — |
| | 3 mg Zn/kg, endotoxin | 0·29 | 0·02 | 14·4 | 0·8 | 9·2 | 1·2 |

nd, Not detected

Rats given the low-Zn diets (⩽3 mg/kg) exhibited classical signs of Zn-deficiency with reduced food intakes and growth rates. Plasma Zn concentrations were reduced in these rats and in those given endotoxin, irrespective of their dietary Zn supply. Liver MT-I concentrations were zero in Zn-depleted rats but were increased in pair-fed control rats and in those injected with endotoxin, although this increase was less marked in Zn-depleted rats given endotoxin.

Plasma MT-I was not detectable in Zn-depleted rats but increased in pair-fed and endotoxin-treated rats, particularly those of normal Zn-status. A Zn deficiency state is indicated only if both plasma Zn and MT levels are low. Assay of MT concentrations in plasma should therefore permit the differentiation of low plasma Zn caused by Zn deficiency or infection.

Mehra, R. K. & Bremner, I. (1983). *Biochemical Journal* **213**, 459–465.
Mehra, R. K. & Bremner, I. (1984). *Biochemical Journal* (In the Press).

**An association between lamb mortality and copper status in different breeds of sheep.** By J. A. WOOLLIAMS, C. WOOLLIAMS and G. WIENER, *AFRC Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ* and D. G. JONES and N. F. SUTTLE, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

The blood copper status of an interbred Scottish Blackface (B) × Welsh Mountain (W) flock was manipulated by selecting for low ('Low' line) and high ('High' line) plasma Cu concentrations for three generations (Wiener & Woolliams, 1983). In order to show that susceptibility to clinical Cu deficiency had also been altered, the High and Low lines were grazed, together with unselected B and W sheep, on improved hill pastures. Within each breed group and line, 1 g cupric oxide needles (May and Baker Ltd) was given to half the lambs at 4·5–7·5 weeks of age. The ewes and lambs were separated into two grazing sets according to age, each set containing all breed types and treatments. In view of early high mortality in set 1, lambs chosen for Cu treatment in the younger set (set 2) were given 0·5 mg Cu by injection (Cujec; TVL Ltd) 4 weeks before receiving the needles.

*Number of lambs dying according to breed type and age*

| | | | Set 1 | | Set 2 | | | |
|---|---|---|---|---|---|---|---|---|
| Age . . . | | 0–1 d | 2 d – 6 weeks | 6–12 weeks | 2 d– 2 weeks | 2–12 weeks | 12–24 weeks | Total |
| Breed/line | No. born | | | | | | | |
| B | 98 | 2 | 23 | 7 (1)• | 2 | 1 | 10 (3) | 45 |
| B × W | | | | | | | | |
| Low | 162 | 5 | 10 | 2 | 15 | 3 (2) | 13 | 48 |
| High | 126 | 3 | 1 | 0 | 6 | 0 | 3 (1) | 13 |
| W | 82 | 2 | 0 | 0 | 0 | 0 | 2 | 4 |
| Significance of | | | | | | | | |
| Breed type | | NS | $P<0.01$ | $P<0.01$ | $P<0.05$ | NS | $P<0.01$ | |
| Cu treatment | | — | — | $P<0.01$ | — | NS | $P<0.01$ | |

•Values in parentheses are the number of deaths of lambs supplemented with Cu.

The mortalities at different ages are detailed in the table. The results indicate that lambs of the 'low Cu' breed types (B and Low line) had higher mortality rates at all ages. Swayback was a cause of death in twelve of the eighty-two lambs which died before weaning at 10·5–13·5 weeks of age, but the bulk of the pre-weaning mortality was due to a variety of pathogenic organisms including *Escherichia coli* and *Pasteurella haemolytica*. Post-weaning mortality was predominantly due to delayed swayback (eighteen cases). Cu treatment alleviated the hypocupraemia which was present in all breed types throughout the study but which was particularly frequent and severe in the low-Cu breed types. Cu treatment also decreased the risk of mortality pre-weaning and prevented delayed swayback. These findings are in agreement with previous experimental studies in mice (Jones & Suttle, 1983) in which Cu deficiency enhanced the susceptibility to infection. Cu deficiency may lead to significant losses in sheep on improved hills, but under these conditions attention to the heredity of the sheep may considerably reduce the level of risk.

Jones, D. G. & Suttle, N. F. (1983). *Journal of Comparative Pathology* **93**, 143–149.
Wiener, G. & Woolliams, J. A. (1983). *British Society of Animal Production* Occasional Publication No. 7.

**Growth responses to copper and selenium in lambs of different breeds on improved hill pastures.** By N. F. SUTTLE and D. G. JONES, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH* and J. A. WOOLLIAMS, C. WOOLLIAMS and G. WIENER, *Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ*

Theoretical grounds for an interaction between copper and selenium can be deduced (e.g. Barry *et al.* 1981) but evidence in ruminants is scanty. The detection of concomitant hypocupraemia and hyposelenaemia in lambs on improved hill pastures prompted the following investigation. In a 2×2 factorial experiment, responses to Cu and Se were investigated using 141 lambs divided almost equally between the four treatments and between two breeds, Scottish Blackface (B) and Welsh Mountain (W). The Cu treatment was 1 g cupric oxide needles (May and Baker Ltd), given at 6 weeks of age, and the Se treatment was 25 mg Se, given subcutaneously as barium selenate (Deposel, Rycovet Ltd) at 12 weeks of age. Glutathione peroxidase (GSHPx; *EC* 1.11.1.9) activity in whole blood haemolysates was assayed at 37° using a lyophilized bovine erythrocyte preparation as an internal standard (Boehringer, UK Ltd).

In untreated lambs, plasma Cu and whole blood GSHPx concentrations declined between 12 and 24 weeks but treatment with Cu and Se produced independent recoveries in Cu and Se status (see table). Both treatments improved growth rate between 12 and 24 weeks of age and there was no significant interaction between them: the mean (with SE) Se effect was 15 (4) g/d and was the same in both breeds. The Cu effect was, however, greater in the breed with the lower plasma Cu concentrations (39 *v.* 8 (SE 5) g/d in B *v.* W). Concurrent studies with low (L) and high (H) plasma Cu lines from an interbred B × W cross (Woolliams *et al.* 1984) showed a similar difference (20 *v.* 6 (SE 3) g/d in L *v.* H) without the complication of different growth potentials. This evidence of a breed × Cu interaction, apparently independent of Se, supports the view that the effects of the Cu and Se deficiencies on growth were largely unrelated. A similar finding has been reported in cattle (Gleed *et al.* 1983). A more detailed analysis of the within treatment responses is in progress.

| | Live-weight gain (kg) | | Plasma Cu (μmol/l) | | Gluthathione peroxidase activity (IU/g haemoglobin) | |
|---|---|---|---|---|---|---|
| Treatment | B | W | B | W | B | W |
| Initial values | — | — | 6·1 | 11·8 | 50 | 64 |
| 0 | 5·4 | 5·1 | 3·1 | 4·8 | 17 | 16 |
| +Cu | 8·3 | 5·4 | 10·9 | 15·0 | 16 | 21 |
| +Se | 5·9 | 6·3 | 2·8 | 5·7 | 243 | 255 |
| +Cu + Se | 9·5 | 7·3 | 13·8 | 15·0 | 198 | 267 |
| | ±0·6 | ±0·6 | ±1·3 | ±0·8 | ±17·5 | ±14·0 |

Barry, T. N., Reid, T. C., Millar, K. R. & Sadler, W. A. (1981). *Journal of Agricultural Science, Cambridge* **96**, 269–282.

Gleed, P. T., Allen, W. M., Mallinson, C. B., Rowlands, G. J., Sansom, B. F., Vagg, M. J. & Caswell, R. D. (1983). *Veterinary Record* **113**, 388–392.

Woolliams, J. A., Woolliams, C., Wiener, G., Jones, D. G. & Suttle, N. F. (1984). *Proceedings of the Nutrition Society* **43**, 102A.

**The absorption of calcium by forage-fed lambs.** By J. K. THOMPSON and A. L. GELMAN, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Data from a series of balance trials with growing wether lambs provided the opportunity to develop a relationship between the proportion of dietary calcium absorbed and the ratio, net requirement:intake. The fifteen dietary treatments in the trials included thirteen fresh, dried or ensiled grass herbages and two diets low in Ca based on barley, oat husk and soya-bean meal. The trials were conducted over 9-d collection periods with, in general, six wether lambs per treatment. The live-weight range of the lambs was 17–36 kg.

The endogenous faecal losses were calculated using the regression from Braithwaite (1982) and the proportion of absorbed Ca was estimated from the difference between intake (*I*) and faecal excretion corrected for endogenous faecal loss. The net requirement (*R*) was estimated from the total endogenous loss and tissue requirement. Tissue requirement of Ca was calculated on the basis of 11 g/kg live-weight gain and live-weight gain was estimated from net energy retention according to the Agricultural Research Council (ARC) (1980).

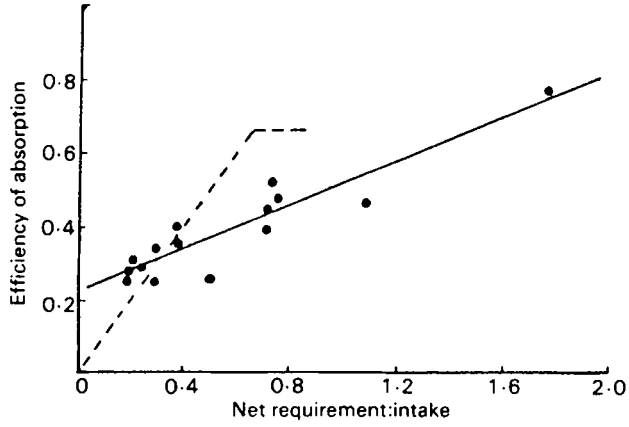


Fig. 1 The efficiency of absorption of Ca in relation to the ratio, net requirement:intake. (– – –) The expected relationship from ARC (1980).

The data (Fig. 1) fit the regression $A = 0{\cdot}222 + 0{\cdot}298\,(R/I)$ ($r$ 0·91), where $A$ is the efficiency of absorption, and support two routes of Ca absorption: a fixed or passive mechanism accounting for 20% of the absorbed Ca and a variable or active mechanism related to $R/I$. The slope for the experimental response is smaller than the expected response. The efficiency of absorption of Ca increased with increased requirement but only reached its highest level with a dietary regimen which probably resulted in significant skeletal depletion.

On the basis of these findings and using a low availability of 0·35, the growing lamb's dietary allowance for Ca is apparently higher than the requirement suggested by the ARC (1980). For example, a 30 kg lamb growing at 100 g/d may need 5·35 g Ca/d compared with the ARC's estimated requirement of 2·32 g/d, to be certain of maintaining its skeletal reserves.

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

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**The triglyceride composition of bovine milk fat and the diet of the cow.** By W. BANKS, J. L. CLAPPERTON, ANNE K. GIRDLER and D. D. MUIR, *Hannah Research Institute, Ayr KA6 5HL*

The melting properties of milk fat can be controlled by adding appropriate fats or oils to the diet of the cow (Banks *et al.* 1983). To date, these changes have been discussed in terms of the fatty acid composition of the milk fat and any contribution from altered triglyceride structure has been ignored. We report here preliminary results in our current study of the relationship between the fatty acids available to the udder and the molecular architecture of the resulting triglycerides.

Triglyceride composition was determined by capillary gas–liquid chromatography using a 25 m×0·32 mm column coated with CP-Sil 8 (Chrompack Ltd, London) using hydrogen as the carrier gas. The sample of fat (2·5 g/l in heptane) was injected on to the column at 60°, the oven was heated rapidly to 250° and then at 4°/min to 340° which was then maintained for 20 min. Under these conditions, there was a reasonable separation of all triglyceride groups containing from 30 to 54 carbon atoms in the fatty acids.

| | Fatty acids (mmol/mol) | | | | Triglycerides (mmol/mol) | | |
|---|---|---|---|---|---|---|---|
| Diet* ... | A | B | C | | A | B | C |
| 4:0 | 97 | 82 | 69 | $C_{30}$ | 10 | 7 | 2 |
| 6:0 | 42 | 53 | 37 | $C_{32}$ | 9 | 17 | 6 |
| 8:0 | 14 | 22 | 13 | $C_{34}$ | 29 | 59 | 25 |
| 10:0 | 20 | 38 | 21 | $C_{36}$ | 111 | 118 | 55 |
| 12:0 | 20 | 36 | 24 | $C_{38}$ | 178 | 154 | 109 |
| 14:0 | 94 | 133 | 109 | $C_{40}$ | 98 | 131 | 108 |
| 16:0 | 372 | 260 | 236 | $C_{42}$ | 40 | 77 | 47 |
| 18:0 | 341 | 376 | 491 | $C_{44}$ | 35 | 57 | 38 |
| | | | | $C_{46}$ | 47 | 63 | 56 |
| | | | | $C_{48}$ | 89 | 79 | 84 |
| | | | | $C_{50}$ | 172 | 99 | 167 |
| | | | | $C_{52}$ | 156 | 94 | 165 |
| | | | | $C_{54}$ | 26 | 45 | 138 |

*A: hay, concentrates, palm oil; B: grass only; C: grass, beet pulp, soya-bean oil

The diets offered to the cows and the results are shown in the table. Compared with the grass only diet, the addition of soya-bean oil reduced all the triglycerides from $C_{30}$ to $C_{46}$ and increased the remainder, especially the $C_{54}$. When palm oil was added to a typical winter diet, there was little effect on $C_{30}$ to $C_{38}$, triglycerides from $C_{40}$ to $C_{46}$ were reduced and the remainder were increased. These changes reflected the changes in milk fatty acid composition.

Banks, W., Clapperton, J. L. & Steele, W. (1983). *Proceedings of the Nutrition Society* **42**, 399–406.

**Energy and nitrogen metabolism in broiler chickens selected for high and low body fat content.** By M. G. MACLEOD, C. C. WHITEHEAD, H. D. GRIFFIN and T. R. JEWITT, *Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS*

Intense genetic selection of broiler chickens for increased growth rate has been accompanied by increased body fat content; this entails loss for both producer and consumer. As fatness is heritable, genetic selection for leanness offers a solution. A breeding programme, based on the assessment of the body fat content of live broilers by measurement of plasma triglyceride concentration, has resulted after three generations in lines with fat contents (g/kg of live weight in females) of 160 (L) and 210 (F) (Whitehead & Griffin, 1984).

Energy (E) and crude protein (CP; nitrogen × 6·25) intake and retention were measured in eight randomly-selected 63 to 70-d-old females from each line in a 5 d period of indirect calorimetry. Two birds from the same line were placed in each calorimeter chamber at 20°. The results are presented in the table.

| | Lean | | Fat | | Statistical significance |
|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | |
| Body-weight (kg) | 2·29 | 0.080 | 2·29 | 0·060 | NS |
| Metabolizable energy intake (kJ/d) | 1971 | 114·6 | 1965 | 69·2 | NS |
| Heat production (kJ/d) | 1194 | 31·4 | 1167 | 38·5 | NS |
| E retention (kJ/d) | 777 | 95·0 | 798 | 82·5 | NS |
| E retained as CP (kJ/d) | 280 | 4·9 | 216 | 30·2 | $P<0·05$ |
| E retained as fat (kJ/d) | 497 | 94·0 | 582 | 57·8 | NS |
| Gross efficiency of E retention | 0·39 | 0·024 | 0·41 | 0·029 | NS |
| Gross efficiency of CP retention | 0·40 | 0·020 | 0·31 | 0·031 | $P<0·05$ |

NS, not significant

Body-weight, metabolizable energy intake, heat production, energy retention and efficiency of energy retention did not differ between lines. Energy retained as CP and efficiency of CP retention were, however, significantly lower in the F line. The critical difference was therefore in the partition of retained energy between fat and protein deposition. The results were consistent with a higher rate of catabolism of amino acids as an energy source in the F line; as heat production did not increase also, the additional energy was available for deposition as fat.

Whitehead, C. C. & Griffin, H. D. (1984). In *Poultry Genetics and Breeding, Proceedings of the 19th Poultry Science Symposium, September* 1983. [D. Hewitt, W. G. Hill and J. Manson, editors]. Edinburgh: British Poultry Science Ltd.

**Heat production and food intake in turkeys given diets of widely differing protein:energy values.** By M. G. MACLEOD and T. R. JEWITT, *Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS*

The domestic fowl, like some mammalian species, increases its energy intake when offered a low-protein or high-energy diet. In both birds and mammals, the 'extra' energy thus consumed has usually been shown to be deposited as fat, although instances of 'diet-induced thermogenesis' have been recorded in both classes (Davidson *et al.* 1968; Rothwell & Stock, 1979; Gurr *et al.* 1980).

The hypothesis that the turkey may be less able than the fowl to alter its energy intake in response to varying protein:energy values was tested by offering three diets differing in metabolizable energy density (ME, MJ/kg) but with identical crude protein content (CP (nitrogen × 6·25), 130 g/kg): ME values were (a) 8·0 (16·25 g CP/MJ), (b) 13·4 (9·70 g CP/MJ) and (c) 18·0 (7·22 g CP/MJ). Two groups of five 42-d-old, individually caged female turkeys were initially fed (*ad lib.*) on diet (b) for 14 d. One group then received diet (a) and one diet (c) (*ad lib.*) for 16 d. Heat production (H) on each diet was measured by respiration calorimetry in two 3-d sessions. Ambient temperature was 20°.

| ME in diet (MJ/kg) | *n* | Food intake (g/kg $W^{0.75}$ per d) | | ME intake (kJ/kg $W^{0.75}$ per d) | | CP intake (g/kg $W^{0.75}$ per d) | | H (kJ/kg $W^{0.75}$ per d) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 8·0 | 5 | 112 | 2·1 | 896 | 16·8 | 14·6 | 0·27 | 655 | 19·9 |
| 13·4 | 10 | 63 | 2·5 | 844 | 33·5 | 8·2 | 0·33 | 716 | 21·0 |
| 18·0 | 5 | 49 | 5·0 | 885 | 90·8 | 6·4 | 0·66 | 625 | 28·6 |
| Statistical difference | | $P \leqslant 0.001$ | | NS | | $P \leqslant 0.01$ | | NS | |

NS, not significant; W, body-weight.

There was no significant effect of CP:ME value on either ME intake or H. However, ME intake and H tended to decrease with time at the highest ME density, possibly because of a limit to fat deposition in the former case and depressed growth in the latter (McCracken, 1983). Rather than adjusting ME intake, growing turkeys therefore tolerated wide variation in the intake of essential nutrients, including amino acids. Neither an increased rate of fat deposition nor diet-induced thermogenesis was therefore used as a mechanism for enhancing the intake of limiting nutrients.

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McCracken, K. J. (1983). *Proceedings of the Nutrition Society* **42**, 4A.

Rothwell, N. J. & Stock, M. J. (1979). *Nature* **281**, 31–35.