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## ABSTRACTS OF COMMUNICATIONS

*The One Hundred and Ninety-first Meeting of the Nutrition Society was held at the London School of Hygiene and Tropical Medicine, Keppel Street London WC* 1, *on Thursday,* 5 *October* 1967, *at* 16.30 *h, when the following papers were read:*

**The economic value of a hypothetical high-lysine barley.** By K. J. CARPENTER, *School of Agriculture, University of Cambridge* and J. H. TAYLOR, *Mond Division, Imperial Chemical Industries Limited, Runcorn, Cheshire*

The development of high-lysine maize has raised wide interest. This paper considers the possible value of a high-lysine barley for animal feeding if resources were used successfully in developing one.

A least-cost mixture for young bacon pigs had lysine as one cost-limiting factor when computed so that the quantity containing 70 lb 'total digestible nutrients' also contained at least 0·69 lb Ca, 0·51 lb P, 17 lb protein, 0·82 lb lysine, 0·57 lb 'methionine+cystine', 0·66 lb isoleucine and fixed levels of six further amino acids. The following ingredients (with prices, £/ton) were used in this calculation: standard barley (22 for UK or 30 for EEC countries), fish meal (72), soya meal (44), DL-methionine (600), L-lysine hydrochloride (560, a likely future price), $CaCO_3$ (2) and $CaHPO_4$ (44). Opportunity prices (i.e. maximum prices for additional ingredients at which they would be brought into the least-cost formula) were then computed for barleys of different composition. Expressed as percentages of the price of standard barley (11% of protein containing 3·4% lysine), they are:

| | Standard barley at £22/ton | | | | Standard barley at £30/ton | | | |
|---|---|---|---|---|---|---|---|---|
| Lysine as % of protein | (3·0) | (3·4) | (4·0) | (6·8) | (3·0) | (3·4) | (4·0) | (6·8) |
| Protein in barley (9%) | 96 | 97 | 99 | 106 | 98 | 99 | 100 | 104 |
| (11%) | 99 | (100) | 102 | 111 | 99 | (100) | 101 | 107 |
| (15%) | 105 | 107 | 109 | 122 | 101 | 102 | 104 | 112 |

Calculations for a 14% protein mix for older pigs, with individual amino acids reduced in proportion, gave essentially the same results; nor were they changed significantly by removing the specification for total protein content. Including synthetic L-lysine hydrochloride at its current price of £900/ton changed results by no more than 2 percentage units.

Normally, high-protein barleys have a lower lysine (expressed as % of protein). If a realistic target for a high-lysine barley were 15% of protein, still containing 4% lysine, our type of calculation indicates that for use in pig rations it could command a price premium of not more than 9% in the UK over standard barley. even though it contained nearly twice as much lysine. Thus, an alternative of 10% increase in yield, with no change in composition, would seem to give a higher economic return per acre.

**The effects of dietary carbohydrate on serum lipid levels.** By A. ANTONIS, CYNTHIA ILES and T. R. E. PILKINGTON, *The Medical Unit, St. George's Hospital Medical School, London, SW1*

There is great controversy about the effects of different carbohydrates on serum lipid levels. Some workers have claimed that sucrose causes elevation of serum triglyceride and that starch and glucose do not have this effect. We have studied fourteen subjects (eleven males and three females). They were first given a diet containing 40% of fat (soya oil), 45% of carbohydrate and 15% of protein. The protein was supplied as fat-free skim-milk powder; this also contains lactose which accounted for 20% of total calories of the diet. The skim-milk powder was given throughout the experiment.

After stable serum lipid levels had been achieved, the fat was isocalorically replaced by glucose, lactose, sucrose, cooked starch and uncooked maize starch in various combinations.

We found that when carbohydrate was thus isocalorically substituted for fat, serum triglyceride levels rose in both men and women. Mono- and di-saccharides such as glucose, sucrose and lactose, or polysaccharides such as cooked or raw starch, all produced similar effects; isocaloric interchange between maize starch and sucrose caused no significant changes in serum triglyceride levels.

The different findings in other studies are best explained by changes in calorie balance, which, unless carefully controlled, can lead to erroneous conclusions.

**Studies on gastric secretion in the pig.** By D. E. NOAKES, P. D. CRANWELL and K. J. HILL, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Full assessment of the role of the stomach in digestion requires detailed information on the factors which influence the secretion and composition of gastric juice. In view of the conflicting evidence about the onset of HCl secretion and the reported proteolytic enzyme deficiency, it is mainly in relation to the young pig that most data are required.

Secretory responses to feeding and to histamine were examined in pigs from 6 to 16 weeks of age with vagally innervated and de-nervated gastric pouches. After fasting for 12–18 h small volumes (1–5 ml/30 min) of juice of low acidity but of high mucus content and proteolytic activity were secreted. The rapid consumption of a small meal was followed by a marked increase in the volume of secretion which reached its maximum in $1\frac{1}{2}$–$2\frac{1}{2}$ h and returned to the prefeeding level after $3\frac{1}{2}$–5 h. The acidity of this juice was high with maximum free acidity of 159 m-equiv./l. and although the pepsin concentration was reduced, total output was increased.

There was continuous secretion of gastric juice in pigs which were fed *ad lib.* although the volume and acidity were reduced during the night period when there was less food and water consumed.

Intramuscular injection of histamine acid phosphate (3 mg) was followed by a rapid increase in secretion which reached a maximum after 70 min and returned

to the pre-injection level after 130 min. The maximal acidity reached was 162 m-equiv./l. and there was the typical inverse relationship between the sodium and potassium concentration in the juice.

The presence of a cephalic phase of gastric secretion was demonstrated in pigs with vagally innervated pouches by teasing them with food after a fasting period. This resulted in an increase in the volume and acidity of the juice secreted.

Gastric secretion in the newborn pig was studied using gastric fistulas prepared a few hours after birth. In the pigs examined the period of achlorhydria varied between 24 h and 14 days after birth. The relationship between the onset of HCl secretion and the gastric flora was also studied.

**The interrelationship between food and water intake and egg laying in light hybrid hens.** By R. S. ANDERSON and K. J. HILL, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Measurements of food and water intake, droppings output and egg production were made on individual hens (Thornber 606, 707 and Shaver 288) receiving a commercial layers diets and water *ad lib.* The intake of food and water and the output of droppings (mean ± SD) in relation to lay of four Thornber 606 hens were as follows:

| Laying status | Pre-lay | Lay | Post-lay | Second lay |
|---|---|---|---|---|
| Age | 18 weeks | 48 weeks | 64 weeks | 78 weeks |
| Water intake (ml/day) | 102±7 | 202±16 | 108±19 | 214±48 |
| Food intake (g/day) | 85±7 | 98±3 | 81±13 | 108±9 |
| Water: food ratio | 1·21±0·02 | 2·04±0·20 | 1·33±0·20 | 1·99±0·60 |
| Droppings moisture (%) | 69±0·8 | 82±3 | 68±5 | 79±6 |
| Water output in droppings (ml/day) | 59±3 | 112±14 | 50±7 | 108±41 |

There was a marked increase (100%) in water intake at the onset of lay, resulting in increased moisture in the droppings. Water turnover and food intake returned to pre-lay values during moult, and subsequently increased at the onset of the second lay (see above).

Daily fluctuations in food and water intake were greater during lay than during the pre-lay period, and there was a significant linear relationship between food and water intake during lay, and also during the pre-lay period. Variation in water intake during constant (restricted) food intake was not significantly different from variation during *ad lib.* food intake, nor was variation in food intake during constant (restricted) water intake different from that during *ad lib.* water intake.

There was a reduction in food intake on 'non egg-forming' days confirming the report of Morris & Taylor (1967) and this was accompanied by a significant reduction in water intake. Peak food intake on 'egg-forming' days usually occurred during the last 2 h of daylight. Food and water intakes were minimal during the hour preceding oviposition.

Cizek (1961) ascribed the linear relationship between food and water intake in the dog, rabbit and rat to the osmotic effect of the diet in temporarily 'loculating'

within the digestive tract the intestinal secretions derived from the extracellular fluid. Our observations that fluctuations in water intake continue when food intake is constant suggest that in the laying hen water intake is not dependent on variation in food intake. The increase in water intake by the hen at the onset of lay is clearly in excess of that necessary to balance the increased output in the egg and it may be that movement of water and electrolyte into the egg (Draper, 1966) and the reproductive tract contribute to the variations in food and water intake which are associated with egg formation.

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**Mobilization of calcium for eggshell formation in the domestic hen.**

By ROSEMARY J. CHADWICK and D. H. SHRIMPTON, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Although considerable attention has been given to studying changes in the concentration of calcium in the plasma of laying hens (Hertelendy & Taylor, 1961; Taylor & Hertelendy, 1961), no information is available on the dynamic equilbria between absorbed dietary Ca, skeletal Ca and the blood Ca pool from which material for the shell is drawn. From observations on the shell, following the feeding of $^{45}Ca$, Tyler (1954) has concluded that the supply of Ca to the shell gland may be made up entirely of Ca from the food, or from the bone, or from varying proportions, depending upon the time of day. To study these relationships and to determine the quantitative contribution of diet and skeleton, a double radioisotope labelling technique was used with Shaver Starcross 288 hens fed on a diet containing 3% Ca.

*Rates of entry into the plasma of calcium of dietary and skeletal origin during shell formation*

| Bird no. | Age (weeks) | Body-wt (kg) | Time in relation to oviposition (h) | Plasma Ca (mg/100 ml) | Entry rate of Ca into plasma (mg/min $W^{0.75}$) | Relative entry rate of Ca (1·0 at oviposition) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Total | Skeletal |
| 1 | 33 | 1·60 | −17 | — | — | — | — |
| | | | −11 | 16 | 1·2 | 1·15 | 3·20 |
| | | | − 5 | 16 | 1·3 | 1·25 | 2·30 |
| | | | + 1 | 16 | 0·9 | 0·70 | 0·95 |
| 2 | 47 | 2·20 | −17 | 20 | 1·8 | 1·45 | 1·65 |
| | | | −11 | 20 | 1·7 | 1·40 | 1·35 |
| | | | − 5 | 17 | 1·5 | 1·20 | 1·30 |
| | | | + 1 | 15 | 1·1 | 0·90 | 0·95 |
| 3 | 47 | 1·56 | −17 | 30 | — | — | — |
| | | | −11 | 26 | 1·9 | 1·75 | 2·45 |
| | | | − 5 | 24 | 1·9 | 1·80 | 2·45 |
| | | | − 1 | 20 | 1·0 | 0·95 | 0·85 |

$^{45}Ca$ was equilibrated with skeletal Ca by fourteen daily intraperitoneal injections of 10 $\mu$c and $^{47}Ca$ was equilibrated with plasma Ca during the period of shell formation by continuous infusion of 0·25 $\mu$c/h into the brachial vein. Samples were withdrawn from the right brachial artery.

The entry rate of Ca into the total plasma Ca pool was not paralleled by changes in concentration of plasma Ca. The mobilization of skeletal Ca was increased during shell calcification but its entry rate into the plasma pool varied independently of that of total Ca and there was no consistent pattern relating maximum skeletal mobilization to the times of feeding and oviposition.

It is concluded that the dynamic equilibria involved in the utilization of Ca for shell formation are more complex than has been previously suggested from studies of plasma Ca concentrations.

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**An experimental procedure for applying conventional balance techniques to the study of calcium balance in the laying hen.** By D. H. SHRIMPTON, K. JACKSON and VALERIE J. LAMBERT, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

The interpretation in nutritional terms of experiments using radioactive markers to determine the relative contributions of skeletal and dietary calcium to the egg-shell requires a knowledge of the nutritional status of Ca in the bird at that time. The balance technique is the direct experimental approach but because of the extensive and quantitatively unpredictable mobilization of Ca by the skeleton previous attempts to use the method in the laying bird have failed (Duncan, 1967).

In the present experiment the conventional balance period of 24 h was abandoned. Instead the period used was that between consecutive ovipositions, and an average 'egg-day', varying between 23 and 30 h, was determined for each bird. Eighteen Thornber 404 and eighteen Shaver Starcross 288 hens were used. Both groups were 43 weeks old at the start of the experiment. A total balance period of 4 weeks was used, the analyses for each 'egg-day' for each of the thirty-six hens being studied separately within the total period of 28 days. Each hybrid group was subdivided so that three levels of Ca could be fed. These were at 2, 3 and 4% of the total diet with a ratio of Ca:P (calculated as available P) of 5:1 in the diet containing 2% Ca. The summarized balance results from the 43rd to the 47th week are shown in the table.

It has been concluded that: (1) A balance technique can be used to study nutritive requirement in the laying hen if the period used is that between consecutive ovipositions. (2) Although the present experiment was concerned with Ca, one might expect that it can be applied to the study of nitrogen requirement, including non-protein sources, and the requirement for individual amino acids. (3) Increasing

| Observation | Hybrid | Apparent gain (+) or loss (−) of calcium in g: Diet 401 (2% Ca) | Diet 402 (3% Ca) | Diet 403 (4% Ca) |
|---|---|---|---|---|
| Average balance for an 'egg-day' period | 288 | −0·14 | +0·12 | +0·28 |
| | 404 | −0·04 | +0·10 | +0·69 |
| Average balance from 4th 'egg-day' periods | 288 | −0·12 | −0·20 | +0·23 |
| | 404 | −0·04 | +0·25 | +0·39 |
| Average turnover of Ca in an 'egg-day' period | 288 | 6·75 | 9·43 | 12·24 |
| | 404 | 6·53 | 10·57 | 12·72 |
| Average intake of Ca in an 'egg-day' period | 288 | 3·26 | 4·80 | 6·29 |
| | 404 | 3·22 | 5·63 | 6·67 |
| Average output of Ca in droppings in an 'egg-day' period | 288 | 1·50 | 2·58 | 3·98 |
| | 404 | 1·48 | 3·04 | 4·13 |
| Average output of Ca in shell in an 'egg-day' period | 288 | 1·95 | 2·06 | 1·97 |
| | 404 | 1·83 | 1·91 | 1·93 |

dietary levels of Ca failed to increase the amount of Ca in the egg-shell. (4) A reasonable prediction of the balance could be obtained from the analysis of the 4th 'egg-day'. (5) Six birds are a minimum for such a study and a 4-week period is necessary for a reliable answer.

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**Plasma insulin and growth hormone measurements in dogs fed diets of different protein value.** By C. R. C. HEARD and PAMELA A. J. HENRY, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC*1, and M. HARTOG and A. D. WRIGHT, *Department of Medicine, Royal Postgraduate Medical School, London W*12

It has been suggested that severe protein-calorie deficiency leads to insulin deficiency accompanied by hypopituitarism (Heard, 1966) but that marginal deficiency leads to increased levels of circulating antagonists to insulin (e.g. growth hormone) with a consequent increase in insulin production (Heard & Turner, 1967). Until recently, the only direct evidence in support of this hypothesis was increased levels of plasma insulin-like activity (epididymal fat pad method) in marginally protein-calorie deficient dogs.

Radioimmunoassay techniques have now been used to measure plasma insulin and growth hormone levels in dogs weaned on to diets of three different protein values (NDpCal% = 10, 7 and 5). The kit supplied by the Radiochemical Centre, Amersham, was used for the insulin assays (Henry & Heard, 1967). The growth hormone assay utilized $^{131}$I-labelled dog growth hormone and antiserum prepared to pig growth hormone (Hartog, Wright, Fraser & Heard, 1967).

After an 18 h fast, plasma insulin levels were measured before and for 30 min after intravenous glucose (0·4 g/kg) and plasma growth hormone levels were measured before and for 150 min after i.v. insulin (0·4 i.u./kg). At the end of these

times, the levels of plasma insulin and growth hormone had returned to fasting values. In young growing dogs, fed the diet of NDpCal% = 10, fasting plasma insulin levels were about 10 $\mu$-units/ml. Litter-mates, in which growth was restricted by feeding diets of low protein value, had higher fasting plasma insulin levels (30 $\mu$-units/ml) and an exaggerated response to i.v. glucose. The same phenomenon also occurred in dogs fed the high-protein diet, at 6 months of age, when the growth rate had fallen. Fasting plasma insulin levels and response to i.v. glucose were subnormal in pups on a voluntary low caloric intake. Insulin production did not correlate with glucose tolerance nor with the degree of hyperglycaemia.

Fasting growth hormone levels were below the limits of sensitivity of the method (10 ng/ml) in most dogs fed diets of NDpCal = 10 or 7% but were elevated in most animals fed the diet of NDpCal = 5%. In the latter group, i.v. insulin led to increased growth hormone levels but not in litter-mates fed the diets of higher protein values. The degree or duration of hypoglycaemia could not explain the differences between the two groups.

The contribution of altered insulin and growth hormone production to the diabetogenic effects and other manifestations of protein-calorie deficiency, and the reversibility of these changes in endocrine function, are still under investigation.

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**Liver folate of infants dying in the perinatal period.** By M. A. HUSSAIN and G. R. WADSWORTH (introduced by B. S. PLATT), *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1*

There seem to be no published accounts in the English language of the amount of folate in the liver of the newborn child. In view of the importance of this vitamin for the multiplication of cells, hence of growth, there is a need for information about the folate status of the human foetus and infant. We have been making determinations of hepatic folate in the course of a study of possible nutritional factors in the causation of perinatal mortality. Some of the results are presented in this communication.

Samples of liver are obtained during post-mortem examinations, and folate is determined by the method described by Chanarin, Hutchinson, McLean & Moule (1966).

There is marked individual variation both in total amount and in concentration of liver folate. Gestational age and body-weight influence the amount of folate as shown in the table.

*Mean quantities of liver folate in seventy-nine infants dying in the perinatal period in England*

| Birth weight within range (kg) | No. | Total (μg) | Standard deviation (μg) | Concentration (μg/g) | Standard deviation (μg/g) |
|---|---|---|---|---|---|
| Gestational age 37 to 44 weeks | | | | | |
| 3·0–3·5 | 15 | 746 | 395 | 4·8 | 1·7 |
| 2·5–3·0 | 7 | 530 | 239 | 4·6 | 1·7 |
| 2·0–2·5 | 11 | 311 | 115 | 3·4 | 0·9 |
| 1·0–2·0 | 2 | 199 | 31 | 3·4 | — |
| Gestational age 28 to 36 weeks | | | | | |
| 2·0–2·5 | 11 | 422* | 264 | 4·1 | 1·3 |
| 1·0–2·0 | 26 | 231† | 146 | 3·5 | 1·8 |
| Less than 1·0 | 7 | 126 | 63 | 3·5 | 1·7 |

*Nine only. †Twenty-two only.

Reduction in the total amount of hepatic folate with decreasing birth weight can be accounted for mainly by a corresponding reduction in the size of the liver.

The present values have been found in dead infants and may not, therefore, be representative of those in healthy surviving infants.

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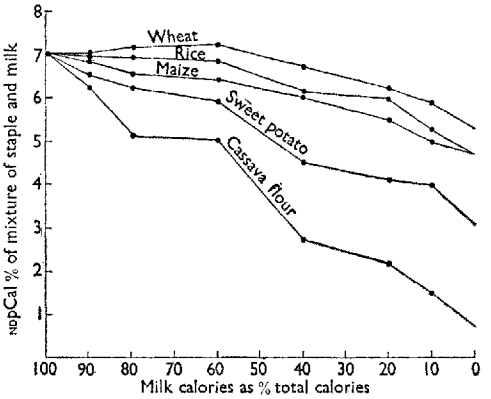
Chanarin, I., Hutchinson, M., McLean, A. & Moule, M. (1966). *Br. med. J.* i, 396.

**The effect of the addition of foods to human milk on the protein value of the infant's diet.** By MARGARET E. CAMERON, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London, WC1*

The nutritional value of diets in terms of protein can be expressed as the proportion of the total calories which are contributed from utilizable protein (Platt, Miller & Payne, 1961). Values so calculated are the net dietary protein calories per cent (NDpCal%). Human milk was found to have a protein value of NDpCal 8·7% (Platt & Miller, 1961).

According to earlier descriptions kwashiorkor occurred characteristically in children from 1 to 4 years old. However, many cases of this form of protein-calorie deficiency disease are now being described in infants under 1 year of age. This can be accounted for by the early introduction of foods of poor protein quality with consequent proportionate, and probably absolute, reduction in the amount of milk taken by the baby. In order to define the limits to which some foods can be introduced with safety into the infant's diet calculations were made of the NDpCal% of isocaloric mixtures of varying proportions of human milk and one other food. The results are shown in the figure.

Using the amino acid composition given by Macy, Kelly & Sloan (1953) human milk was calculated to have an NDpCal% of 7. Additions of appreciable amounts of wheat or rice to the infant's diet seem permissible. But mixtures of milk and even small amounts of cassava would lead to an inadequate intake of protein. Quite




Calculated protein values of varying proportions of human milk and another food in isocaloric mixtures

small amounts of sweet potato or, to a less extent, maize would also reduce the dietary protein level to an undesirable degree.

In circumstances where the infant is liable to be weaned on to an inadequate diet prolongation of the period of breast feeding is to be advocated. However, continuation of breast feeding without attention at the same time to the kinds of food added as supplements may lead to protein deficiency. Indeed protein deficiency may also occur in the first few months of life when some foods are given in addition to human milk, even when the amount of milk by itself would have been adequate.

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**Retention of radioactive iron by the mouse after ingestion of labelled wheat and barley.** By K. K. NARULA and G. R. WADSWORTH, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1*

The view is widely held that the extent to which iron is absorbed from the diet varies with different foods. Poor availability of Fe could explain why some diets lead to Fe deficiency even when the total amount of the element present, as measured by chemical analysis, appears to be adequate. Availability is now usually investigated by determining the uptake of radioactivity from foods which have been labelled during their production. The materials which have been studied in this way in human subjects are egg, vegetables, meat, haemoglobin and legumes (Moore & Dubach, 1951; Walsh, Kaldor, Brading & George, 1955; Callender, Mallett & Smith, 1957; Chodos, Ross, Apt, Pollycove & Halkett, 1957; Zeind, Barrada, El Bayssary & Schulert, 1966). Until the recent work of Hussain, Walker, Layrisse,

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Clark & Finch (1965) and Cowan (reported by Zeind *et al.* 1966) there was no information based on the use of modern techniques about the availability to man of Fe in foods which form the greater part of the diets of communities in which Fe deficiency is common.

We (Hegarty & Wadsworth, 1965) have been investigating whether accumulation of Fe, determined by chemical analysis, in the mouse carcass might be used as a biological test for the nutritive value of Fe in foods. The inquiry has been extended to include measurements of retention of radioactivity from barley and wheat labelled with $^{59}Fe$. The results obtained so far are given in the table; retention is expressed as the percentage of the radioactivity ingested which remains in the bodies of the mice after an interval of 10 days. Radiation is measured in a total body counter.

*Absorption by mice of radioactivity from barley and wheat labelled with radioactive iron*

| | | Retention as a percentage of intake | |
|---|---|---|---|
| Food | No. of mice | Mean for whole group | Range of means for separate groups of five animals |
| Barley | 20 | 14·1 | 12·0–18·3 |
| Wheat | 20 | 12·9 | 8·5–14·5 |

The retention of radioactive Fe by the mice was about three times as much as that retained by human subjects investigated by Hussain *et al.* (1965) and Cowan (1966) using labelled wheat. The present results are about the same as those found by Zeind *et al.* (1966) for absorption of radioactive Fe from beans by rats. Thus the mouse and rat cannot be used as test animals to provide an exact measure of availability to humans of Fe in foods, but this does not preclude their use to differentiate between comparative availability of Fe from different foods and diets.

We wish to thank the ARC unit at Wantage for growing the labelled grains, and the MRC Radiation Protection Service, Sutton, for making the radiation counts.

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