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Protein utilization and in-vitro protein synthesis in young rats given gruels of sprouted white maize supplemented with graded amounts of dried fish

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Sprouted white maize meal was supplemented with 5, 10, 15 and 20% (w/w) sun-dried pelagic fish meal. The nutritive value for young rats fed gruels of these mixtures was determined. The protein, fat, ash, and energy contents of the dried gruels increased when the amount of fish in the diet increased. Both supplemented and unsupplemented samples showed high true digestibilities (above 90%) and there was no statistical difference between them. NPU_{op} and NDp-Cal% increased with increasing amount of fish in the diet. Supplemented maize meals at the 10, 15 and 20% levels were better utilized than the unsupplemented diet ($p < 0.05$). Furthermore, a decreased in-vitro protein synthetic capacity was shown in animals fed low protein diets. There was good correlation ($r = 0.90$) between weight gain and synthetic activity. Supplementation of sprouted white maize meal with 5–10% fish protein was sufficient to optimize the protein utilization in young rats.

INTRODUCTION

Infant feeding poses a problem in many developing countries during weaning. Carbohydrate-rich foods such as maize, millet, sorghum and cassava are used in weaning diets. Although these staples are high in energy, porridges made from them may be poor energy sources due to their bulkiness and high viscosity. By germination (Mosha & Svanberg, 1983; Gopaldas *et al.*, 1986) reduced viscosity allowed for addition of less liquid in the porridges so that energy density was increased. This is an important aspect of the process and product technology.

The staples are either poor in protein and/or deficient in one or more of the essential amino acids. The limiting amino acids in most of these foods are lysine and tryptophan. The protein quality has been shown to be improved in germinated seeds (Tsai *et al.*, 1975; Dalby & Tsai, 1976; Wu & Wall, 1980) and by fermentation (Wang *et al.*, 1978; Hamad & Fields, 1979; Asiedu *et al.*, 1993). However, these improvements are not adequate. It is possible that when energy is sufficient the requirements for other nutrients could be met if a good supplement is added. The importance of ω-3 fatty acids in child health and nutrition has been demonstrated; they are essential in brain development (Weber, 1989). These fatty acids are very low in cereals but present in fish (Hilditch & Williams, 1964; Asiedu *et al.*, 1993). Fish has a good amino acid composition, and appropriate amounts of micronutrients and essential ω-3 fatty acids.

In the present work the effects of adding graded amounts of sun-dried fish (Kapenta) to sprouted white maize were studied.

MATERIALS AND METHODS

Materials

White maize (*Zea mays*) was bought in the open market in Zambia and transported to Bergen, Norway, where sample preparation and analytical procedures were undertaken.

The grains were thoroughly sorted: bad grains, stones and other debris were removed. The grains were washed three times with tap water and then twice with distilled water. They were then soaked in distilled water for 12 h and then spread in 1-cm layers between wet cotton cloth on the floor and watered with distilled

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water each morning. The seeds were allowed to germinate for 72 h at ambient temperature (25°C). The sprouted seeds were frozen and freeze-dried. After drying, they were milled using a laboratory mill and kept in air-tight containers at 4°C prior to preparation of gruels.

Sun-dried small pelagic fish, Kapenta (*Limnothrissa miodon*), from Lake Kariba in East Africa were obtained through FAO/SIDA (Swedish International Development Authority) in Zambia and air-freighted to Bergen, Norway, where analysis took place. The samples were homogenised whole and kept in air-tight containers at 4°C prior to analysis.

Methods

Preparation of gruels

Five porridges were made from the processed maize cereal (one part of water was added to three parts of flour):

1. maize sprouted (no fish)
2. maize sprouted + 5% fish
3. maize sprouted + 10% fish
4. maize sprouted + 15% fish
5. maize sprouted + 20% fish

The porridges, containing 0·5% salt (NaCl), were cooked for 15 min. After cooling, the gruels were freeze-dried and ground to a fine meal.

Proximate analyses

Moisture and ash were determined by gravimetric methods after AOAC (1990) and Mortensen and Wallin (1989), respectively. Protein (N × 6·25) was determined as described by Crooke and Simpson (1971), after digestion in a Tecator block digestor (Høganas, Sweden) at about 370°C, and ammonium was estimated using a Technicon autoanalyser. Gross energy was determined using a Gallenkamp Autobomb (Christopher Street, London) automatic adiabatic bomb calorimeter. Fat was determined using ethyl acetate as the extraction medium, as described by Losnegard *et al.* (1979).

Biological methods

Nitrogen balance experiments were carried out using young male Wistar–Møll rats from Møllegård, Denmark, weighing between 60 and 65 g. Six rats were used in each group. They were kept in metabolic cages in a room with a temperature of 20 ± 1°C and maintained on a 12-h cycle of light and dark. They were given 10 g diets daily. This was eaten completely. Water was available *ad libitum*. There were five days acclimatization, a preliminary period and a five-day experimental period. The rats were weighed at the beginning of the experiment and at the end of the preliminary and balance periods. Access to feed and water was closed 3 h before weighing.

Table 1. Proximate composition of white maize and of the supplemented samples (per 100 g, dry matter basis)

Diet	Dry matter (g)	Protein (g)	Fat (g)	Ash (g)	Energy (kJ)
Maize alone	30·9	9·7	3·2	1·4	1811
Fish alone	90·2	73·1	6·6	11·5	1920
Maize + 5% fish	29·4	12·6	3·5	2·1	1841
Maize + 10% fish	27·3	15·7	3·7	2·6	1853
Maize + 15% fish	26·0	18·2	3·9	3·2	1867
Maize + 20% fish	29·6	21·8	4·1	3·8	1870

Endogenous faecal nitrogen was calculated as 2·02 mg faecal nitrogen per gram feed and endogenous urinary nitrogen was calculated as $W^{0\cdot75} \times K$, where W is the average body weight (g) of the animal during the five-day experiment period and K is 0·645, after Njaa (1963). Faeces and urine were collected and nitrogen was determined in them as well as in the diets. AD, TD, NPU, $\mathrm{NPU_{op}}$ (see note 1), bal% and NDpCal% were calculated (see note 2).

In vitro protein synthesis, using polyribosomes isolated from the gastrocnemius muscle of the rats, was carried out as described by Omstedt and von der Decken (1972). DNA was analysed in homogenates of muscle tissues fluorimetrically at 420 nm (excitation) and 520 nm (emission) (Setaro & Morely, 1976) using salmon DNA as a standard. RNA was extracted from the ribosomes in 0·4 M $HClO_4$ for 18 min at 70°C and the absorbance was read at 260 nm. RNA was calculated on the basis of 34·2 optical density units at 260 nm per mg of RNA (Fleck & Munro, 1962).

To ensure that the sun-dried pelagic fish used to supplement the diets was of good protein quality, it was tested in a balance experiment and compared with a fish meal dried at low temperature (LT-94). Each rat received 10 g feed containing 8% protein from the fish samples daily. The basal diet contained 50 g of fat, 80 g of protein 200 g of sugar, 10 g of cellulose, 10 g of vitamins, 40 g of minerals, made up to 1000 g with partially dextrinized potato starch. The fat contents of the fish meals were taken into account so that the fat content for each group was 5%. Soya bean oil was used to level the fat contents of the diets.

Table 2. Biological utilization of fish protein[a]

Groups	BV	TD	NPU	AD	Bal%
Kapenta	96·0 ± 1·2	98·4 ± 0·7	95·3 ± 0·9	82·6 ± 0·7[b]	66·4 ± 0·9[b]
Herring meal (LT-94)	98·1 ± 1·4	101·9 ± 0·5	99·5 ± 1·3	85·3 ± 0·4	70·8 ± 1·3

[a] $n = 6$, mean ± SEM.

[b] $p < 0\cdot05$ compared with herring meal.

Table 3. Biological utilization of germinated maize protein supplemented with different levels of fish[a]

Diet	AD	TD	Bal%	NPU_{op}	NDpCal%	Weight gain(g)/5 days
Maize alone	83·5 ± 1·2	96·6 ± 3·0	33·7 ± 5·1	59·3 ± 5·4	5·3 ± 0·5	6·6 ± 1·57
Maize + 5% fish	85·5 ± 2·2	95·7 ± 5·5	42·6 ± 4·6	61·1 ± 4·5	7·0 ± 0·5	17·7 ± 0·90
Maize +10% fish	86·3 ± 0·9	94·3 ± 2·5	52·3 ± 3·7[b]	67·6 ± 3·7	9·6 ± 0·5	20·7 ± 1·67
Maize + 15% fish	85·9 ± 1·2	92·9 ± 3·0	52·8 ± 4·0[b]	66·5 ± 4·1	10·9 ± 0·6	24·0 ± 1·60
Maize + 20% fish	84·4 ± 2·4	90·2 ± 5·9	59·1 ± 3·9[b]	70·5 ± 3·8	13·8 ± 0·7	24·6 ± 1·10

[a] $n = 6$, mean ± SEM.
[b] $p < 0{\cdot}05$ compared with maize alone.

Statistical analysis

Data are presented as means ± SEM and were statistically evaluated by ANOVA and the Student's *t*-test.

RESULTS AND DISCUSSION

Proximate analyses of the five experimental diets and of the fish used are given in Table 1. All parameters increased with increasing amount of fish in the diet.

The results of the balance experiments with the fish protein source and of the supplemented diets are given in Tables 2 and 3, respectively. The statistical comparisons were based on AD and Bal% as the other items contain estimated quantities besides the measured quantities. Thus, AD is taken to show the variability in TD and Bal% is taken to show the variability in NPU.

The protein quality of the Kapenta used in the supplementation experiment showed good biological value, digestibility and NPU, with values slightly lower than for the LT-94 meal.

In developing countries grain is the main source of protein in the diet. Table 3 shows the results of the supplementation experiment. High NPU_{op} and NDpCal% were observed with increasing amounts of fish in the diet. Bal% and NPU_{op} of the dietary proteins increased regularly as the amount of fish increased ($p < 0{\cdot}05$). There was good correlation between Bal% and weight gain ($r = 0{\cdot}67$). Thus, supplementation with fish improved the protein quality of the maize gruel. There was no significant difference between maize gruel alone and the 5% fish diet. However, significant differences ($p < 0{\cdot}05$) were observed between maize gruel alone and the 10%, 15% and 20% fish diets. Between the three last diets there were no significant differences.

NPU, which takes both digestibility and nitrogen balance into consideration, is a good measure of protein requirement. According to the Protein Advisory Group of the United Nations System (1975), infant supplementary food items with NPU values below 60% are unacceptable. Ebrahim (1983) asserted that NDpCal%, which takes into account quality and quantity, of an ideal diet should provide values between 7 and 8. The results in Table 3 indicate that the maize gruel should be supplemented with between 5 and 10% fish (3·5 and 7% fish protein) to fulfil these requirements. Statistical treatment of the difference between maize alone and the 5% fish diet was not significant ($p > 0{\cdot}05$); between maize and the three other diets the differences were significant ($p < 0{\cdot}05$) and between the last three diets the differences were insignificant.

Between digestibilities there were no statistical differences between the supplemented and the unsupplemented diets. The present study shows TD-values for protein of 90–96%. These higher values were found for maize protein as well as for the supplemented diets, and contrast with the values of 85% and 76% TD reported in children and adults, respectively, by Pellett and Young (1980) and FAO/WHO/UNU (1985). Amino acid analyses (Asiedu *et al.*, 1993) show that maize is low in lysine and tryptophan, thus explaining the poor utilization of the unsupplemented diet. The amino acid contents (Table 4) were calculated based on amino acid composition of maize alone (Asiedu *et al.*, 1993) and that of the fish used (Steiner-Asiedu *et al.*, 1993). Supplementation with fish (Table 4) improves lysine and tryptophan; hence, the improvement in maize utilization. The suggested pattern of amino acid for infants (FAO/WHO/UNU, 1985) is attained for lysine with 10% addition of fish. For tryptophan, however, the FAO pattern is not reached even with 20% addition of fish (Table 4). It is only breast milk which can meet these requirements. Therefore it is very important to continue breastfeeding when supplementary foods are introduced.

Great gains in rat body weight and protein efficiency when fish flour was added to milled wheat, corn and rice were reported (Sure, 1957*a*). In these experiments the diets were given *ad libitum*. It was also found that the growth rate increased with increasing level of fish added to whole yellow corn, whole wheat, milled rye, grain sorghum and yellow corn. Fish protein added up to 3% in the basal diet led to a significant improvement in PER (Bressani, 1961). This may be a consequence of increasing the protein content in the diet at the same time as diets were given *ad libitum*. Other means of supplementing maize include the addition of the

Table 4. Calculated lysine and tryptophan contents in the diets

Diet	Lysine (mg/g protein)	Tryptophan (mg/g protein)
Maize alone	27·1	7·7
5% fish	45·6	9·0
10% fish	56·4	9·6
15% fish	65·8	10·4
20% fish	84·2	12·5
FAO/WHO/UNU (1985)	66 (53–76)	17 (16–17)

Table 5. Protein synthesis and ribosomal RNA and DNA in gastrocnemius muscle of rat fed with maize supplemented with fish[a]

Diet	Protein synthesis (pmol 1-^{14}C g wet wt/min)	RNA (mg/g wet wt)	DNA (mg/g wet wt)(g)	RNA/DNA	Weight gain/10 days (g)
Maize alone	6·8 ± 1·0	0·59 ± 0·04	0·60 ± 0·04	0·98 ± 0·07	10·2 ± 0·57
Maize +5% fish	8·6 ± 1·2	0·68 ± 0·02	0·59 ± 0·05	1·15 ± 0·05	25·0 ± 0·97
Maize +10% fish	8·9 ± 1·3	0·56 ± 0·03	0·62 ± 0·06	0·94 ± 0·10	33·0 ± 0·69
Maize +15% fish	10·3 ± 0·9[b]	0·70 ± 0·05[b]	0·64 ± 0·36	1·12 ± 0·11	39·1 ± 1·34
Maize +20% fish	11·1 ± 1·1[b]	0·82 ± 0·09[b]	0·62 ± 0·04	1·33 ± 0·11	41·9 ± 1·26

[a] $n = 6$, means ± SEM.
[b] $p < 0·05$ compared with maize alone diet.

limiting amino acids, lysine and tryptophan, and this showed significant improvement in weight gain (Elias & Bressani, 1972). The defatted fish flour added as 1, 3 and 5% in rations (Sure, 1957*b*) proved to be a much more efficient protein supplement to the proteins in the cereal grains than dried brewers' yeast, soybean flour or peanut meal (Sure, 1948).

In Table 5 the weight gain data show, even more clearly than the Bal% data, the improvement of the gruel by fish protein supplementation at all levels. Parallel to the weight gains the activity of the in-vitro protein synthesis increased ($r = 0·90$); the differences between the supplemented diets were, however, not significant. In-vitro protein synthesis increased as the fish content in the diets increased (Table 5). Rats fed low protein diets had low synthetic activity compared with the well-nourished group. Similar findings were observed by Young and Alexis (1968). The DNA content increased but did not show any statistical significance between diets. The DNA content is a measure of the number of cells in the tissue, while the protein content is an index of cell size. This suggested that the number of cells in the muscles were the same for all groups. Growth takes place in phases. First there is an increase in the number of cells, followed by an increase in the size of the individual cells. Inadequacies in protein quality will inevitably affect growth. ANOVA shows differences between treatments; the effect was due to the supplementation at the 15% and 20% fish levels (Table 5). Rats that received cereals alone had lower concentrations of RNA in the muscle. Thus, low dietary protein intake reduces the concentration of RNA in the muscle (Asheley & Fisher, 1967; Young & Alexis, 1968; von der Decken & Omstedt, 1972). Consequently, the synthetic activity of ribosomes isolated from the muscle of animals with low dietary protein (maize alone) was decreased.

Changes in chemical composition of body tissues due to low dietary protein quality and quantity have been reported (Cabak *et al.*, 1963; Garrow *et al.*, 1965). These changes were significant in muscle tissues during protein energy depletion since these tissues are the major protein mass. This is evident in both animals and humans (Waterlow & Mendes, 1957; Halliday, 1967).

Based upon the proximate composition, biological and biochemical results it may be inferred that the addition of fish (5–10%) to the white maize-based porridges has the potential to improve protein quality and thereby promote growth. Weaning diets of this type can therefore be improved by supplementation of small amounts of fish protein.

NOTES

1. The difference between NPU and NPU$_{op}$ is that the former is determined at a standard protein level, e.g. 8 or 10%, while the latter is determined at the practical protein level.
2. AD = Apparent digestibility = $(I–F)/I \times 100\%$; TD = true digestibility = $[I–(F–F')]/I \times 100\%$; Bal% = nitrogen balance = $(I–F–U)/I \times 100\%$, NPU= net protein utilization = Bal % + $100(F'/I + U'/I)$, where I = nitrogen intake, F = faecal nitrogen, U = urinary nitrogen, and U' and F' = obligatory loss of nitrogen in urine and faeces, respectively. NPU$_{op}$= Food or diet fed as it is consumed without dilution or addition. NDpCal% = Net dietary protein calories % = protein calories % (kcal) × NPU$_{op}$ (as fraction).

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