MEASUREMENT OF THE PROTEIN QUALITY OF SOYBEAN-ENRICHED FLOUR MIXTURES IN GROWING RATS

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ABSTRACT

Soybean-enriched cassava, maize and yam flours were incorporated into diets and their protein quality measured in growing rats. Cassava flour enriched with 11.1% soybean permits ideal growth when supplemented with a balanced protein (casein) and about 80% growth, compared to a control diet, when supplemented with animal protein (fish). Maize and yam flour enriched each with 25% soybean favour 68 and 60% growth, respectively.

RESUME

Des farines de manioc,maïs et igname enrichies au soja ont été incorporées dans des régimes et leur valeur proteïque mesurée chez des rats en croissance. La farine de manioc enrichie avec 11,1 % de soja permet une croissance idéale lorsqu'elle est supplementée avec une protéine équilibrée (caseine), et une croissance égale à 80 % de celle d'un régime témoin lorsqu'elle est supplémentée avec une protéïne animale (poisson). Les farines de maïs et d'igname enrichies chacune avec 25 % de soja donnent 68 et 60 % de croissance, respectivement.

INTRODUCTION

A native of Asia, the soybean *(Glycine max)* is now widely used for oil extraction. Because of its nutritive potential, and particularly its protein content, the soybean is also used in various food dishes to mitigate the shortage of protein supplies, especially in the developing countries. In China, for example, soybean milk is now a popular breakfast item, thanks to the work of Tso (1928), while CHI-YUAN CHOU (1983) has studied and developed soybean infant formulas. Likewise, in India, an attempt has been made to combine soybean (deficient in methionine) and whey, a dairy by-product rich in sulphur-containing amino acids, for the production of balanced protein milk-like products for infant and pre-school children (KAPOOR and GUPTA, 1981).

The introduction of soybean to Cameroon was started about five years ago by UCCAO under the national soybean project. Since then farmers, especially those in the Western Province, have been taught to grow it, and many soybean-based food-dishes and beverages have been formulated (CHAPELIER et al., 1984). Studies on the chemical composition and natritive value of some of these dishes as well as on their acceptability have been made (FOTSO et al., in press).

In this paper we report the results of a study undertaken to determine the protein quality in growing rats of some soybean-enriched flours highly appreciated by panelists during an acceptability test in which they were presented as «fufu», «pap» and «akara».

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MATERIALS AND METHOD

Raw Materials

The following enriched flour mixtures were used:-

Cassava-soybean (89.9:11.1) flour with 5.5% total crude protein.

Maize-soybean (75:25) flour containing 17.8% total crude protein.

Yam-soybean (75:25) flour containing 15.2% total crude protein.

The cassava and maize flours were provided by UCCAO, whereas soybean flour was prepared according to CHAPELIER et al., (1984). The yam flour was prepared at the Centre for Nutrition from the species *Dioscorea dumetorum* by sun-drying according to TRECHE et al. (1983). Smoked fish (mackerel) was obtained from the local market and ground into fine powder. It contained 77.2% crude protein.

Animal diets

The following five diets were used in this experiment:-

- a control diet (C) containing casein as source of protein
- a diet (CCS) containing casein and enriched cassava flour
- a diet (FCS) containing fish powder and enriched cassava flour
- a diet containing enriched maize flour (MS)
- a diet containing enriched yam flour (YS).

The detail composition of the diets are given in table I. The mineral and vitamin mixtures were prepared such that by adding the amounts indicated the rat requirements were met according to BERNHART et al., (1966) and PAWLAK et al., (1968), respectively. Non-nutritive cellulose was added to the other diets to bring their fibre content to that in diet MS.

Table I . Composition of diets fed growing rats (g/kg).

DIET	C	CCS	FCS	MS	YS
Enriched flour	--	731.6	731.6	670.0	789.8
Casein (90% protein)	132.6	88.4	—	-	-
Fish powder	-	--	103.6	--	--
Wheat flour	606.0	20.0	--	219.3	64.8
Soybean oil	71.6	4.4	15.6	6.9	26.3
Agar-agar	30.0	30.0	30.0	30.0	30.0
Cellulose	87.6	54.6	50.4	--	20.4
L Cystine	2.5	1.6	—	--	-
L Arginine	0.96	0.64	--	-	-
Mineral mix	48.0	48.0	48.0	48.0	48.0
Vitamin mix	20.8	20.8	20.8	20.8	20.8
Crude protein (%)	11.93	11.98	12.02	11.99	12.00
Energy (Kcal/kg)	4445	4381	4312	4420	4454

Experimental design

Weanling male rats of the Wistar strain Ico: WI(IOPS AF/Han) were fed a standard diet until they were about 70g of weight. They were then divided into five groups of 6 animals each with an average weight of 73 ± 1g. The rats were housed individually in plastic metabolic cages in a room with constant temperature of 22°C, 65° relative humidity and a 12-hour light-dark cycle. One of the groups received the control diet while the others were assigned to one of the test diets. Following a 7-day acclimatization, animals were fed the diets in semi-liquid form and given distilled water ad libitum for further ten days. Feed intake and body weights were recorded everyday and every three days, respectively. During the last five days urine and fecal collections were made daily from each animal, pooled and frozen. One millilitre of N H_2S04 was added as preservative to each container before collecting urine.

Chemical analysis

Before analysis, feed and feces were dried in a vaccum oven and ground into fine powder, and urine was centrifuged at low speed and the supernatant collected. Total nitrogen was determined on duplicate samples by Kjeldahl procedure (AOAC, 1975), using 5.71, 6.38 and 6.25 as conversion factors for soybean, casein and other proteins, respectively. Dry weight of samples was obtained by drying at 107°C to constant weight. Fibre and gross energy were determined by formic acid method (GUILLEMET et al.,) and bomb calorimetry, respectively.

Evaluation and statistical analysis

Growth (G), protein efficiency ratio (PER), apparent protein digestibility (AD), and biological value (BV) were the criteria used to evaluate protein quality and were computed by the following formulas:

$$G = \frac{\text{Weight gain}}{\text{Time of growth}}$$

$$PER = \frac{\text{Weight gain}}{\text{N intake}}$$

$$AD = \frac{(\text{N intake} - \text{fecal N}) \times 100}{\text{N intake}}$$

$$BV = \frac{(\text{N intake} - \text{fecal N} - \text{urinary N}) \times 100}{\text{N intake}}$$

where N = Nitrogen.

Mean values were tested for significant difference by the student-Fisher test at the 5% level.

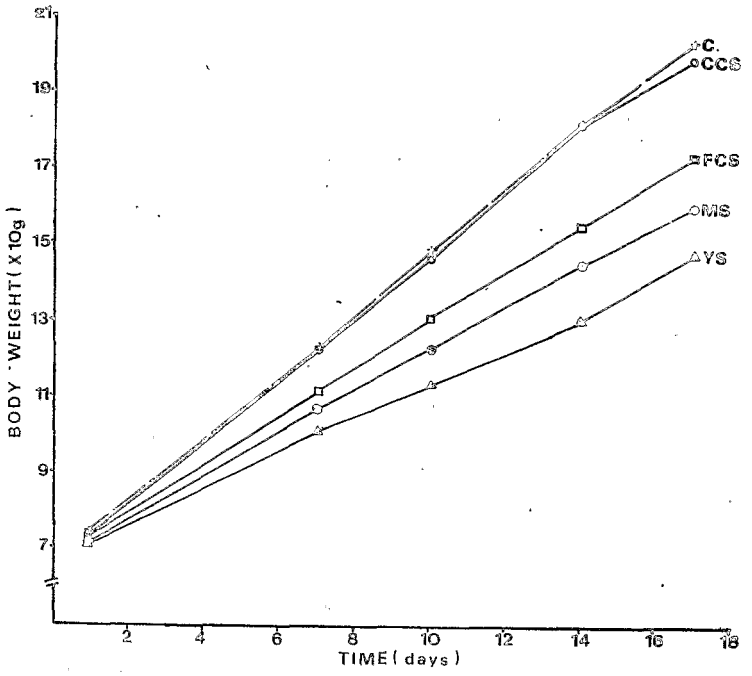

Figure1: Growth curves of rats fed diets containing soybean-enriched flour mixtures: C= control diet, CCS= Casein-Cassava-Soybean; FCS= Fish-Cassava-Soybean' FCS= Fish-Cassava-Soybean' MS= Maize-Soybean; YS= Yam-Soybean.

RESULTS AND DISCUSSION

RESULTS

Diet Intakes and Body Weights

As shown in figure 1, all five groups of animals are in the exponential growth phase. Diets C and CCS give the highest growth, followed by FCS and MS. Diet YS gives the lowest. There is no significant difference in feed intake, weight gain, protein intake and protein efficiency ratio between groups C and CCS, which have the highest values (table II). Groups FCS and MS are similar in all respects whereas group YS has the lowest values, similar to MS in all respects, and to FCS for feed and protein intakes only.

Table II : Feed intakes and body weights of growing rats fed diets containing soybean enriched flour mixtures : C= Control diet; CCS= Casein-Cassava-Soybean; FCS= Fish-Cassava-Soybean; MS= Maize soybean; YS= Yam-Soybean

DIET	C	CCS	FCS	MS	YS
Feed intake g/day	19.58 ± 1.47^{ab}	21.40 ± 2.65^{a}	17.59 ± 3.06^{bc}	16.34 ± 2.51^{c}	15.38 ± 1.37^{c}
Weight gain g/day	7.82 ± 0.91^{ab}	8.26 ± 1.48^{a}	6.25 ± 1.60^{bc}	5.31 ± 0.71^{cd}	4.67 ± 0.63^{d}
Protein intake g/day	23.48 ± 1.77^{ab}	25.67 ± 3.17^{a}	21.10 ± 3.56^{bc}	19.61 ± 2.94^{c}	18.45 ± 1.64^{c}
P E R	3.33 ± 0.18^{a}	3.22 ± 0.18^{a}	2.96 ± 0.34^{b}	2.71 ± 0.13^{bc}	2.53 ± 0.15^{c}

Mean ± SEM. Means with different letters on the same line are significantly different at the 5% level.

Nitrogen Balance

Results on nitrogen balance in rats are given in table III. It can be seen that apparent protein digestibility is similar for diets CCS, FCS, and MS while diets C and YS show significantly higher and lower values, respectively. The biological value is similar for diets C and CCS on the one hand, and for FCS and MS on the other hand. Diet YS has the lowest value.

Table III : Nitrogen balance in growing rats fed diets containing soybean-enriched flour mixtures: C= Control diet; CCS= Casein-Cassava-Soybeans; FCS= Fish-Cassava-Soybean; MS= Maize-Soybean; YS= Yam-Soybean

DIET		C	CCS	FCS	MS	YS
Total N	mg	2031 ± 189^{a}	2145 ± 306^{a}	1796 ± 388^{ab}	1689 ± 262^{b}	1604 ± 142^{b}
Fecal N	mg	235 ± 26^{a}	366 ± 68^{b}	300 ± 67^{abc}	285 ± 58^{ab}	404 ± 37^{c}
Urine N	mg	522 ± 114^{a}	566 ± 121^{a}	585 ± 175^{a}	554 ± 110^{a}	544 ± 51^{a}
App. digest.	%	88.4 ± 0.32^{a}	83.0 ± 0.92^{b}	83.3 ± 0.48^{b}	83.2 ± 1.32^{b}	74.8 ± 1.64^{c}
Biol. value	%	71.2 ± 3.69^{a}	68.2 ± 5.69^{a}	61.2 ± 4.40^{b}	60.7 ± 2.85^{b}	54.6 ± 1.05^{c}

Mean ± SEM. Means with different letters on the same line are significantly different at the 5% level.

DISCUSSION

As mentioned earlier the soybean-enriched flour mixtures used in this expriment were those that obtained the highest scores in an acceptability test (FOTSO et al., in press), and their levels of protein enrichment do not correspond to the biological optimum. Beside, only crude protein content was considered in formulating the diet and there was no supplementation with amino acids except of diets containing casein.

Diets MS and YS, the only ones with crude proteins exclusively from enriched flour mixtures (table I), show lowest values by all four criteria for protein quality and differ from each other with regard to apparent protein digestibility and biological value. This difference is due to higher fecal loss of nitrogen in rats fed the YS diet.

The cassava-soybean flour could not be used as the exclusive protein source as it would have given a diet with at most 4% protein, too low for growth of rats. Hence diets CCS and FCS were formulated and tested to determine the effect of supplementing this flour with a balanced protein (casein) and a relatively cheap and available animal protein (fish), respectively. It has been shown that diet FCS has lower protein digestibility, biological value and protein efficiency ratio than the control C, but shows no difference with respect to growth. The performance of diets C and CCS are similar in all respects except apparent protein digestibility, which may lead to think that the cassava-soybean protein is of high quality, considering that the two diets have the same 8% casein base and differ by their additional 4% casein and 4% cassava-soybean proteins, respectively. But it is more likely that the good performance of diet CCS is largely due to its 8% casein as this is close to the 9.09% casein level recommended for control diets in rat studies involving animal proteins (DERSE 1962). However, in our case we used 12% protein level because the test proteins were of plant origin.

CONCLUSION

Cassava flour enriched with 11.1% soybean permits ideal growth when supplemented with a balanced protein such as casein, and 80% growth, compared to a control diet, when supplemented with animal protein such as fish. Maize and yam flours supplemented with 25% soybean give 68 and 60% growth, respectively. The latter is unsatisfactory, indicating that the yam soybean mixture requires another complementation.

The above results obtained on rats are only of qualitative significance to man and should therefore be interpreted with precaution. The reasons being that the amino acid requirements are quantitatively different for rat and man, and the former consumes a diet of constant composition at random while the latter's diet varies in quantity and quality over a given period. However, studies with human subjects under controlled conditions have shown that, in general, the results obtained are similar to those with rats (BRESSANI et al.,).

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