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Ameliorative effects of boiling and methionine supplementation of raw soyabeans given to weanling rats

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The effects of boiling soyabean (*Glycine wightii L.*) and methionine supplementation in ameliorating the effects on anti-nutritional factors in raw soyabean were determined using Wistar rats. Six soyabean-based diets made up of three cooking durations of the seeds (uncooked, and boiled for 15 and 30 minutes) and two supplementation levels (none and supplementation with methionine) were compared. Boiling and methionine supplementation improved the nutritional value of the soyabeans as indicated by the relatively higher weights of liver, spleen, adrenal, the whole brain and brain parts of the rats. Boiling and methionine supplementation also eliminated hyperthyroidism. The relative thyroid weights of the rats on heated and/or supplemented soyabeans were 47–63 per cent of those of the rats on raw un-supplemented soyabeans. Boiling for 15 min. was sufficient to inactivate the trypsin inhibitors present in unheated soyabean. The nutritional improvement of the heated soyabeans by methionine was greater than that of the unheated soyabeans similarly supplemented.

Key words: Soyabean, boiling, methionine, organs, brain, rat.

Raw soyabean contains biologically active substances which have several anti-nutritional effects when consumed by animals. These anti-nutritional factors include protease or trypsin inhibitors, haemagglutinin, saponin, tannin and phytic acid (Rachis, 1974). Most of the deleterious effects of raw soyabean are, however, due to the trypsin inhibitors (Collins and Beaty, 1980). The trypsin inhibitors retard the liberation of free methionine, thereby preventing the use of methionine for effective protein synthesis, cause hypertrophy of the pancreas and thyroid, and have adverse effects on weight gain of rats (Collins and Beaty, 1980; Liener and Kakade, 1980).

The trypsin inhibiting activities of raw soyabean are easily destroyed by autoclaving or moist heating, resulting in enhanced nutritive value of the diet (Naim *et al.*, 1982; Burn, 1987). The addition of amino acids, especially methionine and cystine, to unheated soyabean also improves protein utilization to essentially the same extent as proper heating (Gumbmann and Friedman, 1987). However, the amino acid supplementation of raw soyabean does not increase its nutritive value to the same level as that of heated soyabean similarly supplemented (Saxena *et al.*, 1962).

Most of the studies on the nutritive value of soyabean have concentrated on the effects of the diets on overall growth rates and on the effects on the thyroid and the pancreas (Dollet *et al.*, 1985; Gumbamann and Friedman, 1987). However, as indicated by Booth *et al.* (1960), improvement in the overall growth rates of rats on soyabean meals does not always indicate the effects of the meals on individual body organs of the animal. This information is required for a better understanding of the use of soyabean in animal nutrition.

This study was designed to determine the effects of raw soyabeans and the roles of heating and methionine supplementation on

the relative weights of body organs, whole brain and brain parts of rats.

Materials and Methods

Feeding experiment

The experiment was carried out with weanling rats of the Wistar strain obtained from the Department of Zoology, University of Ibadan, Nigeria. Thirty rats were used. They were individually housed in cages.

The rats were assigned to six dietary treatment groups of five rats each such that the mean group weights were identical. The six dietary treatments are as shown on Table 1.

The raw soyabean seeds were divided into three sets. Two sets, with adequate amounts of water to totally immerse the seeds, were boiled for 15 min. and 30 min. respectively. The seeds were sun-dried and milled. The third set was sun-dried and milled. The other feed ingredients were added and thoroughly mixed depending on the dietary treatment (Table 1).

Inclusion of soyabean gave diets containing 16 per cent protein. Water and feed were provided *ad libitum*. The experiment lasted for 28 days.

Dissection of the rats

At the end of the experiment, the rats were killed with chloroform. After decapitating the m, the thyroid, spleen, adrenal and liver were excised, trimmed free of connective tissues, and weighed fresh. After weighing the whole brains, the cerebral cortex, cerebellum, amygdala, pons varolii, hypothalamus and medulla oblongata of each of the brains were carefully excised and the individual parts weighed. These weights were converted into relative weights by dividing with the final weights of the rats from which they were taken.

Table 1: Gross composition of the diets

Ingredient	Diets* 1	2	3	4	5	6
Corn starch	25,0	24,7	25,0	24,7	25,0	24,7
Soyabean (Raw)	45,0	45,0	–	–	–	–
Soyabean (Cooked for 15 mins)	45,0	45,0	–	–	–	–
Soyabean (Cooked for 30 mins)	–	–	–	–	45,0	45,0
Methionine	–	0,3	–	0,3	–	0,3
Sucrose	10,0	10,0	10,0	10,0	10,0	10,0
Non-nutritive cellulose	5,0	5,0	5,0	5,0	5,0	5,0
Groundnut oil	10,0	10,0	10,0	10,0	10,0	10,0
Mineral supplement	1,5	1,5	1,5	1,5	1,5	1,5
Vitamin mixture	1,0	1,0	1,0	1,0	1,0	1,0
Bone meal	2,0	2,0	2,0	2,0	2,0	2,0
Oyster shell	0,5	0,5	0,5	0,5	0,5	0,5
Total	100,0	100,0	100,0	100,0	100,0	100,0

* Diets and treatments
Diet 1: Raw soyabean un-supplemented;
Diet 2: Raw soyabean supplemented with methionine;
Diet 3: Soyabean cooked 15 min un-supplemented;
Diet 4: Soyabean cooked 15 min supplemented with methionine;
Diet 5: Soyabean cooked 30 min un-supplemented;
Diet 6: Soyabean cooked 30 min supplemented with methionine.

Data analysis

Data were subjected to an analysis of variance (ANOVA) (SAS, 1986). The mean weights of the organs, whole brains and brain parts were compared by Duncan's multiple range test at 5 per cent level of significance.

Results

The relative weights of organs, whole brain and the brain parts of the rats given the different diets are presented in Table 2. There were no significant differences between the treatments in weights of the liver and the spleen. The heaviest adrenals were, however, recorded for rats on diets 4 and 5 and the lightest for rats on diets 1 and 6. There was a significant dietary effect on the weights of the thyroids. A significantly heavier thyroid was obtained from rats on diet 1 (raw and un-supplemented) than from rats on the other diets. There was a progressive decrease in the weights of the thyroid from diet 2 through diet 5, but the differences were not significant. Rats on diet 6 (cooked 30 min. and supplemented) had the lightest thyroid which was significantly less than the thyroid of rats on the other diets respectively.

There were significant differences between diets in the weights of the whole brain and the brain parts, except for amygdala and pons varolii where there were no significant differences between dietary treatments due to their high coefficients of variation (42 and 58 per cent respectively). Rats on methionine supplemented diets (diets 2, 4 and 6) had significantly heavier whole brains than rats on un-supplemented diets (diets 1, 3 and 5). The heaviest whole brain was obtained in rats on diet 6, but this was not significantly heavier than the whole brain of rats on diets 2, 4 and 5 respectively.

There were progressive increases in the weights of the different brain parts from all diets. In all cases the greatest weights were obtained in rats on diets 4, 5 and 6, and the lowest weights of brain parts, in most cases, were obtained on rats on diet 1.

Discussion

The highest liver, spleen and adrenal weights obtained with rats on diet 4 (cooked 15 min. and supplemented) are indications of the improvement in the nutritive value of the diet when soyabean is heated for 15 min.

Table 2. Dietary effects of soyabean diets on the weights of organs, whole brain and brain parts of rats after 28 days.

Organ	Diets* 1	2	3	4	5	6
			(g 100g^{-1} BW^2)			
Liver	3,3^{a}	3,1^{a}	3,3^{a}	3,5^{a}	2,8^{a}	3,2^{a}
Brain	1,5^{b}	2,0^{ab}	1,6^{b}	2,3^{ab}	2,2^{ab}	2,2^{a}
			(mg 100g^{-1} BW^2)			
Spleen	174,7^{a}	137,8^{a}	172,5^{a}	222,1^{a}	192,0^{a}	197,8^{a}
Adreanal	19,3^{c}	25,6^{ab}	24,2b^{c}	33,2^{a}	30,0^{ab}	20,2^{c}
Thyroid	40,2^{a}	25,2^{b}	24,7^{b}	20,6^{b}	20,4^{b}	19,0^{c}
Cerebral cortex	99,6^{bc}	105,8^{bc}	87,4^{c}	177,8^{a}	170,0^{ab}	167,1^{ab}
Cerebellum	47,6^{c}	73,8^{bc}	75,1^{bc}	100,2^{ab}	112,3^{ab}	124,8^{a}
Amygdala	109,1^{b}	126,5^{ab}	106,1^{b}	162,2^{ab}	190,9^{ab}	204,0^{a}
Hypothalamus	47,1^{a}	47,4^{a}	60,4^{a}	103,2^{a}	106,5^{a}	112,0^{a}
Pons varolii	81,1^{bc}	58,3^{c}	75,3^{bc}	137,7^{ab}	133,0^{ab}	155,4^{a}
Medulla oblongata	60,8^{ab}	53,9^{ab}	45,8^{b}	91,1^{ab}	96,0^{a}	100,5^{a}

^{abc}Values in a row not followed by common superscript letter are significantly different by Duncan's multiple range test, $P<0.05$.

* Diets and treatments: Same as in Table 1.

^{2} = Body weight.

This is similar to the results of Njike *et al.* (1975) and Hancock *et al.* (1990) who reported improved nutritive value of soyabean meal and better performance of chicks and rats when the diet was heated for 15 min. compared to 20 min. or the raw diet. However, Collins and Beaty (1980) reported that heating over shorter durations of 3–9 min. seemed to be a good compromise in terms of anti-nutritional factor inactivation and protein damage.

The nutritive value of soyabean is adversely affected by over-heating as the amino acids are liable to destruction and inactivation by heat (Sibald, 1980). Methionine supplementation is known to alleviate the adverse effects of over-heating on the amino acids in the soyabean meal (Gumbmann and Friedman, 1987). The significant reductions in the weights of the thyroid from all diets indicate the elimination of hyperthyroidism or soyabean goitre by boiling and methionine supplementation.

The higher weights of whole brains and brain parts of rats on soyabean diets cooked for 15 or 30 min and supplemented with methionine than on the raw diets similarly supplemented corroborate the results of most workers that supplementation of raw soyabean with methionine or an assortment of amino acids does not improve the nutritive value of raw soyabean to the same level as properly heated soyabean meal similarly supplemented (Saxena *et al.* 1962; see Gumbmann and Friedman, 1987).

Boiling and methionine supplementation of soyabean diets increased their capacity for supporting growth and development of body organs and encouraged rapid growth of the brains of the rats. These effects result from the improvement of the diets and synthesis of new protein and organ constituents in the animals. This has been demonstrated with the liver (Collins and Beaty, 1980) and the pancreas (Gumbmann and Friedman, 1987). Cooking soyabean for 15 min. seemed to be sufficient to inactivate or denature heat-labile anti-nutritional factors in the raw seeds, and hence, improve the utilization of the diets. More research is, however, needed to evaluate shorter heating periods and to provide more evidence that would suggest a specified practice, taking into consideration the cost of methionine and of heating. Pathological and histological examinations of the organs would be necessary to identify specific metabolic disorders.

REFERENCES

BOOTH, A.M., ROBBINS, D. J., RIBELIN, W. E. AND DEEDS, F. 1960 Effects of raw soyabean meal and amino acids on pancreatic hypertrophy in rats. *Proceedings of the Society of Experimental Biology and Medicine* **104**:681–83.

BURNS, R. A. 1987 Protease inhibitors in processed plant foods. *Journal of Food Proteins* **50**:161–166.

COLLINS, J. L. AND BEATY, B. F. 1980 Heat inactivation of trypsin inhibitor in fresh green soyabeans and physiological responses of rats fed the beans. *Journal of Food Science* **45**:542–546.

DOLLET, J. M., DECK, B., VAILLAUME, C., MAX, J. P. AND DEBRY, G. 1985 Progressive adaptation of the endocrine pancreas during long-term protein deficiency in rats: Effects on blood glucose homeostasis and on pancreatic insulin, glucagon and somatostatin concentration. *Journal of Nutrition* **115**:1581–1588.

GUMBMANN, M. R. AND FRIEDMAN, M. 1987 Effect of sulfur amino acid supplementation of raw soy flour on the growth and pancreatic weights of rats. *Journal of Nutrition* **117**:1018–1023.

HANCOCK, J. D., POE, E. R. JR., LEWIS, A. J., CHIBA, L. I. AND CRESHAW, J. D. 1990 Effects of alcohol extraction and heat treatment on the utilization of soyabean protein by growing rats and pigs. *Journal of the Science of Food and Agriculture* **52**:193–205.

LIENER, I. E. AND KAKADE, M. 1980 Protease inhibitors. In I. E. Liener (ed.), *Toxic Constituents of Plant Foodstuffs* 2nd Edition., 7–57, Academic Press, New York.

NAIM, M., GERTLER, A. AND BIRK, Y. 1982 The effect of dietary raw and autoclaved soyabean protein fractions on growth, pancreatic enlargement and pancreatic enzymes in rats. *British Journal of Nutrition* **47**:281–288.

NJIKE, M. C., MBA, A. U. AND OYENUGA, V. A. 1975 Effect of soyabean protein levels on nutritive value of soyabean meal fed to growing chicks. *East African Agricultural and Forestry Journal* **40**:366–371.

RACHIS, J. J. (1974) Biological and Physiological factors in soyabeans. *Journal of the American Oil Chemical Society* **51**:161A–174A.

RACHIS, J. J. AND GUMBMAN, M. R. 1981 Protease inhibitors: physiological properties and nutritional significance. In: R. L. Ory (ed.), *Antinutrients and Natural Toxicants in Foods*, 302–307, Food Nutrition Press Inc., Westport, CT.

SAXENA, H. C., JANSEN, B. S. AND MCGINNIS, J. 1992 Failure of amino acid supplementation to completely overcome the growth depression effect of raw soyabean meal in chicks. *Journal of Nutrition* **72**:259–263.

SAS (1986). *SAS for Linear Models*. SAS Institute Inc. USA.

SIBALD, I. R. 1980 The effect of heat on the clearance time, true metabolizable energy and true available amino acids of raw soyabean flakes. *Poultry Science* **59**:2358–2360.