

## Effect of Raw and Cooked Soyabean Based Diet Supplemented with or without Methionine on the Growth and Histomorphometric Characteristics of Rat Testes

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**ABSTRACT:** Heat treatment of soya bean for improved absorption is an already established process, but information on the optimal duration of cooking and the effects on sexual development are still limited. This study investigated the duration of cooking and methionine supplementation on testicular parameters and growth rate of rats. Raw soya bean significantly ( $P<0.05$ ) depressed growth rate, testicular weights and seminiferous and spermatogonia diameter. Heat treatment for 30 minutes produced higher growth rate and gonadal characteristics than cooking for 15 minutes. The inclusion of 0.3% methionine further enhanced ( $P<0.05$ ) growth rate and testicular development.

**Key words:** Testes, Soya bean, Rat, Methionine and Histomorphometry

### Introduction

Inspite of high protein content and high nutritional value of raw soya bean, their utilization has been severely hindered by the presence of toxic anti-nutritional constituents (Ham and Sandstedt, 1944). The anti nutritional effect of phytate is not restricted to reduced utilization of major minerals and trace elements from feedstuffs. Protein and amino acids also form complex bonds with these anti nutritional factors and becomes difficult to absorb. In the highly acidic stomach region, amino acids, in particular lysine, methionine, arginine and histidine are bonded directly to phytates creating practically insoluble phytate-protein complexes (Buttin, 2000). Although heat treatment improves the nutritional quality of these beans, the problem of overcooking has been known to produce nutritionally and functionally damaged proteins (Amadi and Hewitt, 1975). Studies with raw or improperly cooked soya beans have shown among other toxic effects, the inhibition of protein synthesis (Jaffe and Camejo, 1961), pancreatic acinar, atrophy and hyper secretion of pancreatic enzymes (Kakade *et al.*, 1965). Supplementation of either raw or cooked soya beans with methionine, lysine and other essential amino acids has been established to improve the digestibility and utilization of their proteins in rats (Polichetti *et al.*, 1996; Ologboho and Adejumo, 1988). However, the effects of such fortification on their reproductive efficiency have not received much attention. This study therefore seeks to determine the effects of dietary raw and differently cooked soya beans with and without methionine supplementation on growth and some histomorphometric characteristics of the rat testes.

### Materials and Methods

A total of thirty-six (36) Winstar strain weanling (21 days old) albino rats were randomly distributed into six (6) groups with six animals each and in individual steel metabolic cages.

There were six Diets in all (Table 1). Diet 1 consisted of raw soya beans as the sole source of protein, while Diet 2 consisted of raw soya bean supplemented with 0.3% methionine. Diet 3 consisted of soybeans cooked for 15 minutes, while Diet 4 also consisted of soya beans cooked for 15 minutes, but with methionine supplementation. Diet 5 consisted of soya beans cooked for 30 minutes, while Diet 6 also consisted of soya beans cooked for 30 minutes but with methionine supplementation. Feed and water were given *ad libitum* for the 28 days that the experiment lasted.

Body weight and feed consumption were determined twice weekly. At the end of the experiment, the rats were sacrificed; the testes, epididymis and seminal vesicles were dissected out, freed of adhering tissues and individually weighed. The organs were thereafter processed for histological analysis and measurements. The tubular diameter ( $\mu\text{m}$ ) from each individual organ slide prepared, were measured in 20 fields per slide using a calibrated stage micrometer. The data obtained were subjected to analysis of variance procedure of SAS (1999) and treatment means compared by Duncan procedure of the same software.

### Results

Table 2 shows the summary of the results of growth performance of the experimental rats. While all the rats gained weight, the least growth rate was observed with rats on Diet 1. Significantly ( $P<0.05$ ) higher weight gain were observed in rats fed soya beans cooked for 15 minutes. The highest growth rates were recorded for rats fed Diets containing soya bean cooked for 30 minutes. Methionine supplementation significantly ( $P<0.05$ ) improved feed intake and growth rate in rats fed Diets with cooked soya beans. However, rats fed Diets containing soybeans cooked for 30 minutes showed highest feed intake with methionine supplementation having no significant effect. Methionine supplementation therefore appears to have nutritional significance only in soybean cooked for 15 minutes. In a similar trend, methionine supplementation significantly ( $P<0.05$ ) improved feed conversion efficiency in raw soya beans. Heat processed Diets irrespective of cooking duration, resulted in significantly ( $P<0.05$ ) higher feed conversion than raw soya bean based Diets. Methionine supplementation did not have any significant ( $P<0.05$ ) effect on heat treated soya bean Diets. Table 3 shows the summary of the results of paired testes weight/100g body weight, seminiferous tubule diameter and spermatogonium diameter. Paired testes weight was significantly ( $P<0.05$ ) depressed in rats fed raw soya beans and increased with methionine supplementation. Heat treated soybean Diets improved testicular weights significantly ( $P<0.05$ ) and methionine supplementation further increased testicular weights. Raw soya beans significantly ( $P<0.05$ ) depressed both seminiferous tubule diameter and spermatogonia diameter. Methionine supplementation of cooked soya bean Diets increased the tubular and spermatogonia diameters. Heat treated soya bean Diets were similar for these parameters.

**Table 1: Gross Composition of Experimental diets**

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Soybean (Raw)	45.0	45.0	0.0	0.0	0.0	0.0
Soybean (cooked 15 minutes)	0.0	0.0	45.0	45.0	0.0	0.0
Soybean (cooked 30 minutes)	0.0	0.0	0.0	0.0	45.0	45.0
Corn Starch	25.0	24.7	25.0	24.7	25.0	24.7
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	1.50	1.50	1.50	1.50	1.50	1.50
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
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
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
Calculated Crude Protein (CP%)	21.15	21.13	22.95	22.92	22.95	22.92
Gross Energy (Kcal/kg)	3321.20	3310.10	3501.20	3490.90	3501.10	3490.90

\*Vitamin Mineral Premix Supplies per kg of diet: Vit A. 13,340 i.u, Vit D<sub>3</sub> 2,680 i.u, Vit E 10 i.u; Vit K 2.68mg; Calcium Pantothenate, 10.68mg; Vit B<sub>1</sub> 20.022mg; Folic acid 0.668mg; Choline chloride, 400mg, Chlortetracycline, 26.68mg; Manganese 133.34 mg; Iron, 66.68mg; Zinc, 55.34mg, Copper 3.2mg; Iodine 1.86; Cobalt 0.268mg. Selenium 0.108mg.

**Table 2: Effect of Raw and Cooked Soybean on Feed Intake, Growth Rate, and Feed Conversion Efficiency in Rats**

PARAMETERS	EXPERIMENTAL			DIETS		
	1	2	3	4	5	6
Average Initial Live weight(g/Rat)	43.50±1.63	44.80±0.42	43.80±1.96	43.10±1.11	44.20±0.88	43.40±1.54
Final Live weight (g/Rat)	80.41±2.07 <sup>d</sup>	97.80±3.74 <sup>c</sup>	165.30±2.46 <sup>b</sup>	174.5±3.42 <sup>ab</sup>	183.60±2.89 <sup>a</sup>	186.90±4.62 <sup>a</sup>
Total Feed Intake (g/Rat 4weeks)	212.40±5.78 <sup>c</sup>	236.70±7.50 <sup>c</sup>	331.80±4.60 <sup>b</sup>	346.80±8.80 <sup>ab</sup>	366.70±9.50 <sup>a</sup>	365.80±5.30 <sup>a</sup>
Weight gain (g/rat/day)	1.28±0.04 <sup>b</sup>	1.89±0.17 <sup>b</sup>	4.34±0.15 <sup>a</sup>	4.62±0.18	4.80±0.14 <sup>a</sup>	4.99±0.12 <sup>a</sup>
Feed Conversion Efficiency (%)	37.85±1.62 <sup>c</sup>	41.32±2.43 <sup>b</sup>	49.81±0.65 <sup>a</sup>	50.32±1.11 <sup>a</sup>	50.06±0.87 <sup>a</sup>	51.09±0.19 <sup>a</sup>

Values are means ± standard error of the means. Values in the same row bearing different superscripts differ significantly (P=0.05)

**Table 3: Effect of Raw and Cooked Soybean on Testicular Weight and Histomorphometry of Rat Testes**

PARAMATER	EXPERIMENTAL		DIETS			
	1	2	3	4	5	6
Paired Testes Weight (PTW /100g/Rat weight)	0.41±0.07 <sup>d</sup>	0.75±0.07 <sup>c</sup>	0.96±0.06 <sup>b</sup>	1.18±0.07 <sup>a</sup>	0.86±0.02 <sup>b</sup>	1.12±0.16 <sup>a</sup>
Seminiferous Tubule Diameter (μm)	47.14±1.70 <sup>e</sup>	55.22±2.0 <sup>d</sup>	59.73±2.0 <sup>bc</sup>	62.08±2.80 <sup>b</sup>	57.27±2.1 <sup>cd</sup>	66.05±1.30 <sup>a</sup>
Spermatogonium Diameter (μm)	3.66±0.29 <sup>e</sup>	3.99±0.20 <sup>b</sup>	4.28±0.16 <sup>a</sup>	4.48±0.16 <sup>a</sup>	4.18±0.41 <sup>ab</sup>	4.40±0.19 <sup>a</sup>

Values are Mean ± Standard Error of means. Values in the same row bearing different superscripts differ significantly (P<0.05)

## Discussion

The results in this study indicate that heat processed soya bean was better utilized by rats than the raw beans. It also appeared that cooking for 30 minutes was more effective in increasing body and testicular weights than cooking for 15 minutes. This is in agreement with earlier reports of Udedibe and Carlini (1988); Borchers and Ackerson (1950) that cooking soya beans and similar legumes for 30 minutes gave better protein efficiency than cooking for 15 minutes. Although it is generally believed that some other factors apart from the known anti nutritional (toxic) factors may be partly responsible for the poor nutritional value of the raw soya bean. Cooking for 15 minutes is apparently enough to inactivate the several heat labile toxic constituents. Due to the marginal improvements observed in cooking soya beans for 30 minutes, over 15 minutes cooking in most of the parameters studied, it would appear that cooking for 15 minutes is adequate as other Authors (Rackis, 1974) have recommended shorter cooking time but at relatively higher temperatures, because prolonged heating reduced the availability of lysine, methionine and tryptophan (Clendenin *et al.*, 1974). Leathern (1959) observed limited testicular growth, reduced spermatogenesis and secretion of gonadotropins in rats fed low protein Diets. This present results agree with these findings especially as regards paired testes weight. It should be noted that while the least testicular weight was recorded in rats fed raw soya beans, the highest weight was recorded in rats fed cooked soya beans with methionine supplementation.

The principal drawback in the utilization of soya bean protein is its deficiency of the sulphur-containing amino acids (Kakade *et al.*, 1974; Liener, 1971). In this present study, both raw and cooked soya bean supplemented with methionine showed improvements in testicular parameters, but the improvements were more pronounced in the heat processed soya bean Diets as results obtained indicated significant ( $P<0.05$ ) increase in testicular weight and histomorphometry of rats fed heat processed soya bean with or without methionine supplementation. The assumption therefore is that methionine supplementation of raw soybean is not enough to improve its nutritional value to optimal levels unless the anti-nutritional constituents are first eliminated through heat treatment. The larger seminiferous tubule diameter and spermatogonia diameter observed in rats fed heat processed soya bean for up to 30 minutes, are not excessive and are in fact advantageous to testicular development.

Fortification of cooked soya beans with methionine, therefore, is nutritionally beneficial to the animal as it not only improves growth rate and feed conversion ratio, but also enhances fertility and reproductive capacity of the animal through enhancement of testicular development.

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