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An assesment of the oestrogenic effect of soyprotein on female rabbits

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Abstract

The oestrogen effect of soyprotein on female rabbits, fed for a period of 6 weeks, was investigated. Results indicate significant increase ($P<0.05$) in uterus weight and serum albumin of soyprotein-fed rabbits when compared with the control. There was a significant positive correlation between total soyprotein intake and uterus weight in test animals ($r=0.808$, $P<0.05$). Furthermore, serum luteinizing hormone (LH) levels were significantly reduced ($P< 0.05$) in soyprotein- fed rabbits compared to the control. As similar reduction ($P< 0.05$) in serum total cholesterol levels was observed in soyprotein-fed rabbits compared to the control. However, there were no significant differences ($P<0.05$) in the average daily food intake or body weight-gain of the test animals compared to the control. These results suggest a cholesterol-lowering capacity of soyprotein and provide strong evidence of its oestrogenic effect on female rabbits. Its consumption may therefore be a risk factor in female infertility.

Keywords: Soyprotein; Cholesterol; Luteinzing hormone; Uteius; Estrogenic effect

1. Introduction

Soyabeans, which are an important source of protein in the diet of Nigerians, (Adejuwon, Antony, & Ologhobo, 1988) and have been found to contain naturally occurring phytoestrogens which share with steroidal oestrogen, the ability to activate oestrogen receptors (Adlercreutz, 1990). The phytoestrogens found in soybeans are mainly isoflavones, including genistein [4,5,7-trihydroxyisoflavone (Adejuwon, Antony, & Ologhobo, 1988)], daidzein [4′,7-dihydroxyisoflavone (Adejuwon, Antony, & Ologhobo)] and glycitein (4′,7-dihydroxy-6-methoxyisoflavone). These isoflavones are present primarily as the glucosides, but also as malonyl- and methylglucosides, as well as the aglycones.

Studies have shown the advantageous roles that non-steroidal phytoestrogens play in human health, especially in the prevention of coronary heart diseases and cancer (Adlercreutz, 1990; Barnes, Messina, Persky, & Setchell, 1994; Dreosti, McInerney, & Record, 1995). Their deleterious effects have also been investigated as regards female infertility (Adams, 1990), since phytoestrogens may alter ovarian cycles and inhibit oestradiol action in the early stages of follicular development through the inhibition of 17β-hydroxysteroid dehydrogenase or even ovarian aromatase (Chinzi, Ienczowski and Pelissero, 1996; Kostian, Makela, & Poutanen, 1998). The specific activities of these phytoestrogens and their roles in human physiology and health are thus currently of concern to scientists (Arnold, Collins, & McLachlan, 1997; Baird, Lansdell, & Unbach, 1995) and there is a need to evaluate and establish their role in human reproduction.

This study represents a preliminary investigation into the direct effects of dietary soyprotein on the female reproductive system in the rabbit.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents used were of analytical grade.

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2.2. Animal management

Ten female rabbits weighing between 0.7 and 1.7 kg were used. The animals were divided into two groups of five. They were kept in well ventilated cages and allowed free access to food and water before and during the experiments.

The animals in Group A (control) were fed on normal rabbit pellets (devoid of soyprotein), purchased from Ladokun Feed Limited, Ibadan, Nigeria (Table 1), while Group B rabbits were fed on rabbit pellets and raw soybeans, mixed in the ratio 1:1 by weight.

2.3. Preparation of serum

Blood was obtained from the animals by ear puncture, allowed to clot and then centrifuged at 3000 ***g*** for 10 min using an MSE bench macrocentrifuge to give serum. The animals were sacrificed by cervical dislocation. The uteri were removed and weighed using a nettler electronic balance. Serum total cholesterol was determined by the method of Parekh and Jung (1990), while serum albumin was assayed by the modified method of Doumas, Watson, and Biggs (1971).

Determination of serum luteinizing hormone was done by the Enzyme-Immuno Assay (EIA) technique. Briefly, 100 μl of sample/standard and 100 μl working suspension of magnetic antibody were added to a tube, vortex-mixed and incubated at 37 °C for 30 min. The tubes were washed by adding 500 μl diluted wash buffer and then placed on a magnetic separator for 5 min. This was followed by addition of 300 μl diluted enzyme-labelled antibody, vortex mixing and re-incubation at 37 °C for 2 h. The residues were then twice re-washed and placed on a magnetic separator for 5 min. Colour development was achieved by adding 500 μl substrate solution to all tubes, including the substrate blank, vortex-mixing and then incubating at 37 °C for 1 h. The reaction was stopped by adding 1 ml diluted stop buffer and placing on the magnetic separator for 10 min. LH values were determined automatically by a zero 11 reader, provided by WHO (1999).

Table 1
Composition of basal and soybean-containing diets

Component	Percentage of component (%)	
	Basal diet	Soybeans diet
Maize	50.65	35.65
Ground cake	32.03	22.03
Non-nutritive fiber	5.27	5.27
Corn oil	4.5	4.5
Vitamin-mineral premix	2.0	2.0
Oyster shell	2.0	2.0
Bone meal	3.0	3.0
Methionine	0.3	0.3
Sodium chloride	0.25	0.25
Soybeans	0.0	25.0

3. Results

The effect of soyprotein treatment on body weight, average daily food intake and uterus weight is shown in Table 2. Soyprotein had no effect on body weight or average daily food intake. However, soyprotein intake significantly increased ($P<0.05$) uterus weight in the test rabbits compared to the control. Serum LH and total cholesterol were significantly reduced ($P< 0.05$) in soyprotein-fed rabbits compared to the control (Table 3). In

Table 2
Effect of soyprotein treatment on body weight[a]

Group	Body weight (kg)		Weight gain (kg)	Average daily food intake (g)	Uterus weight (g)
	Initial	Final			
Control	0.90±0.07	1.30±0.07	0.40±0.07	84.2±4.6	1.28±0.09
Test	1.37±0.06	1.62±0.47	0.37±0.27	90.8±5.9	5.44±0.58*

[a] Values are expressed as mean ± S.D.
*Value is statistically significant at $P<0.05$.

Table 3
Effect of soyprotein treatment on serum luteinizing hormone and total cholesterol levels[a]

Group	Serum total cholesterol (mg/dl)		Serum albumin (g/dl)		Serum LH (IU/l)	
	Before feeding	After feeding	Before feeding	After feeding	Before feeding	After feeding
Control	180±4.47	396±2.24	2.95±0.54	3.48±0.51	0.81±0.33	1.60±0.28
Test	125±5.71	190±18.91*	3.78±0.34	4.95±0.37*	0.60±0.08	0.11±0.03*

[a] Values are expressed as mean ± S.D. of five animals.
*Values are statistically significant at $P<0.05$.

contrast, soyprotein significantly increased serum albumin ($P<0.05$) in the test animals compared to the control.

4. Discussion

In the female, LH is important in ovulation. The follicle, which has been previously ripened by follicle stimulating hormone (FSH), requires an optimum FSH/LH ratio for ovulation (Cline, Hughes, & Tansey, 1998). It is also known that LH release is stimulated by small amounts of oestrogen and inhibited by large doses (Botella, 1973). In the present study, the decreased serum LH, observed in soyprotein-fed rabbits, serves as an indicator of possible oestrogen activities of soyprotein (Ergon, 1999). Ergon (1999) has also implicated oestrogen activity in plants as the major cause of menopausal symptoms in young females. The study also indicated that soyprotein caused significant increase in uterus weight of the female rabbits. This observation is in consonance with the findings of Santell, Change, Nair, and Helferich (1997) that oestrogen activity has a direct effect on the size and composition of the uterus, thereby making it unstable for implantation and foetal development.

The observed oestrogen effects of soyprotein are attributed to the relatively large quantities of isoflavones in soy foods (Block and Reinli, 1996; Fenwick and Price, 1985), since dietary isoflavones have been linked to the infertility syndrome (Adam, 1995; Setchell and Welsh, 1987).

Our results indicate that soyprotein-fed rabbits have lowered serum total cholesterol levels compared to the control. This fits in with the studies of Burke, Anthony, and Vitolins (1996), Dodge, Glasbrook and Merge (1996) and Sierksma, Westrate, and Meyer (1999) showing that soyprotein have a beneficial role in the prevention of cardiovascular diseases.

In conclusion, despite the nutritive value of soyprotein and its established protective effect against cardiovascular diseases, and even cancer, Its deleterious effect on female reproduction, due to the oestrogenic activities if the isoflavones, is hereby supported. However, further work is required to clarify the possible link between the oestrogenic activities of soyprotein and female infertility risks.

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