**EFFECT OF DIETARY SUPPLEMENTATION WITH SOYBEAN ON REPRODUCTIVE HORMONES OF MALE WISTAR RATS**

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**ABSTRACT**

Dietary soybean has been shown to have variable effects on the reproductive system of male rodents, non-human primates and humans. These effects can affect subsequent fertility. Soybean intake in men has been advocated due to its protective effect in the prevention of prostate cancer, cardiovascular disease and osteo-arthritis. However, the protection it offers in the prevention of prostatic cancer by altering the levels of male reproductive hormones may lead to infertility. This study aimed at determining the effect of dietary supplementation with soybean on reproductive hormones of male Wistar rats. The study was a randomised controlled trial. Adult male Wistar rats were randomly assigned to a control group and two study groups. The rats in the control group were fed on soy-free diet while rats in Group 1 and Group 2 of the study were fed on 20% and 50% soybean supplemented diet for four weeks. Serum concentration of FSH, LH and Testosterone were estimated. Data obtained was analysed using statistical package for social sciences (SPSS) version 20.0. There was no statistically significant difference (p<0.05) in the level of FSH (p=0.783), LH (p=0.815) and Testosterone (p=0.330) between the study and control groups. Dietary supplementation with of soybean has no effect on the level of FSH, LH and Testosterone in male Wistar rats and therefore may not affect their reproductive function.

**Keywords: Soybean, FSH, LH, Testosterone, Wistar Rats**

**INTRODUCTION**

Soybean is a species of leguminous plants that belong to the Fabaceae group of plants and is a rich dietary source of bioactive phytoestrogens. Phytoestrogens are subdivided into isoflavones, flavones, lignans and coumestans. The phytoestrogen content of soybean belongs to the isoflavone group of phytochemicals. Each gram of soybean contains approximately 3.5mg of isoflavone (Massina et al., 2006). Genistein is the main isoflavone of soybean. Phytoestrogen is a natural selective oestrogen receptor modulator (Weber et al., 2001). Phytoestrogens occur naturally in an inactive glycosidic form but are hydrolyzed by intestinal bacteria to form biologically active aglycone products that can be absorbed into the blood stream. It has all the physiological and physicochemical characteristics of oestrogen (Moderasi et al., 2011; Crouse et al., 1999). It is non-steroidal and has diphenolic structure. It has weak oestrogenic activity being able to bind oestrogen receptor α with an affinity 100-1000 times lower than estradiol (Adlercreutz et al., 2000) but bind strongly to membrane oestrogen receptor to exert non-genomic actions potentially deleterious to male fertility (Serag El Din et al., 2011).

Phytoestrogens have been reported to have a protective effect in prevention of development of cardiovascular diseases, osteoporosis and hormone-dependent cancers like breast and prostate cancers (Adlercreutz et al., 2000; Knight et al., 1996; Adlercreutz and Mazzur, 1997). The anticancer effect of phytoestrogens is associated with several possible mechanisms. They can inhibit tyrosine kinase, growth factors, and DNA topoisomerase and steroidogenic enzymes. They also can act as antioxidant and anti-angiogenic agents. All these properties prevent carcinogenesis (Knight et al., 1996; Price and Fenwick, 1995; Setchell 1998).

Despite the possible enormous health benefits that can be derived from dietary intake of soybean, it has been implicated as a possible cause of male infertility (Fraser 2006, Atanassoun et al., 2000). Soybean has been...
associated with impaired development of reproductive organs following intrauterine exposure and abnormal spermatogenesis and low sperm concentration following exposure in adult males leading to male factor infertility (Hamilton-reeves et al., 2007). Some studies have demonstrated no effect on concentration of serum testosterone, follicle stimulating hormone, luteinizing hormone, and serum concentration of testosterone in men (Liang and McGrath, 2001; Perry et al., 2007).

On the other hand, some studies (1) reported some adverse effects following dietary ingestion of soybean on the reproductive physiology in both human and animal models of research. These adverse effects include decrease in serum free testosterone, lower sperm concentration, increase in serum estradiol (E2) and oestrone (E1) and adverse effect on spermatogenesis (Song et al., 1999; Lund et al., 2004; Goodin et al., 2007; Dillingham et al., 2005).

This study sought to determine the effect of dietary supplementation with soybean on FSH, LH and Testosterone levels in male Wistar rats.

MATERIALS AND METHODS

Study Design
The study was a randomised controlled trial. The control group was given soy free diet. The experiment groups were given different concentration of soy-based diet as explained under randomization section below. It was conducted at the Department of Human Physiology Bayero University, Kano. The laboratory tests were conducted at the Department of Chemical Pathology, Aminu Kano Teaching Hospital, Kano.

Ethical Consideration:
Approval to conduct the research was obtained from the Research and Ethics committee of the College of Health Sciences, Bayero University Kano (BUK/CHS/REC/01/41). Ethics of use of animals for research was adhered to.

Study Animals
A total of 30 Inbred male post pubertal Wistar rats (three months old) weighing 120g±20g were obtained from the animal house of Bayero University, Kano. They were kept in plastic cages with solid floor, saw dust bedding/nesting, at a room temperature of 25 – 27°C and 12/12 hours light/dark cycle to acclimatize over a period of 2 weeks.

Randomization
After the period of adaptation, the animals were randomly assigned (using random number table) to three groups. Each group had a total of 10 rats. Group 1 was the control group and Groups 2 and 3 were the experimental groups. Each group had two cages with five rats per cage. The cages were labelled 1 to 3 indicating the group. The rats in each cage were numbered based on cage number and number in the cage. Thus, the rats were labelled 1.1 to 1.10 in group 1, 2.1 to 2.10 in group 2 and 3.1 to 3.10 in group 3.

Formulation of Study Diet
Soybean was obtained from a local market in Zaria, Kaduna state. The soybean was fermented in water for 12 hours to break the oligosaccharides within it. It was then cooked at 120°C for 18-20 minutes. This will decrease the anti-nutrient content within it which includes trypsin inhibitors, phytin, lectins, saponins and haemagglutinins. It was then dried and ground into powder.

Twenty percent Soybean supplemented diet was prepared by mixing 20g of the grounded soybeans with 80g of Growers mash while 50% soybean supplemented diet was prepared by mixing 50g of the grounded soybean with 50g of Growers mash.

Animal Feeding
Rats in group 1 (control) were fed on Growers mash and given water ad libitum while rats in the study group were fed on twenty percent (group 2) and fifty percent (group 3) soybean supplemented diet prepared as stated above and given water ad libitum. Rats in each cage (5) were given 100 g of the respective feed per day. The animals were fed on these diets for 6 weeks. The period represented at least four reproductive cycles.

Blood Sample Collection and Processing
After the period of experiment, trunk blood was obtained from all animals. A sterile blade was used to make an incision on the trunk. The blood was collected directly into plain bottles. The blood was spun at 1000 rpm for 5 minutes to separate the serum from the cells. The serum was stored in plain bottles labelled with rats’ identification number at -20°C and used for the test.

Assay Technique
LH, FSH test kit (obtained from Wuhan Fine Biotech Co., Ltd. Wuhan, China) principle is based on Competitive Enzyme Linked Immunosorbent Assay technique (ELISA) detection method. The microtiter plate provided in this kit has been pre-coated with target. During the reaction, target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to target. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate
reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve and Testosterone were measured using an Enzyme Linked Immunosorbent Assay (ELISA) based assay technique. Further details available on the company website (www.fn-test.com).

The DEMEDITEC Testosterone rat/mouse ELISA Kit(obtained from Demeditec Diagnostics GmbH, Kiel Germany) is a solid phase enzyme-linked immunosorbent assay(ELISA), based on the principle of competitive binding. An unknown amount of testosterone present in the sample and a defined amount of testosterone conjugated to horseradish peroxidase compete for the binding sites of testosterone antiserum coated to the wells of a microplate. After one-hour incubation on a shaker the microplate is washed four times. After addition of the substrate solution the concentration of testosterone is inversely proportional to the optical density measured. Further details available on the company website (http://www.demeditec.com)

Statistical Analysis
Data obtained were analysed using SPSS (version 20.0). Mean values of all the assayed hormones in each group were calculated. Analysis of Variance was done to test for any significant differences among the groups. The level of significance was set as p<0.05.

RESULTS
The result showed no significant difference (P<0.05) in the serum level of LH (p=0.815), FSH (p=0.783) and Testosterone (p=0.330) between the control group (no soy diet) and the experiment groups (20% and 50% soy diet).

Table 1: Effect of Dietary Supplementation with Soybean on Reproductive Hormones Levels

<table>
<thead>
<tr>
<th>Hormone Group</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.92 ±0.18</td>
<td>2.21 ±0.00</td>
<td>7.83 ± 0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.89 ±0.27</td>
<td>2.09 ± 0.23</td>
<td>6.95 ± 0.77</td>
</tr>
<tr>
<td>3</td>
<td>0.90 ±0.46</td>
<td>2.19 ± 0.00</td>
<td>7.73 ± 0.01</td>
</tr>
<tr>
<td>ANOVA (F)</td>
<td>0.206</td>
<td>0.247</td>
<td>1.155</td>
</tr>
<tr>
<td>P-value</td>
<td>0.815</td>
<td>0.783</td>
<td>0.330</td>
</tr>
</tbody>
</table>

Values as Mean ±SD; Significance= P<0.05

DISCUSSION
The mean value of Follicle Stimulating Hormone in the control group of this study was 2.21±0.001. A randomised study on effect of soybean on fertility of male and female albino rats (Serag El Din et al., 2011) reported a mean value of Follicle Stimulating Hormone among the control group to be 4.69±0.23. In the same study (Serag El Din et al., 2011) the mean values of Follicle Stimulating Hormone among male albino rats fed on 30, 60 and 90 gm cooked soybeans/70 kg human body weight (b.w.) for three months were 3.63±0.23, 2.86±0.17 and 2.60±0.38 respectively which showed that the higher the soy bean content, the lower the mean hormone levels. In this study, the mean values of Follicle Stimulating Hormone among the Wistar rats fed on 20% and 50% soy diet was 2.09±0.23 and 2.20±0.00 respectively. This showed that from our study, the study group that was fed on 20% soy diet had the lowest mean Luteinising Hormone levels. In a similar study (Serag El Din et al., 2011) where the mice fed on higher concentration of soy diet had lower mean Follicle Stimulating Hormone values. However, the mean value of Follicle Stimulating Hormone in that study was almost twice of that reported in this study. This may be due to the difference in the species of rats used in the studies. Even though differences were observed in the mean values of Follicle Stimulating Hormone among the different study groups from our study, these differences were not statistically significant (Table 1). This finding agrees with the findings of Moderasi et al (Moderasi et al., 2011) but is contrary to other findings in the literature where soybean was reported to cause a significant decrease in the levels of Follicle Stimulating Hormone.

The mean value of Luteinising Hormone among the control group in this study is 0.92±0.18, whereas that of the groups that had 20% and 50% soybean was 0.89±0.27 and 0.90±0.47 respectively. This showed that the study group that was fed on 20% soy diet had the lowest mean Luteinising Hormone levels. In a similar study the levels of Luteinising Hormone among male albino rats fed on 30g/70 kg body weight, 60g/kg body weight and 90g/kg body weight
This showed that the higher the dietary soybean content, the lower the mean Luteinsing Hormone levels with the control group having the highest mean value. The mean values obtained in this study are slightly higher than that obtained by the study on albino rats. Moderasi et al. reported lower values among those fed on low soy diet (Moderasi et al., 2011). Their finding of lower mean value of Luteinsing Hormones among rats fed on 20% soy diet is similar to the finding in this study. However, they reported a higher value of Luteinsing Hormone among experimental groups that were fed on higher concentration of soy diet. Even though differences were observed in the mean values of Luteinsing Hormone among different study groups from our study, these differences were not statistically significant. This finding agrees with the findings of Moderasi et al. where they reported that there was no significant difference in mean values of Luteinsing hormone between rats fed on soy free diet when compared to rats fed 20% soy diet but contrary to their other findings that reported a significant increase in serum Luteinsing Hormone levels among rats fed on high concentration (30% and 50%) of soy diet when compared to control group. Soybean has also been reported to cause a significant decrease in values of Luteinsing Hormone by other authors (Serag El Din et al., 2011).

The mean level of Testosterone among the control group in this study was 7.83±0.00 while those in the 20% soy diet and 50% soy diet were 6.96±0.77 and 7.73±0.01 respectively. This showed that the group fed on 20% soy diet had lower mean Testosterone level when compared to the groups that had soy free diet and 50% soy diet. Lower mean values of Testosterone due to ingestion of soy diets have been reported in both human and animal studies (Moderasi et al., 2011; Serag El Din et al., 2011). A similar study (Moderasi et al., 2011) reported a lower value of Testosterone among rats fed on 20% soy diet as was observed in this study but higher values were recorded with higher concentration (50%) of soy diet which is in contrast to our finding. In another human study (Goodin et al., 2007) ingestion of soy diet led to a significant decrease in serum concentration of testosterone, however, the effect was reversed within two weeks of stoppage (Goodin et al., 2007). Even though differences were observed in the mean values of Testosterone among the different study groups from our study these differences were not statistically significant. This is contrary to other findings in the literature where 20% soy diet was said to significantly decrease the serum testosterone values among rats when compared to control while 30% and 50% soy diets were said to significantly increase the serum testosterone values among rats when compared to control (Moderasi et al., 2011). The findings in this research work with regards to the effect of dietary intake of soybean on serum testosterone is in keeping with studies reported by some authors in both human and animal experiments (Liang et al., 2001; Perry et al., 2007). Perry et al. conducted an animal study on Cynomolgus monkeys over a period of three months (Perry et al., 2007). The control group were fed soy free diet while the experiments group were fed soy-based diet. They concluded that there was no significant difference in serum levels of testosterone and sperm counts in all study groups. The researchers also confirmed presence of phytoestrogen in serum of experiment animals during the period of research. A Meta-analysis involving 32 reports involving 36 treatment groups in humans also reported that soy food and supplements do not alter the bioavailability of testosterone in men (Hamilton-reeves et al., 2007).

Even though, at molecular level, soybean phytoestrogens have the ability to inhibit enzymes involved in steroidogenesis leading to decrease in testosterone resulting in a negative feedback on Gonadotrophin Releasing Hormone (GnRH) with subsequent cessation of production of FSH and LH, this effect was not evident in this research work. A direct explanation may not be evident. However, some factors may play a role in this finding. The process of preparation of the soybean may reduce the phytoestrogen content of the soybean thus limiting its effect on the reproductive hormones. Also, Soybean contains some anti-nutrients within it which includes trypsin inhibitors, phytin, lectins, saponins and haemagglutinins (Serag El Din et al., 2011) which are affected by the way soybeans is processed before consumption. It may be possible that while processing the soybean for dietary consumption, the composition of the various nutrients and other substances is affected which may in turn affect the bioavailability of the phytoestrogen content of soybean. The effects of these anti-nutrients content in relation to reproductive functions in males may also affect the role dietary soybean plays in male reproductive function. The effect of dietary soybean may also be affected by a
background abnormality in steroidogenesis. This may further be worsened by ingestion of soybean. Modaresi et al also suggested that males with metabolic syndrome or those who are overweight should take precautions using oestrogenic compounds such as soy bean since such compounds decrease the expression of oestrogen receptors on testes tissue, occupy the active site of these receptors and have a negative role on hypothalamic-pituitary-gonadal axis. However, these factors may not be evident from this study.

CONCLUSION
Dietary Supplementation with 20% and 50 % soybean did not cause significant alteration in the serum FSH, LH and Testosterone levels suggesting that soybean does not disrupt reproductive function in male Wistar rats.

RECOMMENDATION
1. It is also recommended that the effect of methods of processing soybean on the phytoestrogen content of soybean be studied to determine whether it affects the overall effect on male reproductive hormones. Also, the role of the other contents of soybean (trypsin inhibitors, phytin, lectins, saponins and haemagglutinins) on reproductive function may shed more light on the association between soybean and male reproductive dysfunction. This is particularly important especially in our environment where soybean provides a rich source of cheap protein and has rightly been named the Cinderella crop of Africa.
2. Future research on the effect of dietary soybean on sperm quality and subsequent conception is also recommended.

LIMITATION
The major limitation of this study is that it is a translational study and as such may not be directly applicable to humans. The research was time bound using personally financed resources which limited its scope.

AUTHOR CONTRIBUTION STATEMENT
All authors contributed to the conceptualization, study design, analysis, result discussion and final manuscript.

CONFLICT OF INTEREST
The Authors declare that no conflicting interests exist.

REFERENCES


