Effect of Long Term Administration of Citrullus Lanatus on Fertility Hormones of Male and Female Albino Rats.
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Abstract
This study was done to highlight the diverse benefits of Citrullus lanatus (egusi melon) seeds with the principal focus on its highly nutritive and calorific values. This makes Citrullus lanatus necessary in diet and in the fight against malnutrition. It also indicated the effect of Citrullus lanatus on Follicle Stimulating Hormone (FSH), Luteinizing hormone (LH) and Testosterone which are responsible for ensuring preparation for and maintaining of reproductive fertility. A total number of forty wistar rats, 30 males and 10 females were used for the study. They were fully acclimatized for two weeks and grouped into the control group (group I) and the test group (group II) which was further subdivided into three: starved, normal feed and egusi supplement administered groups. Test animals were sacrificed and blood samples collected for hormone analysis. The result showed that malnourishment induced through starvation increased FSH and LH levels and lowered testosterone concentration in both male and female wistar rats. The egusi supplement had more effect on FSH and LH levels for the female rats while it had more effect on testosterone levels for the male rats. This is because FSH and LH are primarily female fertility hormones while testosterone plays a greater role in males. Steady effects were observed for FSH and testosterone levels for the male and female wistar rats respectively while that of LH had alternating effect. Based on the result, further work is therefore recommended be done to shed more light in the details of mechanism of action of egusi supplement on male and female fertility hormones.

Key Words: Citrullus lanatus,Follicle Stimulating Hormone,Luteinizing Hormone,Testosterone,wistar rat

Introduction
Melon refers to the seeds of a type of watermelon widely found in West Africa, called Citrullus lanatus. It is popularly used in soups in West and Central Africa. These seeds have nutritive and calorific values which make them necessary in diets and can be considered as an important source of plant proteins, lipids and calcium. (Fokou et al, 2004). Used in preparing the popular Nigerian egusi soup, it is used in making cakes and other fruit snacks. Very healthy cholesterol free oil is also made from the seeds. They have a very long shelf life and are quite versatile in the range of uses. They can be put into human and livestock feeding, as well as in manufacturing. It is made up of about 28.4% protein (60% in the defatted), 52.0% oil, 8.2% carbohydrate, 3.6% ash and 2.7% fibre. (Uruakpa and Aluko, 2004) They are also important sources of major minerals. In terms of vitamins, it contains alpha-tocopherol, a component of vitamin E that helps in maintaining smooth young skin and good fertility. Egusi melon also contains palmitic, stearic, linoleic, oleic acids important in protecting the heart too. Egusi Melon seeds are annual, herbaceous, monoecious plants with climbing stems. It has forked tendrils, hairy stems and three-lobed hairy leaves. The fruits are indehiscent smooth berries which enclose many seeds and they belong to the Cucurbitaceae family. (Fokou et al, 2004) They look like pumpkin leaf in appearance and are often swapped for them where not available. Melon prominently features in most African foods especially Nigerian, Ghanaian, Sierra Leonian and Congolese soups. They are also largely grown in Northern Namibia. They are very rich in protein and oils. The common names include: Egusi, Egunsi, Ikon, Agushi, Guna shanu, Ikpoghiri, Neri niri, Ibara.

There are several species of melon seeds among which are; Cucumeropsis mannii, Cucurbita maxima, Cucurbita moschata, Lagenaria siceraria, Cucumis savitus. (Fokou et al, 2008.). Protein energy malnutrition (PEM) is a disease caused by intake of food deficient in protein and energy.(Allen,1995). The World Health Organization (WHO) defines malnutrition as the cellular
imbalance between the supply of nutrient and energy and the body’s demand for them to ensure growth, maintenance and specific functions. It is a disease of the poor, undernourished and chronically ill patient. Ojieh and Oluba (2008) showed that egusi melon compares favorably with the known protein diets such as soybean, cowpeas, pigeon peas and pumpkin. The proximate amino acid and mineral composition of Citrullus lanatus (Egusi melon) flour were determined using standard analytical procedures. The proximate composition analysis of egusi melon showed that the seed contained (% dry weight): moisture (4.6 ± 0.3), ash (3.7 ± 0.1), ether extract (45.7 ± 0.1), crude protein (23.4 ± 0.2), crude fibre (12.0 ± 0.1) and total carbohydrate (10.6 ± 0.2). The result of the amino acid analysis showed that egusi melon seed contained good quantities (g/100g protein) of arginine (9.0), isoleucine (4.8), leucine (4.2), and phenylalanine (3.2) which are essential amino acids as well as glutamic acid (16.9) and aspartic acid (16.3). The mineral analysis (mg/100g) of the flour included: Na (13.0 ± 0.2), K (96.1 ± 0.4), Ca (28.2 ± 0.2), Mg (31.4 ± 0.2), Mn (1.7 ± 0.1), Cu (0.4 ± 0.1), Zn (1.2 ± 0.1), Fe (1.3 ± 0.2) and P (125.3 ± 3.1).

With this nutrient profile, egusi melon compares favorably with the known protein rich foods such as soybean, cowpeas, pigeon peas and pumpkin. Hence, comprising 50% oil and 35% protein (Jack, 1972), the seeds have both nutritional and cosmetic importance. The seeds contain vitamin C and B2, minerals, riboflavin, fat, carbohydrates and protein (Lazos, 1986).

Oluba (2008) stated that citrullus lanatus when fed as supplement to a cholesterol enriched diet, decreases serum total, free and esterified carbohydrate levels as well as serum levels of lecithin and isolecithin. Commonly, melon can be used widely as food ingredients, soup thickener and the melon seed can be fermented to produce ‘ogiri’ (Oke 1965, Oyenuga and Feliga 1975).

Moreover, Anderson (2002) conducted a study to determine if a change in protein/carbohydrate ratio influences plasma steroid hormone concentrations. Testosterone concentrations in seven normal men were consistently higher after ten days on a high carbohydrate diet (468 ± 34mg/dl, mean ± S.E) than during a high protein diet (3.71 ± 23mg/dl, p<0.05) and were accompanied by parallel changes in sex hormone binding globulin (32.5 ± 2.8nmol/l vs. 23.4 ± 1.6nmol/l respectively, p<0.01). By contrast, cortisol concentrations were consistently lower during the high carbohydrate diet than during the high protein diet (7.74 ± 0.71ug/dl vs. 10.6 ± 0.4ug/dl respectively, p< 0.05) and there were parallel changes in corticosteroid binding globulin concentrations (635 ± 60nmol/l vs. 754 ± 31nmol/l respectively, p< 0.05). The diets were equal in total calories and fat. These consistent and reciprocal changes suggest that the ratio of protein to carbohydrate in the human diet is an important regulatory factor for steroid hormone plasma levels and for liver-derived hormone binding proteins.

Furthermore, Adam (2010) discovered an alternative fuel to fossil petrol and diesel from Citrullus lanatus seeds. Egusi seed oil has the potential to be processed into petrol and diesel for commercial use as it is lighter, harvesting of the seeds is easy and more economical compared to palmoil and jathropa, among the sources of biodiesel. This research has also found the egusi melon (citrullus lanatus) oil to be low in fatty acid melts easily and increases combustion.

Herbert (1980) conducted a study on growth patterns and hormonal profile of male rats with protein calorie malnutrition and the result showed that Serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone were lower in the malnourished rats. A condition of protein-calorie malnutrition was precipitated in young Sprague-Dawley male rats at 20 days of age using an 8% low protein diet (LPD). At five-day intervals for up to 50 days of age, the rats were studied to determine the effect of an LPD on the reproductive axis of the endocrine system. Daily monitoring of the body weight as well as the consumption of food, kilocalories and protein was conducted. The same parameters were followed over the identical time period in a group of animals designated as controls which were fed a standard laboratory diet (SLD) containing 27% protein. The controls showed a linear growth over the 30-day experimental period. In comparison, the malnourished rats grew more slowly so that by 50 days of age, their mean body weight was 68.9 ± 3.1g for the controls. The daily food, kilocalorie and protein intake by the
experimental animals were also appreciably less. Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL) and testosterone were lower in the malnourished animals at all ages studied.

Moreso, Giwa et al, 2010 illustrated that citrullus lanatus seeds can be used as a potential biodiesel feedstock. Crude egusi melon seed oil was transesterified using sodium methoxide as the catalyst at 60°C and an oil/methanol ratio of 1:6 to produce its corresponding methyl esters. Egusi melon oil methyl ester (EMOME) yield was 82%. Gas chromatographic analysis of EMOME showed that it was composed mainly of palmitic, stearic, oleic, linoleic and linolenic esters which is similar to a profile of sunflower, soybean and safflower oil. All the measured fuel properties of EMOME satisfied both the ASTM D6751 and the EN 14214 biodiesel standards. Fuel properties of EMOME were identical with those of soybean, safflower and sunflower biodiesel. Remarkably, the kinematic viscosity of ENOME was measured to be 3.83mm2/s, a value lower than most biodiesel fuels reported.

Incledon and Gross, (2006) carried out research to find out the effect of protein diet on testosterone levels. They stated that fasting for 5 days can lower Luteinizing hormone and Testosterone by 30-50% and that this decrease could be a contributing factor to the loss in lean body mass that occurs with fasting. When male subjects were overfed in an attempt to induce weight gain, there was a decrease in testosterone levels. Protein liquid meals have been shown to decrease testosterone in resistance-trained males. They suggested that this may be due to an increased uptake by tissues or decreased responsiveness of the testes to produce testosterone.

Materials and Method

The following methodology was employed in this study to investigate the effect of long term administration of egusi melon supplement on the fertility hormones of male and female wistar rats.

The egusi supplement was identified and supplied by Dr S. E Ofodile, Department of Chemistry, and Faculty of Science. The supplement was brought in the form of defatted flour. Forty healthy albino rats: 30 males and 10 females were used in this investigation.

A wooden cage divided into 10 compartments with iron mesh for ventilation was used to house the animals in the University of Port-Harcourt animal house.

The rats were kept for a period of two weeks so as to be accustomed with the new environment. Within this period, the rats initially weighing about 60g (for males) and 40g (for females) gained weight of about 20g.

After the two weeks acclimatization period, the rats were divided into specific groups and fed accordingly.

1. Control group: The rats were fed at will with rat feed throughout the duration.
2.
3. Test group.
   i. Starved: These groups were starved for a week without feed but were given enough water.
   ii. Normal feed group: they were put back on the normal feed after the one week starvation.
   iii. Egusi supplement group: they were administered the egusi supplement orally plus the normal feed after the one week starvation.

The supplement was prepared by mixing 5g of egusi flour with 50ml distilled water after which it was sieved. The filtrate was then administered (2ml every other day) while the chaff was incorporated into their feed.

The blood samples were collected weekly after the wistar rats were killed. This was done by putting the rats in the desiccator with cotton wool soaked with chloroform to make them unconscious without them dying entirely. The reason for this is to ensure the collection of blood before it clotted. They were then cut open to expose the heart after which the blood was collected with the aid of sterile hypodermic 2ml syringes. The blood was introduced immediately into the lithium oxalate anti-coagulant bottles properly labeled to differentiate
between the male and female and also, the experimental groups. The blood samples collected from the male and female Wistar rats were centrifuged and the serum separated. Afterwards, the blood samples were used for the hormone analysis using the different hormone EIA test kit. The instrument used includes Printer (EPSON 300 + II), Washer STAT FAX-2600 and Reader (STAT FAX -2100) The LH ELISA kit is used for the quantitative measurement of LH in human serum or plasma.

Microwell coated with LH MAb (12x8x1 wells), LH Standard: 6 vials (0.7ml each) i.e 0, 3.1, 6.25, 12.5, 25, 50mIU/ml, Enzyme Conjugate: 1 bottle (12ml), TMB Substrate: 1 bottle (12ml), Stop Solution: 1 bottle (12ml), 20X Wash Concentrate (25ml)

Prior to assay, the reagents were allowed to stand at room temperature and gently mixed before use, the desired number of coated strips were placed into the holder and pipette 50uL of LH standards, controls and patient's sera.

The FSH ELISA kit was used for the quantitative measurement of FSH in human serum or plasma. The FSH is a solid state direct sandwich ELISA method. The samples and diluted anti-FSH-HRP conjugate are added to the wells coated with MAb to FSH beta subunit. FSH in the patient's serum binds to the anti-FSH MAb on the well and the anti-FSH-HRP second anti-body then binds to FSH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of substrate, the intensity of color is proportional to the concentration of FSH in the samples. A standard curve is prepared relating color intensity to the concentration of the FSH.

Materials provided
Microwells coated with FSH MAb(12x8x1 wells) i.e 96 wells, FSH Standard: 16 vials 0, 5, 10, 25, 50, 100mIU/ml (0.7mL each), Enzyme conjugate: 1 bottle (12mL), Stop solution: 12mL, Wash concentrate: 1 bottle (25mL), 20X concentrate. Prior to assay, reagents were allowed to stand at room temperature. Gently mix all reagents before use.

Testosterone EIA was used for the quantitative determination of Testosterone concentration in human serum. The testosterone EIA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 10uL of Testosterone standards, controls, patient samples, 100uL Testosterone-HRP conjugate reagent and 50uL rabbit anti-Testosterone reagent at 37oC for 90 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the endogenous Testosterone in the standard sample or quality control serum for a fixed number of binding sites of the specific testosterone antibody. Thus, the amount of peroxidase conjugate immunologically bound to the well, progressively decreases as the concentration of Testosterone in the specimen increases. Unbound Testosterone peroxidase conjugate is then removed and then wells washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 20 minutes resulting in the development of blue color. The color development is stopped with the addition of 1N HCl and the absorbance is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The testosterone concentration of the specimens and controls run concurrently with the standards and can be calculated from the standard curve.

Reagents-Goat anti-Rabbit IgG-coated microtiter wells (96 wells), Testosterone Reference Standards: 0, 0.1, 0.5, 2.0, 6.0 and 18.0ng/ml. Liquids, 0.5ml each, Rabbit anti-Testosterone Reagent (pink color), 7ml, Testosterone HRP Conjugate Reagent (blue color), 12ml, Testosterone Control 1, Liquid, 0.5ml (Range < 2.0ng/ml), Testosterone Control 2, Liquid, 0.5ml (Range 7.3-17.0ng/ml), TMB Reagent (One-step) 11ml, Stop Solution (1N HCl), 11ml

Materials provided : Precision pipettes: 10ul, 50ul,100ul and 1.0ml, disposable pipette tips, distilled or deionized water, vortex mixer or equivalent, absorbent paper or paper towel, linear-linear graph paper, microtiter plate reader.
RESULTS

The results obtained from this study are summarized in the table below:

<table>
<thead>
<tr>
<th>Sample (In weeks)</th>
<th>Group</th>
<th>FSH mlU/ml</th>
<th>LH mlU/ml</th>
<th>TESTOSTERONE ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>Control Male</td>
<td>1.0</td>
<td>1.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Control Female</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Starved Male</td>
<td>2.0</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Starved Female</td>
<td>2.3</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Week 1</td>
<td>Control Male</td>
<td>1.1</td>
<td>1.0</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Egusi Male</td>
<td>1.4</td>
<td>1.2</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Egusi Female</td>
<td>1.6</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Week 2</td>
<td>Control Male</td>
<td>1.0</td>
<td>0.9</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Egusi Male</td>
<td>1.4</td>
<td>1.0</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Egusi Female</td>
<td>1.7</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Week 3</td>
<td>Control Male</td>
<td>1.2</td>
<td>0.8</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Egusi Male</td>
<td>1.5</td>
<td>1.1</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Egusi Female</td>
<td>1.8</td>
<td>1.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Week 4</td>
<td>Control Male</td>
<td>1.1</td>
<td>0.9</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Egusi Male</td>
<td>1.6</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Week 5</td>
<td>Control Male</td>
<td>1.0</td>
<td>1.0</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Egusi Male</td>
<td>1.6</td>
<td>1.1</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Fig. 1
**Effect of long term administration of melon on FSH levels of male and female wistar rats**

- **Week 1**: Control male - 1.2, Egusi male - 1.6, Egusi female - 1.4
- **Week 2**: Control male - 1.3, Egusi male - 1.8, Egusi female - 1.5
- **Week 3**: Control male - 1.1, Egusi male - 1.9, Egusi female - 1.6
- **Week 4**: Control male - 1.0, Egusi male - 1.7, Egusi female - 1.3
- **Week 5**: Control male - 0.9, Egusi male - 1.6, Egusi female - 1.5

* = Significant difference from control is P<0.05
# = Significant difference from control is P<0.05
** = Significant difference from control is P<0.01

**Fig. 2**

**Effect of long term administration of egusi melon on LH levels of male and female wistar rats**

- **Week 1**: Control male - 0.8, Egusi male - 1.2, Egusi female - 1.5
- **Week 2**: Control male - 0.7, Egusi male - 1.3, Egusi female - 1.4
- **Week 3**: Control male - 0.6, Egusi male - 1.1, Egusi female - 1.2
- **Week 4**: Control male - 0.5, Egusi male - 1.0, Egusi female - 1.1
- **Week 5**: Control male - 0.4, Egusi male - 0.9, Egusi female - 1.0

* = Significant difference from control is P<0.05
# = Significant difference from control is P<0.05

**Fig. 3**
Data Analysis

Analysis of FSH values
The FSH concentration of the control male before starvation was 1.0mIU/ml while that of the control female was 1.2mIU/ml. FSH levels increased in both male and female from 1.0 and 1.2 mIU/ml to 2.0 and 2.3mIU/ml respectively after the rats were starved for one week. In week 1, 2 and 4, the normal feed rats (control 1) had reduced FSH levels while the egusi supplement group for both male and female maintained an increase from week 1 to week 3. In week 5, the normal feed males (control 2) had the same FSH level with the control.

Analysis of LH values
The initial LH concentration for the male control before starvation was 1.0mIU/ml while that of the female control was 1.1mIU/ml. After starvation, the LH levels in both male and female increased from 1.0 and 1.1 mIU/ml to 1.4 and 1.6mIU/ml respectively. There was decrease in LH levels in weeks 2, 3 and 4 of the normal feed (control) males. The egusi supplement group maintained an increase all through except in week 4. The 2nd and 3rd weeks of the normal feed group had slight increases in LH concentration.

Analysis of testosterone values
The testosterone concentration for the initial male and female control before starvation was 3.2mIU/ml and 1.2mIU/ml respectively. There was decrease in testosterone concentration from 3.2mIU/ml and 1.2mIU/ml to 2.0 and 0.7mIU/ml after the one week starvation in the male and female rats respectively. The testosterone levels increased in weeks and increased all through the weeks for the egusi supplement males while that of the females had alternating effects. Significantly, the testosterone concentration for the males were higher than those of the females all through the weeks.

Discussion

![Graph showing effect of long term administration of egusi melon on testosterone levels of male and female wistar rats.](image)

* = Significant difference from control is P<0.05
** = Significant difference from control is P<0.01

Fig. 4
Follicle stimulating hormone (FSH)
The male control (Cm) had a higher value than that of the female before starvation, the FSH concentration increased to about twice the original concentration. This increase is as a result of the one week starvation. This therefore depicts that malnutrition alters FSH secretion by causing an increase. For week 1, the rats administered the egusi supplement in addition to the normal feed had higher FSH concentration than those on normal feed only(control). This suggests that the protein supplement favoured the increase in FSH concentration. It is important to note that the egusi supplement had effects on the male and female wistar rats but was more significant in the female wistar rats. In weeks 2,3,4 and 5, there was a steady increase in FSH levels. Conclusively, the egusi supplement was adequate to balance the effect of the malnutrition induced earlier by bringing their concentrations to almost that of the male and female control in week one and increasing them thereafter.

Luteinizing hormone (LH)
After the period of one week starvation, LH concentration increased favorably in both male and female. This suggests that malnutrition causes increased secretion of Lutenizing hormone in the anterior pituitary.In weeks 1,2 and 3, the egusi supplement had alternating effect in both the male and female egusi group.Comparing the male egusi supplement (Sm) with the female supplement (Sf), LH concentrations were much higher in Sf than the Mf. This shows that LH has more effect on females than males. This is because LH is primarily a female fertility hormone and plays a vital role in females.Week 4 and 5 showed no change in LH levels for the male wistar rats. This means that there was no significant effect on the males. The egusi had alternating effect on the females too.

Testosterone
Testosterone level of the initial male control group was much more higher than that of the female. After starvation; there was decreased concentration of testosterone for both the male and female. This agrees with the study previously carried out by Incledon and Gross which stated that fasting for 5 days can lower testosterone level by 30-50%. This decrease in testosterone level as a result of starvation is a contributing factor to the loss in lean body mass that occurs with fasting.In weeks 1,2,3,4 and 5, the control male group had a lower testosterone concentration than that of the egusi male. The testosterone levels of the egusi male increased consistently suggesting that the supplement was effective in increasing testosterone level. It had more effect on the male as a result of the significant role played by testosterone in males.In weeks 1,2 and 3, the female result showed negligible and inconsistent changes in testosterone levels suggesting that its effect was primarily on the male group.

Conclusion
From the results of the study, Citrullus lanatus(egusi melon), highly rich in protein, consistently increased testosterone levels in male wistar rats, caused a steady increase in FSH levels in the female wistar rats but caused negligible and inconsistent changes on LH levels.

Recommendation
Based on the result of this research, further work should be carried out on Citrullus lanatus(egusi melon) in relation to its effect on fertility hormones to investigate deeper and understand its mechanism of action.

References


