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# The effects of various yam diets on the reproductive hormones of experimental rats

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**ABSTRACT**

*White Guinea yam (Dioscorea rotundata) 'Ehuru' consumed in Ibarapa Local Government Area of Oyo State, South West region of Nigeria has been claimed to be responsible for the high incidence of multiple births in the area. This study assessed the effects of various locally prepared yam diets consumed in the study area on the reproductive hormones of experimental animals. Freshly harvested yams, purchased from local farmers, were compounded into the various yam diets. Six groups of diets which included the control which was normal rat chow (RC), a mixture of pounded yam, soup and stew (PYSS), boiled yam with stew (BYS), fried yam with stew (FYS), boiled yam with palm oil (BYPo) and boiled yam with chow (BYC) were prepared and fed to the six groups of rats respectively. The soup in PYSS was prepared with the tender leaves of Hibiscus abelmuschus (okro) known locally as 'Ilasa' and BYC was a mixture of equal proportion of boiled yam and rat chow. Each group had 4 female and 2 male rats, fed 20g of the diet per day/rat for 11 weeks. Water was given ad libitum. The female rats were separated on observation of pregnancy, and the feeding continued until they littered. Both the male and female rats were sacrificed after the weaning of the litters. Samples were collected from each rat by cardiac puncture using sterile syringes. Result of the hormonal profiles showed Luteinizing hormone (LH) ranging from 2.60 ± 0.12mlu/ml for RC to 6.67 ± 0.38 mlu/ml for PYSS. Follicle stimulating hormone (FSH) ranged from 12.57 ± 0.19mlu/ml for RC to 16.67 ± 0.87mlu/ ml for PYSS. Prolactin levels ranged from 5.00 ± 0.31ng/ml for RC to 7.10 ± 0.61ng/ml for FYS. PYSS caused a significant elevation (P<0.05) in FSH, LH, Prolactin and estradiol in the female rats.*

**Keywords:** *(Dioscorea rotundata), Hibiscus abelmuschus,* Luteinizing hormone, Follicle stimulating hormone, Prolactin and estradiol.

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## INTRODUCTION

Yam (*Dioscorea rotundata*) tubers constitute the stable food for majority of Nigerians. It is a rich source of carbohydrate, dietary fat and minerals and is low in saturated fat *[1]*. Consumption of yam is common in Nigeria and can be prepared in many different ways depending on the locality of the consumer. In some parts of Nigeria, especially Ibarapa local government area of Oyo State, there is a high incidence of twin births *[2-3]; [4]*. It has been hypothesized that Dioscorea species possess dehydroepiandrosterone (DHEA) – like properties and act as a precursor to human sex hormones such as estrogen and progesterone *[5]*. Pregnancy and fertility are chiefly under endocrine control and so there may be some components of the yam that modulate the endocrine environment. A preliminary survey carried out in the local government showed that 82.37% of the respondents claimed to have multiple births in their families and 13.03% of these were non-indigenes who had no previous history of twins in their families. The link between consumption of Dioscorea rotundata and increased incidence of multiple births has been suspected and claimed in the South West region of Nigeria but not scientifically proven. Endocrine environment is known to play a role in modulating pregnancy and impact of diet on pregnancy can be assumed indirectly by its effect on reproductive hormones. This research work evaluated the effects of the various yam diets consumed in Ibarapa on the reproductive hormones of experimental animals.

## MATERIALS AND METHODS

Freshly harvested yam (Dioscorea rotundata) were purchased from farmers in Igboora, wrapped in cartons and transported to Calabar. Similarly, fresh young leaves of okro plant (Hibiscus abelmuscus) commonly called 'Ilasa' by the people of Ibarapa were purchased, wrapped in grease proof paper and also transported to Calabar to prepare the soup for one of the diets. The yams were stored in a dry place and the leaves in a cool place ready for use.

**Treatment of vegetable and preparation of "Ilasa" soup**

The soup was prepared following the traditional method of the Ibarapa people of Oyo State. 300g of the fresh vegetable were rinsed in clean tap water to remove sand and dust particles. It was then drained of excess water and in a colander and chopped with a kitchen knife into fine pieces on a wooden chopping board. Meanwhile, one litter of clean tap water was put into an aluminium pot and set on fire to boil. On boiling, the vegetable was added and allowed to boil for about 2 minutes before the melon, dawadawa (locust bean) and other ingredients were added. The pot was then left to boil for another 2 to 3 minutes with stirring occasionally before it was removed from the fire and allowed to cool (recipe in table 1).

**Preparation of the stew**

The stew was prepared using the traditional method of the Ibarapa people. The tomatoes, onions and pepper were blended together and set aside. 155g of palm oil was placed in a stew pot and fried for 10 minutes then the blended tomatoes, onions and pepper were added and allowed to boil for another 20 minutes. The meat and offals which were previously cleaned with tap water, salted and boiled forty minutes until tender enough to homogenise were then added. The knorr cubes and salt were added for seasoning and the pot was allowed to boil again for a further 10 minutes before setting it down (recipe in table 2).

**Table 1 RECIPE FOR ILASA SOUP**

| | | |
|---|---|---|
| Ilasa leaves | - | 300g (finely chopped) |
| Dawadawa | - | 22g (ground) |
| Melon (Egusi) | - | 83g (ground) |
| Potash (Kaun) | - | 3g (crushed) |
| Salt | - | 9g |
| Water | - | 1L |
| 1 Knorr cube for seasoning | | |

**Table 2 RECIPE FOR STEW**

| | | |
|---|---|---|
| Cow leg | - | 180g |
| Meat | - | 380g |
| Tripe | - | 125g |
| Spleen | - | 62g |
| Intestine | - | 83g |
| Tomatoes | - | 160g |
| Pepper | - | 21g |
| Onions | - | 12g |
| Palm Oil | - | 155g |
| Knorr cube | - | 2g |
| Salt | - | 7g |

**Preparation of the yam diets**

**Pounded yam, soup and stew**

10kg of yam were washed in clean tap water to remove sand, peeled with a kitchen knife and cut into chunks. These were boiled in tap water in an aluminium pot without salting until soft enough to pound with a local mortar and pestle. Thereafter, the soup "*Ilasa*" and stew were added, homogenized in a Kenwood food processor, dried in Gallenburg oven at $55^0$C for 24 hours and pelleted for easy consumption by the rats.

**Boiled yam and stew**

The same quantity of yams were washed in clean tap water to remove sand, peeled with kitchen knife, cut into slices and boiled in salted water until soft enough to eat. The yam was then homogenized with another pot of prepared stew, dried in the same oven at $55^o$C and made into pellets. This was stored in a refrigerator for subsequent use.

**Fried yam and stew**

10kg of yams were washed to remove sand, peeled with kitchen knife and cut into medium slices for quick frying. 300g of palm oil was allowed to fry in a deep frying pan and the yam slices were added in small quantity at a time.

200mls of water was gradually added to the frying oil and yam at interval to soften the yam and fried until golden brown. The fried yam was then homogenized with the already prepared stew and the homogenate dried in the Gallenburg oven at 55$^{o}$C for about 24 hours. This was stored in a cool dry place for subsequent use.

**Boiled yam with palm oil**

10kg of yams were washed in clean tap water to remove sand. The yams were then peeled with kitchen knife, washed again before cutting into slices. The slices were then put in an aluminium pot, salted and boiled until soft enough to eat after which they were homogenized with 126g of palm oil. The homogenate was dried in the oven at 55$^{o}$C for 24 hours, cooled and stored in a cool dry place.

**Boiled yam with normal rat chow**

10kg of yams were washed, peeled and cut into slices. They were then put in an aluminium pot; water added and boiled until soft enough to eat. The yams were further cut into smaller pieces, dried in Gallenburg oven at 55$^{o}$C for 24 hours. After drying, they were left to cool, blended and thoroughly mixed in the ratio of 50:50 with the normal rat chow and stored in a cool dry place.

**Feeding Trial:**

An initial thirty-six weanling albino Wistar rats (24 females and 12 males) were obtained from the Animal House of Pharmacology Department, University of Calabar, Calabar, Nigeria. The animals were then conveyed to the Biochemistry Departmental animal house and were fed until they weighed between 120g to 150g. During this period, the males were separated from the females to avoid mating. On attaining this weight, the animals were further allowed to acclimatize for another 10 days before the start of the experiments. The animals were kept in the animal house facility under standard animal laboratory conditions of adequate ventilation, room temperature of 28 ± 5$^{o}$C, relative humidity of 65 ± 5% and light (12hour day light circle)

At the completion of the acclimatization, male and female rats were randomly cohabited in the mating ratio of 1:2 and on the basis of their average weight to give six (6) mating study groups of six animals each (2 males and 4 females). This was to produce pregnant animals, to allow for assessment of litter size at the end of the feeding.

Vaginal smears of females were examined daily for the presence of spermatozoa at gestation day zero. Soon after separation into these mating groups, each of the study groups was placed on a specific diet until the females littered.

*Group 1* - *reference diet (normal animal chow (RC))*
*Group 2* - *pounded yam with soup and stew (PYSS)*
*Group 3* - *boiled yam with stew (BYS)*
*Group 4* - *fried yam with stew (FYS)*
*Group 5* - *boiled yam with palm oil (BYPo)*
*Group 6* - *boiled yam with rat chow (BYC)*

During the mating period, the animals were closely observed for any signs of pregnancy. Pregnant animals were then isolated into separate cages in their different groupings until the pregnancy came to term (littering). The number of litters was then recorded for each animal in each group and was left until the litters were weaned. The parent animals were removed and sacrifice.

**Collection of tissue and blood samples**

At the end of the experimentation, each of the adult rats was anaesthetized in chloroform vapour in a dessicator and dissected using surgical forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into plain sample tubes and were allowed to stand for 2 hrs at room temperature to clot, after which they were centrifuged at 3000rpm for 10 minutes using a bench top centrifuge, (MSE England), to obtain the serum. The sera obtained from the respective samples were carefully removed using Pasteur pipettes, into respective, dry, labeled plastic specimen bottles and stored frozen in a deep freezer until ready for analysis.

**Statistical analysis**

The experimental data were analyzed for statistical significance by one-way analysis of variance (ANOVA) using the SPSS computer-based programme. All data were expressed as mean ± SEM and the probability tested at 95% level of significance ($P<0.05$) so as to establish research hypothesis.

## RESULTS

The results of the hormonal concentration in rats fed *Dioscorea rotundata* are presented in table 3. The data showed that the prolactin levels in the rats ranged from 5.00 ± 0.31ng/ml to 7.17 ± 0.42ng/ml. Luteinizing hormone (LH) ranged from 2.60 ± 0.12 to 6.67 ± 0.38mlU/ml with PYSS fed rats recording the highest concentration. FSH showed

significant (P<0.05) increases between the control and the test diet groups. PYSS fed rats again recorded the highest concentration of FSH (16.67 ± 0.87mlU/ml). The FSH concentration for BYC fed rats 13.20 ± 0.12mlu/ml is similar (P>0.05) to the control group. Testosterone concentration in the control group 0.90 ± 0.06ng/ml was significantly higher (P<0.05) than the test diet groups. The highest estradiol concentration was in PYSS (22.40 ± 0.61pg/ml) with the control having the lowest level 16.87 ± 0.23pg/ml. Progesterone concentration ranged from 31.80 ± 0.61ng/ml for PYSS to 44.43 ± 0.41ng/ml for FYS.

**HORMONAL INDICES OF FEMALE ALBINO WISTAR RATS FED ON CONTROL AND VARIOUS YAM DIETS**

| | Samples | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **RC** | **PYSS** | **BYS** | **FYS** | **BYPo** | **BYC** |
| Prolactin ng/ml | 5.00 ±0.31 | 6.87 ±0.72 | 6.47 ±0.32* | 7.10 ±0.61* | 5.80 ±0.26 | 7.17 ±0.42* |
| LH mIU/ml | 2.60 ±0.12 | 6.67 ±0.38 | 5.10 ±0.20* | 3.80 ±0.06* | 4.00 ±0.12* | 3.33 ±0.09* |
| FSH mlU/ml | 12.57 ±0.19 | 16.67 ±0.87* | 14.37 ±0.27 | 14.60 ±0.23 | 14.30 ±0.17 | 13.20 ±0.12 |
| Testosterone ng/ml | 0.90 ±0.06 | 0.60 ±0.06* | 0.33 ±0.03* | 0.33 ±0.03* | 0.53 ±0.03* | 0.43 ±0.03* |
| Estradiol pg/ml | 16.87 ±0.23 | 22.40 ±0.61* | 19.03 ±0.47* | 18.57 ±0.29* | 21.67 ±0.35* | 17.83 ±0.45 |
| Progesterone ng/ml | 36.63 ±0.95 | 31.80 ±0.61 | 37.10 ±0.70 | 44.43 ±0.41* | 35.47 ±0.41 | 40.17 ±0.99* |

*Values are expressed as mean ± SEM; *P<0.05 vs RC.*

## DISCUSSION

The hormonal concentration of female rats fed the Igboora varieties of *Dioscorea rotundata* or any other varieties is being reported for the first time. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentration levels in the test diet containing 'Ilasa' soup were highest when compared to the other diets and the control. LH is a hormone produced by the anterior pituitary gland. In female, an acute rise in LH triggers ovulation and development of the corpus luiteum *[6]*. Follicle stimulating hormone (FSH) is a glycoprotein that stimulates the growth and maturity of between 10 to 20 follicles in the ovaries each menstrual cycle [7]. In the study on the effect of yam ingestion in healthy post menopausal women, [8] reported no significant changes in LH and FSH levels. [9] reported increase in LH and FSH levels in rats fed on peppermint teas. These reports are in agreement with the results reported in this study. Prolactin stimulates milk secretion but also reduces gonadal activity [6]. The results of the testosterone levels in this study were in agreement with [10] which reported testosterone levels of rats as 2.14 ± 0.32mmol/L on the effect of ovarietomy of the level of plasma sex hormones in albino rats and also in agreement with normal testosterone levels for male adult 3.0 to 10ng/ml and for female in follicular phase 0.2 to 0.8ng/ml, luteal phase 0.2 to 0.8ng/ml and post menopausal phase 0.08 to 0.35ng/ml.

## CONCLUSION

This study showed that with the exception of testosterone which decrease in the rats fed the diets compared with the control, other reproductive hormones showed increases. PYSS recorded higher values for both LH and FSH in comparison to the other diets.

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