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Effect of fluted pumpkin leaf extract on testicular morphometry and testicular homogenate glucose concentration on rabbit bucks

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Target Audience: *Animal physiologists, Rabbit farmers and students.*

Abstract

A study involving 20 grower rabbit bucks which lasted a period of 12 weeks was carried out in a completely randomized design to evaluate the testicular morphometry and testicular homogenate glucose concentration of rabbit bucks fed Fluted Pumpkin (Telfairia occidentalis) Leaf Extract (FPLE). The rabbit bucks were allotted to 4 Treatments containing 0ml of FPLE/litre of drinking water (Treatment 1), 50ml of FPLE/litre of drinking water (Treatment 2), 100ml of FPLE/litre of drinking water (Treatment 3) and 150ml of FPLE/litre of drinking water (Treatment 4). Each treatment was replicated 5 times. The same concentrate diet was fed to the rabbits in all the treatment groups. At the end of the feeding trial, 3 rabbits per Treatment were slaughtered and the reproductive organs of each animal were removed intact and completely and the testes and epididymides were separated. Each epididymis was further dissected into caput (head), corpus (body) and cauda (tail) and each testis, epididymal section and ductus deference were then weighed individually using a sensitive scale. Testicular homogenate glucose concentration (THGC) was evaluated using the homogenate method. Data derived from the study was subjected to analysis of variance (ANOVA) for completely randomized design. The result of the study showed that, Testicular Morphometry of the Rabbit Bucks was significantly ($P<0.05$) different for the left caput and the right ductus among the treatment groups. Testicular homogenate glucose concentration was similar ($P>0.05$) among Treatment groups and increased with increasing levels of FPLE. The study concluded that, FPLE at any level (from 50ml/litre of water) is detrimental to testicular growth.

Keywords: *Rabbit Bucks; Fluted Pumpkin; Testicular Morphometry; Testicular homogenate glucose concentration*

Description of Problem

The Food and Agriculture Organization (1) of the United Nations recommended a minimum of about 56 grams of protein intake per person per day to be consistent with good living of which, about 50 percent (or 28 grams) should come from such animal protein sources as meat, milk, eggs and fish. However, most Nigerians consume less than 10 grams of protein per person per day out of which only about 3.2 grams is animal protein. This is because the growth in the population of livestock products in sub-Saharan Africa is not keeping pace with the growth in human population (2). The expected role of animal production is to fill the wide gap of about 25 grams of daily animal protein intake per person in Nigeria (3).

The demand for protein of animal origin in Nigeria is greater than the supply (4) and there is acute shortage of animal protein

supply in Nigeria hence the need to increase livestock production (5).

The Food and Agriculture Organization (1) has estimated increases of about five to seven percent annually in meat consumption but such increases cannot be met easily by large animals because of their slow production cycles. They may however be met by short-cycle animals like rabbits, poultry and pigs. Poultry and pigs require food sources which are in serious competition with man but rabbits can be produced on forage alone although, production can be improved by the addition of other food by-products (6). The advantages of using the rabbit as the animal of choice for providing sufficient protein for man abound in literature (6).

It has been observed that in the tropical environment, animals reared under the extensive system of animal management seem to thrive better and are usually able to resist some of the endemic diseases to which intensively managed animals continually remain susceptible (7). This apparent resistance has been attributed at least in part to adequate exercise and the possibility of selective and unrestricted grazing whereby, a wide variety of edible forages are available to the animals of which some have been shown to provide not only nutritional but also medicinal benefits (8).

For centuries, plant and plant base products have been used as a valuable and safe natural source of medicines for treating various ailments and the therapeutic potential of most of these plants could be ascribed to their anti-cancer, anti-diabetic, hepatoprotective, cardio-protective, anti-plasmodic, analgesic and various other pharmacological properties (9). The utilization of plant and leaf extracts in animal production has found wide spread scientific and commercial acceptance as a strategy to improve the health status and performance of the animals (10). Leaf extracts have appetizing and digestion-stimulating properties and antimicrobial effects (11). According to Ifon and Basir, (12) leaf vegetables supply minerals, proteins and vitamins which could complement the inadequacies of most feedstuff. (13) reported that, leaf proteins have the potential for supplying good quality food proteins than would be obtained with cereals, legumes and oil seeds and are also, rich in potassium, calcium and magnesium.

Fluted Pumpkin *(Telfairia occidentalis*) is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. The protein from Fluted Pumpkin (*Telfairia occidentalis*) leaves can be harvested and fed to farm animals as solution in form of protein concentrates (14). Fluted Pumpkin is known to be helpful in the protection against atherosclerotic cardiovascular disease (15), stimulation of bone marrow to produce blood cells and maintenance of body resistance to infection and in remedy of all cases of anaemia (16); treatment of hypercholesterolemia, liver problems and impaired immune system (17); treatment of malaria (18) and management of diabetes mellitus (19).

Body and testicular biometric parameters are very important for establishing reproductive patterns and consequently, the development of protocols for assisted reproduction in different species (20). Reproductive organs are not unconditionally necessary for the individual life but they have essential role in the reproduction and genesis of species (21). The knowledge of basic morphometric characteristics of the reproductive organs have been found to provide valuable information in the evaluation of breeding and fertility potential of the animals (22).

Although Fluted Pumpkin may have proved well as a nutrient source, very little is

known on its effect on testicular morphometry and testicular homogenate glucose concentration of rabbit bucks. Therefore, this study was carried out to assess the effect of Fluted Pumpkin Leaf Extract on the testicular morphometry and testicular homogenate glucose concentration of rabbit bucks.

Materials and Methods

Location and experimental site

The experimental site was at the Rabbitary unit of the research farm of the Federal University of Agriculture Livestock Teaching and Research Farm, Makurdi, Benue State. Makurdi is geographically located on latitude 7.73^0 North of the equator and longitude 8.53^0 East of the prime meridian on the map of the world.

Experimental animals and their management

A total of twenty (20) grower rabbit bucks of mixed breeds were used for the experiment. The rabbits were sourced for within Makurdi metropolis. The experimental animals upon arrival on the farm were kept for an adjustment period of two weeks during which they were given dewormers (piperazine) to guard against diseases and treated for ecto and endo parasites using ivomectin (subcutaneous) at the dose of 0.1ml per kilogram body weight. The animals were housed individually in cages, measuring 60x60x50cm, raised 60cm above the ground with a roof over to prevent the direct impact of sun rays and minimize ambient temperature. Drinkers and feeders were provided in each cage and were well fitted to curtail feed and water wastages.

Experimental design

After the acclimatization period was over, the rabbit bucks were randomly allotted to four treatments with five replicates per treatment with each animal serving as a replicate in a completely randomized design.

Preparation of fluted pumpkin leaf extract

Fluted pumpkin leaves for this study were sourced for from Makurdi metropolis. Care was taken to obtain only fresh and tender leaves. The leaves were washed free from sand and other contaminants and drained such that, the water from the washing was negligible. A clean chopping board and a clean knife were then used to chop the leaves into small pieces. Only tender leaves and stalks or vines were used. Chopped leaves and tender vines and stalks were then pound in a mortar and pestle with no water added. After pounding, the extract was got by squeezing in a muslin cloth. The extract was then served to the animals at levels of 0.00ml of FPLE/liter of drinking water to Treatment 1, 50ml of FPLE/liter of drinking water to Treatment 2, 100ml of FPLE/liter of drinking water to Treatment 3 and 150ml of FPLE/liter of drinking water to Treatment 4.

Table 1: Proximate Composition of Fluted Pumpkin (*Telfairia occidentalis*) Leaf Extract

Nutrient	Quantity (%)
Crude Protein	21.42
Ether extract	6.81
Crude fiber	9.86
Ash	9.37
Nitrogen free extract	52.54
Dry matter	5.43
Moisture	94.57

Experimental diet

All the experimental animals irrespective of their treatment group were fed a single basal diet.

The following ingredients were used in compounding the diet; maize, soyabean, rice offals, vitamin and mineral premix, palm oil, salt, bone meal and methionine.

Table 2: Composition of Experimental Diet

Ingredient	Quantity (%)
Maize	30.16
Soyabean	28.12
Rice offal	35.32
Vitamin and mineral premix	0.5
Palm oil	1.0
Bone meal	4.0
Methionine	0.4
Salt	0.5
GE Kcal/kg	2713
CP	15.59
CF	13.88
EE	9.24
Calcium	1.60
Phosphorus	1.10
Ash	8.43
Lysine	0.81
Methionine	0.63
Arginine	1.04

GE= Gross energy, CP= Crude protein, CF= Crude fiber, EE= Ether extract

Table 3: Proximate composition of the experimental diet

Nutrient	Quantity (%)
Crude Protein	15.58
Ether extract	8.61
Crude fiber	11.78
Ash	17.89
Nitrogen free extract	46.14
Dry matter	93.64
Moisture	6.36

Parameters measured.

Testicular morphometry

Testicular morphometry was done as reported by (23). The reproductive organs were removed intact and completely from the animals immediately after slaughter. The testes and epididymides were dissected, trimmed free of fat and adhering connective tissues. The left and right sides of each epididymis were further dissected into caput, corpus and cauda. Each testis, epididymal section and ductus deference were then weighed individually using a sensitive scale and the results were recorded in grams.

Testicular homogenate glucose concentration

Testicular homogenates were obtained

by first taking the weights of each testis, epididymal section and ductus deferens after which, they were separately and completely macerated in normal saline at 0.1g per ml of normal saline according to Bitto and Egbunike (23) and the suspensions mixed and strained through a double layer of gauze into graduated tubes and stored in a refrigerator. Testicular homogenate glucose concentrations were determined by the method of (24). Values were expressed in mmol/L.

Statistical analysis

Data obtained from this study was subjected to the analysis of variance (ANOVA) for completely randomized designed (CRD) and where significant difference(s) occurred, the means were separated using the Duncan multiple range test (DMRT) as outlined by (25).

Results and Discussion

The result of the proximate composition of FPLE is presented in Table 1. The crude protein content of 21.42% obtained in the present study was similar to the values of 21.80%; 21.31% and 22.40% reported by (26); (27) and (28) respectively but lower than the value of 30.50% reported by (29). The crude fiber content of Fluted Pumpkin Leaf Extract in the present study was 9.86%. It was higher than the value of 5.50% obtained by (27) but similar to the values of 10.10% and 8.5% reported by (28) and (29) respectively. The ash content of Fluted Pumpkin Leaf Extract in the present study was 9.37% and it was similar to the values of 8.40% and 10.92% reported by (29) and (27) but slightly lower than the value of 12.60% obtained by (28). The ether content of 6.81% obtained for Fluted Pumpkin Leaf Extract in the present study was slightly higher than the value of 5.50% reported by (27). The discrepancies in values for the proximate composition of Fluted Pumpkin Leaf Extract in the present study to those of other workers may probably have been due to variety, age at which leaves were harvested, processing methods employed on the leaves to get the extract, soil type and soil fertility level (30).

Testicular Morphometry

Table 4: Reproductive Tract Morphometry and Testicular Homogenate Glucose Concentration THGC) of Rabbit Bucks Fed Graded Levels of Fluted Pumpkin Leaf Extract (mean ± SEM)

Parameter (g)	T1(0)	T2(50)	T3(100)	T4(150)
Left testis	2.13 ± 0.12	1.73 ± 0.07	2.03 ± 0.15	1.83 ± 0.12
Right testis	1.87 ± 0.20	1.80 ± 0.06	1.80 ± 0.17	1.87 ± 0.13
Paired testes	4.00 ± 0.25	3.53 ± 0.12	3.83 ± 0.28	3.70 ± 0.25
Left caput	0.27 ± 0.03^{a}	0.37 ± 0.03^{a}	0.20 ± 0.06^{b}	0.30 ± 0.06^{a}
Right caput	0.30 ± 0.00	0.30 ± 0.06	0.30 ± 0.06	0.37 ± 0.07
Paired caput	0.57 ± 0.03	0.67 ± 0.09	0.50 ± 0.12	0.67 ± 0.09
Left corpus	0.17 ± 0.03	0.17 ± 0.03	0.17 ± 0.03	0.13 ± 0.03
Right corpus	0.17 ± 0.03	0.17 ± 0.03	0.13 ± 0.03	0.13 ± 0.03
Paired corpus	0.33 ± 0.07	0.33 ± 0.07	0.30 ± 0.00	0.27 ± 0.03
Left cauda	0.40 ± 0.10	0.27 ± 0.03	0.30 ± 0.00	0.33 ± 0.03
Right cauda	0.43 ± 0.03	0.37 ± 0.03	0.33 ± 0.07	0.37 ± 0.03
Paired cauda	0.83 ± 0.12	0.64 ± 0.07	0.63 ± 0.07	0.70 ± 0.06
Left ductus	0.23 ± 0.03	0.27 ± 0.07	0.30 ± 0.00	0.30 ± 0.06
Right ductus	0.30± 0.00^{a}	0.20 ± 0.00^{b}	0.27 ± 0.03^{a}	0.33 ± 0.07^{a}
Paired ductus	0.53 ± 0.03	0.47 ± 0.07	0.57 ± 0.03	0.63 ± 0.12
THGC	49.43 ± 5.11	55.00 ± 4.72	57.17 ± 5.15	58.08 ± 8.99

a,b = Means on the same row with different superscripts differed significantly ($P < 0.05$)
SEM = Standard Error of Mean
THGC = Testicular Homogenate Glucose Concentration
0, 50, 100, 150 = Levels of inclusion of FPLE in milliliters

The result of the testicular morphormetry of rabbit bucks fed graded levels of FPLE is as shown in Table 4 above. For the testes, the result shows that there were no significant effects (P>0.05) of FPLE on the weights of the left testis, right testis and paired testes however, rabbit bucks which were not given FPLE (Treatment 1) recorded the highest values for the left testis, right testis and paired testes while for the epididymis, FPLE had a significant effect (P<0.05) on the weights of the left caput and the right ductus with no significant effects (P>0.05) on the weights of right caput, paired caput, left corpus, right corpus, paired corpus, left cauda, right cauda, paired cauda, left ductus and paired ductus. In the left caput, Treatment 3 was significantly lower than (P<0.05) all the other Treatments while in the right ductus, Treatment 2 was significantly lower than (P<0.05) Treatment 1, Treatment 3 and Treatment 4. Body and testicular biometric parameters are very important for establishing reproductive patterns and consequently, the development of protocols for assisted reproduction in different species (31). Testis size is a good indicator of the present and future spermatozoa production of an animal (32). Larger testes (without any abnormality) have been reported to produce more spermatozoa than smaller testes (33). With regard to reproductive characteristics, a positive correlation has been reported between testicular weight and sperm production (34). Tubular measurement is thus, the approach traditionally used as indicator of spermatogenic activity in investigations of testicular function (35).

The findings of this study showed that, Fluted Pumpkin had a reducing effect on testicular size. In testicular parameters where significant differences (P<0.05) occurred, Treatment 1 which served as the control though similar (P>0.05) with some treatments, was significantly higher (P<0.05) than others. This finding is in accordance with the findings of (36) which showed that, Fluted Pumpkin Leaf Extract when administered to wista rats, had a reducing effect on weights of the testes and also, a reducing effect on semen quality, plasma testosterone values and had potentials for testicular cell damage in a dose dependent fashion. Though Fluted Pumpkin Leaf Extract had no significant effect (P>0.05) on the left testis, right testis and paired testes, these parameters showed apparent decline in weights with increasing levels of Fluted Pumpkin Leaf Extract inclusion.

The significant effect of Fluted Pumpkin Leaf Extract on the weights of the left caput and right ductus may affect the proper functioning of the epididymis as (37) reported that, sperm motility and the ability of the buck to store spermatozoa was negatively affected as the weight of the epididymis reduced in rabbit bucks fed graded levels of neem (*Azadirachta indica*) leaf meal.

Testicular homogenate glucose concentration

The result of the testicular homogenate glucose concentration is also shown in Table 4. Testicular homogenate glucose concentration in the present study showed no significant difference (P>0.05) between treatments. Values ranged from 49.43 ± 5.11 to 58.07 ± 8.99 mmol/L and followed an increasing trend across treatments.

According to (38), glucose has been reported to be beneficial to sperm for optimal capacitation and fertilization. Sperm capacitation is a complex process which involves profound structural and functional changes in male gamete thereby, preparing it for acrosome reaction and egg fertilization (39). Fluted Pumpkin had no significant effect (P>0.05) on the testicular homogenate

glucose concentration in spite of the apparent increase in testicular homogenate concentration with increasing levels of Fluted Pumpkin Leaf Extract inclusion.

Conclusion and Applications

1. The findings of this study showed that, the introduction of Fluted Pumpkin Leaf Extract failed to cause any significant increase in testicular weight.
2. The inclusion of Fluted Pumpkin Leaf Extract had significant effects on the weights of the left caput and right ductus. In all segments of the epididymis studied, the Rabbit Bucks in the control group had similar weights with Rabbit Bucks in the other groups and where significant differences ($P<0.05$) occurred, the Rabbit Bucks in the control group had higher weights than some of the treatments.
3. The inclusion of Fluted Pumpkin Leaf Extract in the drinking water of rabbit bucks had no significant effect on testicular homogenate glucose concentration although, there was an increasing trend in values across treatments as the level of Fluted Pumpkin Leaf Extract increased.
4. It is not recommended to give Fluted Pumpkin Leaf Extract at any level to breeding rabbit bucks as shown by reduced testicular growth as well as reduced growth of all the segments of the epididymis.
5. To reduce the cost of livestock feeding, further studies are recommended on the huge quantity of by-products generated from the preparation of Fluted Pumpkin Leaf Extract.

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