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The reproductive tract morphometry and gonadal sperm reserve of rabbits (bucks) fed cassava peel meal diet as replacement for maize in the south- south region of Nigeria

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Target Audience: *Nutritionists, Animal Reproductive Physiologists*

Abstract

The reproductive tract morphometry and gonadal sperm reserve of Rabbits (Bucks) fed cassava peel meal diet as replacement for maize in the South- South region of Nigeria was determined, with 60 crossbred pre-pubertal rabbit of mixed sexes (30 bucks and 30 does) with an initial weight of 698.34±0.02g were used in a feeding trial that lasted for 9 weeks for the growth phase and another 16 weeks for pubertal/ Reproductive phase. Five experimental diets were formulated during the two phases. The diets Comprise 0, 25, 50, 75 and 100% replacement levels of cassava peel meal for maize and were assigned to T_1 (Control), T_2, T_3, T_4, and T_5 respectively. The rabbits were randomly distributed to different treatments groups after equalizing for weight; using a completely randomized design (CRD). All data obtained were subjected to the one way analysis of variance (ANOVA). Results for the Epidydimal and Testicular morphometry showed a non-significant (P>0.05) effect for absolute and relative weight values, while the total sperm reserve was significantly (P>0.05) affected by dietary treatment with treatment 5 having the least total sperm reserve. However, the testes volume showed a non-significant effect. The study concludes that the cassava peel meal diet as replacement for maize, adversely affected the total sperm reserve and testes volume as the level of replacement increased in the study.

Keywords: *Rabbits, Buck, Epidydimal, Morphometry, Testis.*

Description of problem

The quality of feed and plain of nutrition given to a male animals coupled with its physiological status can affect libido and the amount and quality of sperm produced (1). Nutrition is an important component to be considered when assessing the reproductive efficiency of animals, nutrition is considered an important aspect in the adjustment of the body conditions (2). The connection between nutrition and reproduction cannot be overstressed, since their interface is known to have a direct correlation with reproductive performance (3). Watches et al. (4) reported that high productive animals, most be nutritional balanced so as to achieve high reproductive and productive quotient. Feeding is the most critical and expensive part of any research, this is made more complicated with the unending competition in the use of conventional feed resources, due to the astronomical increase in human population. Issues with the affordability of the conventional feed stuff, has prompted researchers to channel their time and energy in seeking for alternative feed resources, as ingredient in ration formulation, ((5);(6)). Availability of these alternative feed resources is a crucial yardstick in determining their usability. In recent years, many novel agro industrial waste that would have posed a nuisance to the environment and add up to the issue of ozone layer

depletion have been harnessed, processed and used as valuable feed resources. Cassava peel an agro industrial waste found commonly around areas with garri and fufu processing mills are cheap and available all year round. Cassava peel meal can be incorporated into the rabbit diets after proper processing (fermentation, sundrying, boiling, frying) which reduces the presence of cyanogenic glucoside, the anti nutritional component of cassava (7). Incorporating it into animal feeding will not only reduce the environmental pollution but also reduce the cost of rabbit production. Cassava peel contains 10-30% crude fiber, 4-7% crude protein, 69 soluble carbohydrate. (8).The pressing issues of food insecurity among poor and rural communities especially in Africa and Asia cannot be addressed using traditional livestock (cow, sheep and goat) due to their long reproductive cycle. Rabbit with its short generation interval can be successfully used to bridge the gap of low protein intake among developing countries (7) thereby reducing the issue of hunger and malnutrition and enhancing livelihood security (9) . rabbit has a short gestation period of 30-31 days, easing their usage for quick multiplication (10). The meat from rabbit has being acknowledged as 'healthy'containing low cholesterol suitable for people with cardiovascular diseases. Rabbit with its added advantage as a pseudo-ruminant is able to digest efficiently waste of high fibre sources such as yam peel, cocoa pod, husk, hay and agro by-products (11). The constant hike in the price of feedstuff in the livestock industries has prompted researchers and feed millers to intensify their search for alternative feed sources (12) The use of rabbit as a source of protein is gaining acceptance among Nigerians (13).. Rabbits with its outstanding qualities can be raised successfully with the use of cassava peel meal diet. The research was aimed at assessing the reproductive tract morphometry and gonadal sperm reserve of rabbit buck fed cassava peel meal diet as a replacement for maize in the humid tropics.

Materials and Methods

Location of the Study:

The study was carried out at the Rabbitry unit of the Teaching and Research Farm, Department of Animal Science, University of Calabar, Calabar, Cross River State, Nigeria. According to the GeoNames geographical database by Google Earth (2012), Calabar is located at 4.9517^0 latitude and 8.322^0 longitude with an average elevation/altitude of 42 metres. The annual rainfall ranges from 3000-3500mm and the average daily temperature is $25^0C/77^0F$ increases to 30^0C (86^0F) in August. The relative humidity is between 70-80% while the wind speed/direction is 8.1km/h West and the cloud is broken at 1000ft with little cumulonimbus at 2200ft. The time zone in Calabar is Africa/Lagos.

Processing of the test ingredient (Cassava Peel Meal, CPM)

Fresh cassava peels were collected from cassava processing locations in Odukpani and Akamkpa, washed and sun dried for seven to eight days and milled using a hammer mill. The milled cassava samples were stored in sterile polythene bags at room temperature of 23^0C and kept to be used as replacement in the test diets. Cassava peel meal served as the test ingredient while the major feed ingredients were maize, full fat soybean, rice husk, palm kernel meal, and crayfish dust. The proximate composition of sun dried cassava peel meal was determined using the (14) method.

Experimental Diets

Five (5) experimental diets were formulated each comprising of 0, 25, 50, 75

and 100% in the ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 maize: sun dried CPM respectively. Each of the dietary treatments were assigned T_1, T_2, T_3, T_4, and T_5 respectively. Treatment 1 served as the control diet. The proximate composition of the experimental diets was carried out based on the procedures outlined by (14). The composition of experimental diets is presented in Table 1.

Table 1: Gross Composition of the Experimental diets (Reproductive phase)

Ingredient	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Maize	30.00	22.50	15.00	7.50	0.00
SBM	12.70	15.60	17.60	18.60	19.79
Cray fish dust	4.00	4.00	4.00	4.00	4.00
PKC	7.00	11.10	12.10	12.80	12.91
Wheat offal	15.00	10.00	9.00	9.20	7.00
Rice husk	25.00	23.00	21.00	19.10	20.00
Cassava peel meal	0.00	7.50	15.00	22.50	30.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Premix	0.20	0.20	0.20	0.20	0.20
Salt	0.10	0.10	0.10	0.10	0.10
Palm oil	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
%CP	16.21	16.95	17.17	17.43	17.33
%CF	9.76	10.05	10.31	10.61	10.47
ME (Kcal/kg)	2,482.05	2,391.53	2,279.28	2,155.90	2,022.02

Gross Composition of Bio –Super Premix per Kg; Vitamin A-1,500,000IU; Vitamin D3-300,000IU; Vitamin E-400mg; Vitamin B2-400mg; Vitamin B12-2,000mg; Nicotinamide-2,000mg; Calcium D Panto thenate-800mg; Choline Chloride-40,000mg; Ferrous Sulphate-2,000mg; Manganate Sulphate-5,000mg; Copper Sulphate-80mg; Zinc Oxide-3,000mg; Cobalt sulphate-10mg; Potassium Iodide-120mg; DL-Methionine-10,000mg and Antioxidant-18,000mg. The premix was manufactured by Bio-Phamachemic Company HCM city, CF-Crude fibre, CP- Crude Protein, SCPM- Sun dried cassava peel meal, SBM- Soybean meal, PKC-palm kernel cake, ME-methabolizable energy. Note well that 0%CPM is the control diet. T1 (0%), T2 (25%), T3 (50%) T4 (75%) T4 (100%)

Experimental Animals and Management:

A total of sixty (60) (30 does and 30 bucks) crossbred weaner rabbits aged between 5-6 weeks old with an average initial weight of 559.58 + 0.42g were used for the study. The rabbits were obtained from standard rabbitries in Uyo and Calabar. Immediately on arrival the rabbits were given vitalyte an anti-stress medication via drinking water. The rabbits were then randomly distributed into five different treatment groups after standardizing for weight, with a stocking ratio of 4 bucks to 8 does. Each rabbit occupied a compartment to make up a replicate. The rabbits were conditioned by providing prophylactic medication using anti-biotics and vitamins against bacterial diseases and also health screening against ecto and endo parasites. The rabbits were fed with commercial diet, before the experiment commenced a small quantity of forage and water was given ad libitum. All these procedures took two weeks within which the rabbits acclimatized to the environment.

The actual feeding trial proceeded thereafter, initially for nine weeks during which their pre – pubertal growth performance was determined. During this period a weighed amount of feed were given daily, morning and evening and leftover weighed to determine the daily feed intake, daily, fresh and clean water was supplied ad libitum. Then the rabbits were now kept for another sixteen (16) weeks for evaluation of haematological and reproductive parameters.

Housing and Equipment

The animals were housed in wooden double tier cages, covered with wire mesh and measuring 65cm X 65cm X65cm and raised 25cm from ground level. The cages and the rabbitry unit were thoroughly washed with disinfectant and allowed for seven days to dry before animals were brought in. A concrete watering trough and a fabricated feeding trough were placed in each hutch.

Experimental Design:

The animals were assigned to experimental diets using a completely randomized design, with 12 rabbits per treatment (6 bucks and 6 does). All data obtained in this study were subjected to one way analysis of variance (ANOVA) using completely randomized design. Means were compared using Duncan New Multiple Range Test. (15).

Reproductive Performance and Gonadal Sperm Reserve

The reproductive parameters determined for the buck included. Weights of testes and epididymis (caput, corpus and cauda), gonadal sperm reserves were determined haemocytometrically by homogenate technique, using a modification of the method of (16), (17), and (18) as described by (19). Daily sperm production was obtained by dividing Gonadal sperm reserve by 3.56 (time in days of the duration of the seminiferous epithelium cycle) while total sperm output was calculated by multiplying sperm volume by the concentration as reported by (20) and (21).

Reproductive Tract Morphometry:

Six rabbits per treatment were dissected and the reproductive tract obtained immediately after slaughter, trimmed of fat and adhering tissues and subjected to morphometric analysis as described by (22). All weights were determined using an electronic precision scale (Kerro model) while linear measurement were obtained with measuring tape and a glass rule respectively.

Result and Discussion

Reproductive tract morphometry of rabbits (Bucks)

Epididymal morphometry:

The epididymal morphometry showed a non- significant ($P>0.05$) effect for absolute and relative weight values. The epididymal weight ranged between 0.45 and 0.59g, 0.22 and 0.30g, 0.18 and 0.27g and 2.32 and 4.33g for the caput, cauda, corpus and paired epididymis respectively. The weight recorded in this study were lower than the values of 1.19 and 1.44g and 1.08 and 1.32g for the left and right epididymis but higher than the values of 2.23 and 2.76g for paired epididymis documented by (23).

The length of the epididymis ranged between 10.50 and 11.00 cm, 3.14and 4.13cm, 3.02 to 4.56cm and 3.11 to4.10cm for entire, caput, cauda and corpus. (24) showed the importance of having a basic idea of the morphometric features of the male reproductive organs, since it is necessary for the evaluation and forecast of the sperm creation, storing ability and

fertilizing ability of the breeder male. The replacement of maize with cassava peel meal in the study did not adversely affect the epididymal morphometry of rabbits. Since the epididymis is the site of gamete maturation and storage before ejaculation, it can therefore influence sperm maturation (23)

Testes morphometry:

The weight of the testis of rabbits in this study were not significantly (P>0.05) affected by dietary treatments. The values ranged between 10.13 and 12.94 g, 0.46 and 0.62 g and 2.24 and 2.78ml for paired testes (absolute and relative) and testis volume respectively. The values for this study were higher than the values of 2.94 -3.26g and 3.89 – 4.17 g and 1.47 -1.80 ml reported by (23) , (25) respectively for rabbits, but similar to the value of 2.13 – 2.22 ml reported by (23) for paired testis volume; (26),(18) opined that testicular weight and sperm production are highly related.

The testicular length was "not significantly (P>0.05) affected by the dietary treatments". The values ranged between 6.19 and 7.80cm. The values were lower than the ranges of 8.07 – 9.99 cm reported by (23). The testicular size of an animal is relative to the age of the animal (27). The testicular size has been identified as a good indicator of sperm producing ability of an animal (28).Therefore the mammalian testes are accurate predictors of spermatozoa production (24).

Table 2: Reproductive Tract Mephometry of Rabbit Bucks fed Varying Levels of Cassava Peel Meal Based Diet.(Pubertal Average Weight of Bucks).

PARAMETER	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)	S.E.M
Relative Weight of Reproductive Organs (% Live Weight Of Bucks):						
Caput Epididymis	0.02	0.02	0.02	0.02	0.02	0.01
Corpus Epididymis	0.02	0.01	0.01	0.01	0.01	0.01
Cauda Epididymis	0.01	0.01	0.01	0.01	0.01	0.01
Paired Testes	0.51	0.46	0.58	0.62	0.46	0.24
Paired Epididymis	0.18	0.10	0.13	0.11	0.19	0.26
Linear Measurement (Cm)						
Testicular Length	6.19	6.29	7.80	6.49	6.68	0.38
Entire Epididymis Length	11.00	10.50	11.26	10.66	10.53	0.68
Caput Epididymis Length	4.13	4.06	3.96	4.10	3.14	0.20
Cauda Epididymis Length	3.02	3.05	4.56	3.45	3.30	0.24
Capus Epididymis Length	4.03	3.60	4.23	3.11	4.10	0.28
(Absolute weight in g)						
Average Live Weight Of Buck (g)	2250±229.12	2170±147.30	1983±104.08	2066.66±115.47	2213.33	80.66
Caput Epididymis (g)	0.59	0.54	0.55	0.45	0.49	0.45
Cauda Epididymis (g)	0.30	0.32	0.22	0.25	0.26	0.30
Corpus Epididymis (g)	0.27	0.18	0.22	0.22	0.19	0.11
Paired Testies (g)	11.57	10.13	11.52	12.94	10.22	0.79
Paired Epididymis (g)	4.31	2.32	2.62	2.40	4.33	0.59
Testes Volume (Ml)	2.24	2.78	2.67	2.63	2.58	0.32
Gonadosomatic Index (GSI) %	0.51^a	0.46^b	0.58	0.62^a	0.46^b	0.21

^{a,b,c} Means in the same column showing different superscripts are significantly different (P<0.05)
S.E.M: Standard Error of Mean

Gonadal Sperm Reserve

The result of the sperm and gonadal sperm reserve characteristics of rabbit buck fed cassava peel meal diet are shown on table 3. Sperm volume was significantly ($p<0,05$) different across treatment groups treatment 1 had a higher volume of 0,80 while treatment 5 had the the least, The volume of sperm decreased significantly as the level of CPM replacement increased, The result obtained in the study were 0,80, 0,75, 0.60, 0.55 and 0.45 for treatments 1, 2, 3,4 and 5 for rabbits fed cassava peel meal diets respectively. The gonadal sperm reserve showed a significant ($p<0.05$) difference between the treatments groups. The results obtained in the study were 581.96, 605.12, 428.66, 515.42 and 512.00 ml for rabbits fed diets1, 2, 3, 4 and 5 respectively. The gonadal sperm reserve in this study, showed a fluctuating trend between treatments group. The total sperm reserve "was significantly ($p<0.05$) affected by the dietary treatment". Treatment 1 has the highest total sperm reserve of 465.47and treatment 5 had the least total sperm reserve of 230.50ml for rabbits fed diets 1, 2, 3, 4 and 5 diets The daily sperm production showed a significant ($p<0.05$) difference between the dietary treatment groups. The results obtained were 163.47, 169.97, 120.4, 144.78 and 143.82 ml for rabbits fed diets1, 2, 3, 4 and 5 respectively. The testes volume was not significantly ($p>0.05$) different among dietary treatments. The values obtained in the study were 2.24, 2.78, 2.67, 2.63 and 2.58ml for rabbit fed diets 1, 2, 3, 4 and 5

Testes volume and gonadal sperm reserve characteristics of rabbit buck fed cassava peel meal-based diets

The testes volume and total sperm reserve in this present study indicated "significant ($p<0.05$) difference between the dietary treatments" . Gonadal sperm reserve and daily sperm production showed a significant ($p<0.05$) difference across dietary treatment though with a fluctuating trend. Testes volume decreased as the level of CPM replacement increased in the study. In domestic animals, there is a close relationship between testicular weight and sperm reserves (28).

Table 3: Sperm and Gonadal Sperm Reserve Characteristics of Rabbit Buck fed Cassava Peel Meal Based Diets

Parameter	T_1(0%)	T_2(25%)	T_3(50%)	T_4(75%)	T_5(100%)	S.E.M
Sperm volume (ml)	0.80^{a}	0.75^{a}	0.60^{b}	0.55^{bc}	0.45^{bc}	0.38
Gonadal sperm reserve ($x10^6$/ml)	581.96^{a}	605.12^{a}	428.66^{b}	515^{b}	512.00^{b}	16.91
Total sperm reserve ($x10^6$/ml)	465.47^{a}	454.11^{a}	255.41^{c}	283.37^{b}	230.50^{b}	17.5
Daily sperm production ($x10^6$/ml)	163.47^{a}	169.97	120.41^{c}	144.78^{b}	143.82^{b}	4.35

^{abc} means on the same row with different superscript are significantly different
S.E.M: Standard Error of Mean

Conclusion and Application

1. The testes morphometry were not adversely affected by the replacement of maize with cassava peel meal.
2. The gonadal sperm reserve was affected as the level of cassava peel meal replacement for maize increased. Treatment five had the least total sperm reserve.
3. Testes volume also decreased as the level of cassava peel meal diet increased in the study

4. 50 percent replacement of cassava peel meal diet for maize is recommended as it does not adversely affect the male reproductive functions.

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