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Growth and Testicular Spermatozoa Reserve Potential of Pre-pubertal Rabbits Fed With Diet Containing 16% Bovine Rumen Digesta-Blood Meal Mixture (50:50) In Combination With 18% Cooked Macuna Bean Meal

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Abstract

The growth response of pre-pubertal rabbits and their gonadal sperm reserve potential at pubertal stage when fed diets containing a combination of 16% (50:50) bovine rumen digesta-blood meal (BRDBM) mixture and 18% cooked mucuna bean (CMBM). This was in replacement of 50% of the maize and 50% of the groundnut cake of a pre-pubertal control diet. Twenty-four male rabbits of mixed breed aged between 6-8 weeks and body weight range 900-915g were randomly allocated to two diets: the control (diet 1) and the experimental (diet 2). Daily feed intake (72.4±7.5g Vs 64.0±9.1g), total weight gain (518±1g Vs 406±18g) and Terminal live weight (1427±48g Vs 1317±41g) were significantly (P<0.05) higher in the control than experimental group. Feed to gain ratio in group 1 was marginally lower (7.5±2.6g Vs 8.5±1.2g) than for diet 2. However, dressing percentage (68.6±7.0% Vs 75.4±9.0%) was significantly higher in the rabbits on diet 2 than diet 1. The cost per kg of diet (N47.0±0 Vs N40.6±0.0) as well as the cost per kg live weight gain (N352.6±0.1 Vs N344.5±0.1) were significantly lower for diet 2 than diet 1. The paired testes weight (1.8±0.6g Vs 1.9±0.4g), daily sperm production (3.6x 10^8 Vs 3.8x 10^8) and testicular spermatozoa reserve (12.2±5.3x10^8) were marginally higher in rabbit on diet 2 (experimental) than diet 1 (control). The paired epididymal weight for rabbits on diet 2 was significantly (P<0.05) higher (0.6±0.3g Vs 1.2±0.3g) than those on diet 1, indicating more efficient sperm storage. The haematological measured were not affected (P>0.05) by the dietary treatments. The non-significant difference (P>0.05) in the efficiency of sperm production and sperm reserve between the two diets confirmed that the inclusion of these materials in the diet of rabbits supports the ventures of increasing animal protein sources through cheaper rabbits production process in Nigeria.

Key words: Cooked mucuna bean meal, Growth, Daily sperm Production, rumen digesta-Blood meal, Sperm reserves.

Introduction

Like many developing countries, Nigeria has for long been plagued with the problem of worsening situation of inadequate consumption of animal protein. With the continuous rise in the cost of beef, chicken and mutton, there is need for a greater emphasis on the development of other less common, but potentially sustainable sources of animal protein for the populace.

A panacea for the protein shortage is to target prolific livestock species with short reproductive cycle, high fecundity but with high (21%) crude protein and low (5.40%) crude fat content in its meat (Das and Bujarbarua 2005). When compared with meat from other species, the fat in rabbit meat contains less stearic and oleic acids but a high proportion of the essential polyunsaturated linolenic and fatty acids (Adrian *et al* 1981). The crude fat in rabbit meat is lowest (8%) when compared with beef (35%), mutton (31%), Pork (29.5%) and Chicken (12%) (Lebas *et al* 1986). Rabbit meat is recommended for cardiovascular patient and those prone to arteriosclerosis (Odunwo, 1992). According to Cheeke (1986) a small rabbitry unit of about 3-6 does and a buck is sufficient to meet the animal protein requirement of an individual family.

Mucuna pruriens, commonly known as velvet bean, is a legume well adapted to hot than cold regions (Bodgan, 1977). According to Iyayi, (2002) mucuna is currently unharnessed but has potentials in livestock feeding.

As a livestock feed, Iyayi and Egharevba (1998) reported on the successful use of the seeds for feeding monogastric animal. Aletor and Aladetimi (1989) reported on the improvement in the nutrient quality of mucuna seeds by soaking, germination and heat treatment. These processing methods these inactivate, reduce or completely destroy the inherent anti-nutritional component, which constitute the major hindrance to the utilization of the legume seeds. Iyayi (2002) reported better performance in layers with roasted or autoclaved mucuna seed and that at 6.0% in diet; it produced a performance that compared favourably with a diet solely based on soybean meal. Cattle rumen content, a waste of high nuisance value in abattoirs, is considered to be higher in proteins and vitamins than the initial ingested fodder on which the animal has been maintained (Mann, 1962). It can be prepared as livestock feed ingredient either by sun drying or ensiling and its nutritive value as well as blood-rumen has been experimented in feeding trials with monogastics without any negative effect on performance (Mann 1984; Dairo and Aina 2000, Adeniji and Balogun 2001, 2002). However, according to Olajide (1990), its high crude fibre content hinders digestibility and availability of other nutrients for growth and development resulting in poor performance.

Bloods meal has been reported to contain 80% protein but has low palatability and digestibility, hence low acceptability (Wahlstrom and Lubal, 1997). When not well processed or when taken in large quantity, it depressed digestibility of crude fibre as well as caused the production of watery feaces, (Adeyanju 1989 ; Mc Donald *et al.*, 1981).

A major aspect of production is the emphasis on the reproductive status of the animal which directly influences stock population increase and hence animal protein availability. Swierstra (1996) had implicated genetic, nutrition and environmental factors as determinants of growth, development and efficient functioning of the reproductive organs in animals. Sexual maturity is delayed by poor nutrition during growth.

This study investigated the feeding value of cooked mucuna bean meal (CMBM) in combination with bovine rumen digesta/blood meal (BRDBM) mixture as an unconventional

feeding stuff for animal feeding and the resultant lowering of cost of production. It was also designed to evaluate the impact of their inclusion in feed on the sperm reserve potential of pubertal rabbits in a derived Savanna environment of Nigeria.

Materials and Methods

Location

The experiment was conducted at the Rabbitry of Ladoke Akintola University of Technology Teaching and Research farm in Ogbomoso. The study area is located on Latitude 8° 07' N and 80 12' N and Longitudes 4° 04' E and 4° 15'E. The mean annual rainfall is 1247mm with a relative humidity of between 75 and 95%. Ogbomoso is situated at about 600m above sea level with a mean annual temperature of 26.2°C. The vegetation is the derived savannah (Oguntoyinbo, 1978).

Animal and their management

Twenty four (24) bucks were used with the ages ranging between 6-10 weeks. The rabbits were weighed, balanced to about equal mean weight in two groups of twelve bucks each. Each group was further sub-divided into two replicates of six rabbits housed individually in metabolic cages. The control (group) received normal compounded rabbit feed while the experimental group received 16% bovine rumen digesta/blood meal (BRDBM) mixture (50:50) in combination with 18% cooked mucuna bean meal. Bovine rumen digesta was collected from freshly dressed cattle; sun dried to a moisture content of below 15% and mixed with dried commercially produced blood meal in the ratio of 50:50, to represent 16% of the experimental diet. The blood meal was purchased from Bovajah Farms and Agro-allied services, a reputable commercial feed mill located in Ogbomoso North Local Government area, Oyo State, Nigeria. The proximate composition of the blood meal used was: dry matter 89.76%, Crude protein 78.85%, Crude Fibre 1.46%, Ether extract 0.72%, Ash 3.96% and Nitrogen free Extract 15.01%. Mucuna beans collected from Iresa Adu in Ogbomoso were cooked at 96°C for one hour, sun dried and milled as cooked mucuna bean meal (CMBM) to represent 18% of the diet. Both ingredients, in combination, replaced 50% of the groundnut cake of the control diet (Table 1). Feeds were offered twice daily to prevent wastage and to give a total of 100g per rabbit per day. Weight of feed remnant was subtracted daily from quantity fed, to record the feed consumed. The rabbits were weighed at the commencement of the study and thereafter at two-weekly intervals, to determine weight gains during the experiment.

Proximate analysis of the feeds was carried out by the procedures itemized by AOAC (1990) and presented in Table 2.

Feed conversion efficiency: This was calculated as the quantity of feed that produced 1kg of weight gain, i.e. $\frac{\text{Total fed intake}}{\text{Total Weight gain}}$

Blood collection and analysis

During the 10th week of experiment, blood samples were collected from the ear vein of the rabbits,

using hypodermic syringe and needle. Blood was collected into tubes containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. The packed cell volume (PCV) was determined by the Cyanomethaemoglobin method (Dacie and Lewis 1991). Heamoglobin concentration was determined by the Cyanomethaeglobin method (Kelly, 1979). Red blood and white blood cell counts were computed using improve Neubauer heamocytometer. Mean corpuscular volume (MCV), Mean Corpuscular Heamoglobin (MCH) and Mean Corpuscular Heamoglobin Concentration (MCHC) were derived as outlined by Jain (1986).

Carcass analysis and organ weights

The rabbits were starved overnight preceding slaughter and weighed at the point of slaughter. The rabbits were slaughtered by cutting the jugular vein. The carcasses were scalded, the viscera organs (kidney, liver, spleen, lungs and heart) were carefully removed, blotted free of blood and weighed. The organ weight were expressed as a percentage of live weight. The left hind leg of each rabbit was cut and the muscle separated from the bones. The muscles were dried at 800c and ground for proximate composition by the procedure of AOAC (1990).

Dressing Percentage: This was calculated as the difference between live weight at the time of slaughter and the post slaughter weight after removal of the fur and inedible offals.

Reproductive organs measurements

The scrotal parameters were measured in order to ascertain if there is any relationship between them and sperm production. The scrotal length and width were taken as the length and width of the testes while in the scrotum, as they form the boundaries on which the veneer calipers were fixed. The scrotal skin fold thickness was taken as the thickness of the skin. The testes were removed immediately after slaughter and trimmed free of the epididymides. They were individually weighed and their separate volumes determined by Archimedes principle of water displacement. The tunica albuginea of each testis was removed and weighed, and the weight of each testis parenchyma was also recorded. The epididymides were weighed. The testes parenchyma was stored in the deep freezer for 48h.

The frozen testes were thawed out and from each testis parenchyma, a known weight was put in a breaker containing saline solution at 200mg/ml and macerated with a pair of sharp pointed scissors (Egbunike *et al*, 1976). The resulting homogenate was strained through cheesecloth solution drops from this were introduced into a Numbeaur heamocymeter, where the spermatozoa and late spermatids were counted in four diagonals and the centre cells of each field. Ten fields were counted and the average recorded. Gonadal sperm reserve (GSR) was estimated as

$$GSR/gm = \frac{\text{No of cells counted x volume used}}{\text{Weight of sample}}$$

GSR=concentration/mg x Testis parenchyma weight

Table 1: Percentage composition of experimental diets

	Diets	
Ingredients	*Control (1)*	*Experimental (2)*
Maize	44.00	22.00
Groundnut cake	30.00	15.00
Fish meal	3.00	3.00
Palm kernel cake	15.00	15.00
BRDBM	-	16.00
CMBM	-	18.00
Premix	3.00	3.00
Salt	1.00	1.00
Oil	2.00	2.50
Molasses	2.00	2.50
Total	***100.00***	***100.00***
Cost/100kg (₦)*	*4701.00*	*4057.00*

BRDBM-Bovine rumen digesta-blood meal
CMBM Cooked mucuna bean meal
₦ 130 = 1 US dollar

Table 2: Proximate Composition of Experimental diets.

	Diets	
Nutrients	*Control (1)*	*Experimental (2)*
Dry Mater (DM)	94.2	94.2
Crude protein (CP)	17.1	15.9
Crude Fibre (CF)	8.0	8.2
Ether Extract (EE)	4.2	4.4
Ash (%DM)	8.1	12.4
Nitrogen Free Extract (NFE)	62.7	59.2

Table 3: Body and testis morphometry of pubertal rabbit fed a diet containing 16% (50:50) BRDBM mixture and 18% CMB meal.

	Diet	
Parameters	*Control (n=12)*	*Experimental (n=12)*
Initial body weight (g)	913	909
Live weight at slaughter (g)	1427 ± 48[a]	1317±41[b]
Total weight gain (g)	518±21[a]	406±18[b]
Daily weight gain (g/day)	9.4±3.3	7.5±1.1
Dressed weight (g)	970±11	993±32
Dressing percentage	68.6±.7.0	75.4±9.9
Daily feed intake (g/day)	72.4±7.5[a]	64.0±9.1[b]
Feed to gain ratio	7.5±2.6	8.5±1.2
Cost/Kg feed (N)*	47.0±0.0[a]	40.6±0.0[b]
Cost Kg/Weight gain(N)*	352.6±0.1[a]	344.5±0.1[b]
Paired testes weight (g)	1.8±0.6	1.9±0.4
Paired tunica albuginea wt (g)	0.2±0.1	0.2±0.0
Paired epididymal wt (g)	0.6±0.3	1.2±0.3[a]
Paired testes parenchyma wt (g)	1.6±0.5	1.70±0.0
Scrotal length (cm)	2.7±0.4[a]	2.1±0.3[b]
Scrotal width (cm)	1.9±0.4[a]	1.6±0.2[b]
Scrotal skin fold (cm)	0.2±0.1[b]	0.4±0.1[a]
Ratio of Left: Right testis weight	1:0.8	1:1.0
Ratio of left: Right tunica Albuginea weight	1:1.2	1:1.9
Ratio of left: Right testis Parenchyma weight	1:1.2	1:1.0
Ratio of Left: Right epididymal weight	1:1.2	1:1.4

[a,b] = Means on same row with different superscripts differ significantly (P<0.05)
BRDBM=Bovine rumen Digesta blood meal
CMB=Cooked Mucuna bean
n=Total number of sample
*N = 1 US dollar

Daily sperm production (DSP)

From the GSR above, DSP of each animal was estimated by dividing the testicular count by a time divisor, given for rabbit as 3.43 (Amann andAlmquist, 1962).

Data Analysis

Data collected in the complete randomized experiment were subjected to analysis of variance (ANOVA) procedure (Steel and Torrie, 1980). Means were computed and significant means were separated using Duncan Multiple Range Test (Duncan, 1955).

Results

Table 2 shows the proximate analysis of the control (diet 1) and experimental (diet 2) diets. The crude protein (CP) of diet 2 (17.1 Vs 15.9) represented 92.9% of diet 1 while the ash content of diet 1 (8.1 Vs 12.4) was only 65.0% of diet 2. Table 3 shows the body and testicular morphometry of the pubertal rabbits. Live weight at slaughter (1427±48g Vs 1317±41g) and total weight gain (518±21gVs 406±18g) were significantly ($P<0.05$) higher in the rabbits on diet 1 than those on diet 2. However, daily weight gain was only marginally higher than those on diet 2. Dressed weight, dressing percentage and fed to gain ratio of rabbit on diet 1 were marginally higher in rabbits on diet 2. The testicular weights were only marginally higher in the rabbits on diet 2 (1.8g Vs 1.9), but epididymal weights (0.6Vs 1.2) were significantly ($P<0.05$) higher. The spermatogenic characteristics of the rabbits are shown in Table 4. There was no significant difference between diets 1 and 2 in the spermatogenic characteristics. The right testes characteristics were however generally superior to the left tests in rabbits on experimental diet but the converse was the case in the control diet. No significant difference ($P>0.05$) was noticed between relative weight of Liver, heart , kidney, spleen and lung (Table 5), crude protein, ether extract, ash and nitrogen free extract of the rabbit muscle (Table 6) and the hematological parameters (Table 7) which were within the normal range value.

Discussion

The higher weight at slaughter, observed in rabbits on diet 1 is related to the higher protein (amino acids) and Nitrogen free extract (NFE) contents of the diet, as well as the lower crude fibre (CF). The high protein of diet 1 encouraged higher feed intake of the rabbits than experimental diet 2. However, protein in the two diets are within the range recommended by (Lang, 1981; Lebas, 1979).

Factors such as crude fibre level, texture, taste and odour have been linked with level of feed consumption (Atteh *et al.*,1995; Nir *et al* 1994; Odunsi and Longe 1995; Odunsi *et al.*, 1996; Ravindan and Ravindan 1988). The presence of anti-nutritional factors which could still remain after the cooking of CMBM, according to Iyayi, (2002) could negatively affect digestibility and availability of nutrient needed for normal growth and development. According to Aletor and Aladetimi (1989) crude protein, calcium and phosphorus of mucuna seeds are

Table 4:Gonadal sperm reserve (GRS) and estimated daily sperm production [DSP] in pubertal rabbits fed cooked mucuna bean meal plus RC/BM mixture during pre-pubertal

Growth period

	Diets	
Parameter	*Control (n=12)*	*Experimental (n=12)*
Paired testes parenchyma wt (g)	1.61±0.5	1.75±0.0
Gonadal index (Relative weight of testis		
To body weight)	0.1±0.0	0.2±0.0
Gonadal sperm reserve ($x10^8$)	12.2±5.3	13.5±4.2
Gonadal sperm reserve/gm testis		
Parenchyma ($x10^8$)	6.9±2.1	6.6±2.0
Daily sperm production/gm ($x10^8$)	3.6±1.1	3.8±1.2
Daily sperm production ($x10^8$) testis		
Parenchyma	2.0±0.1	2.0±0.1
Ratio of GSR (left: right testis)	1:0.1	1:1.1
Ratio of DSR (left: right testis)	1:0.9	1:1.1
Ratio of GSR/gm (left: right testis)	1:0.8	1:1.1
Ration of DSP/gm (left: right testis)	1:0.8	1:1.1

GRS-Gonadal Sperm reserve

DSP – Daily Sperm production

n=Total number of sample

reduced by 2.7% due to loss of soluble or proteineous part of the seeds into the cooking water, although the addition of BRDBM mixture (50:50) improved the CP level and reduced CF in the diet. However blood meal (BM) inclusion above 10% in the diet has been found to reduce feed consumption due to low palatability. The lower body weight of rabbits on diet 2 therefore can be explained in the content of the above facts in conjunction with the lower level of calcium and phosophorus, which may have also been lost during the processing of mucuna (Aletor and Aladetimi 1989). The result was the likely impairment in nutrient utilization. High crude fibre level as well as the bulkiness of BRDBM, which leads to increased rate of passage of digesta in the digestive tract, both reduce digestibility of other nutrients, which could have been available for normal growth and development (Lebas *et al* 1986).

The dressing percentage in this study was superior to 62.8% obtained by (Ukachukwu *et*

Table 5: Relative organ weight of rabbits fed a diet containing 16% (50:50) BRDBM mixture and 18% CMB meal at pre-pubertal growth period.

Parameters	*Diets*	
Organs (% of body weight)	*Control*	*Experimental*
Liver	3.2±0.7	3.3±0.4
Heart	0.2±0.0	0.2±0.0
Kidney	0.9±0.5	0.7±0.1
Spleen	0.1±0.0	0.1±0.0
Lung	0.7±0.1	0.7±0.2

Table 6: Proximate Composition of Muscles of rabbit fed a diet containing 16% (50:50) BRDBM mixture and 18% CBM meal at pre-pubertal growth period.

	Diet	
Nutrient Composition	*Control*	*Experimental*
Crude protein (% Dry Matter)	76.9±1.7	76.8±2.3
Ether extract (%Dry Matter)	14.6±2.9	14.0±2.6
Ash (%Dry Matter)	4.2±0.6	3.2±0.7
Nitrogen Free extract (%Dry Matter)	4.4±2.9	6.1±4.1

Table7: Haematological Parameters of Pubertal Rabbits fed a diet containing 16% (50:50) BRDBM mixture and 18% CMB meal at pre-pubertal growth period.

	Diets	
Parameters	*Control*	*Experimental*
Packed cell volume (%)	40.7±3.4	40.2 ± 3.4
Hemoglobin (g/dl)	13.5 ± 2.0	13.4 ± 1.1
White blood cell x (10^3/mm^3)	8.6 ± 1.0	9.9 ± 1.6
Red blood cell x (10^6/mm^3)	6.8 ± 1.0	6.7 ± 0.6
Mean corpuscular volume (μ^3)	59.9 ± 3.0	60.1 ± 3.1
Mean corpuscular Hemoglobin (ug)	19.9±1.1	20.0±1.1
Mean corpuscular concentration (%)	3.3	3.3

al. 1999) with diets containing mucuna leaf meal. The difference could be due to the nutrient content of the ingredients used, levels of inclusion as well as the genetic make up of the rabbits. The cost/kg of feed for the experimental diets was significantly ($P<0.05$) lower than that of the control. The marginally higher feed to gain ratio of the rabbits on the experimental diet than the control rabbits, suggested a lower efficiency in the utilization of the experimental diet. However, the significantly ($P<0.05$) lower cost per kg weight gain point to the economic advantage of the experimental diet over the control diet.

The fact that the consumption of the experimental feed did not result in any mortality throughout the experimental period also lends credence to the wholesome quality of the experimental feed.

The marginally higher paired testes weight and the higher paired epididymal weight of rabbits of diet 2 are indications of greater probability of the diet in supporting higher sperm production in the testes and corresponding storage (extragonadal reserve) in the epididymides. According to Nkanga and Egbunike (1990) the testes mass is significantly correlated with daily sperm production (DSP). Togun, (1981) had earlier reported significant correlation between daily sperm production (DSP) and epididymal weight and explained that the epdidymal weight, at any given time, is influenced by the quantity of spermatozoa passing from the testes into the epididymides.

The testicular sperm reserve and daily sperm production of rabbits on diet 2 were higher than those on diet 1. This observation can be attributed to the paired weight, which is about the most important factor in the estimation of both parameters. The higher testes weight could serve as the basis for improved breeding potential, which when viewed along with the significantly ($P<0.05$) higher epididymal weight, would account for higher number of mature spermatozoa per ejaculate (Togun, 1981).

The efficiencies of sperm reserve and sperm production were marginally higher in diet 1 than 2. Johnson *et al* (1992) had reported that the efficiency of sperm production is more important than testes size in determining spermatogenic potentials. While the difference between the two group was not significant, the fact that diet1 accounted for 90% and 93% of diet 2 in GSR and DSP respectively while diet 2 accounted for 96% and 97% of their efficiency would support the fact that the experimental diet competed favourably well with the control diet.

Conclusion

The experimental diet, with inclusion of 16% (50:50) BRDBM mixture in combination with 18% CMBM, reduces maize and groundnut cake inclusion in the rabbit diet. Though it resulted in significantly ($P<0.05$) lower total weight gain and live weight at slaughter, it resulted in lower cost per kg of diet and lower cost per kg of live weight gain.

The inclusion of these feedstuff resulted in non-significant ($P>0.05$) differences in major

sperm production characteristics. Paired epididymal weights were significantly ($P<0.05$) higher than the control diet, implying that the diet will impact superior sperm storage capability. Since these materials are of low value in human diet and the BRDBM is relatively cheap to acquire by farmers, being abattoir waste of high nuisance value, their inclusion in rabbit diet has potential usefulness, especially when the focus is on the selection of bucks for breeding programme.

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