

## EPIDIDYMAL SPERM RESERVE EXTRACTION AND LIBIDO RESPONSES OF MALE RABBIT FED GRADED LEVELS OF FERMENTED AFRICAN LOCUST BEAN (*Parkia biglobosa*)

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### ABSTRACT

The experiment was conducted at the Rabbit Unit of the College of Agriculture, Bauchi, in Bauchi local government of Bauchi state, for a period of 8 weeks, to investigate the effects of feeding male rabbit graded levels of fermented African Locust Bean Meal (ALBM) on epididymal sperm reserve and libido responses. Completely randomized design (CRD) was used to allot the experimental diets to the experimental animals. Five dietary treatment levels were formulated as T1, T2, T3, T4 and T5. Each treatment was replicated four times, with two rabbits per unit which made a total number of 40 experimental bucks. The bucks weighed between 1.5-2.0 kg. The experimental diets were fed twice a day (morning and evening) and lasted for 8 weeks and clean drinking water was provided ad-libitum. The data obtained were subjected to statistical analysis of one sample t-test using a statistical package (SPSS) version 25, 2017. The means were separated using turkey range test. Hematological analysis such as PCV, HB and WBC were determined also including serum biochemistry analysis for only globulin and albumin to view the blood profile at a glance after experimental feeding. Result obtained for testicular weight shows no significant difference at ( $P > 0.05$ ). T5 (100 %) shows the highest mean value of ( $1.88 \pm 0.13$ ), while T1 (0.0 %) shows the lowest mean value of ( $1.25 \pm 0.32$ ). The epididymal length, paired epididymis shows no significance difference at ( $P > 0.05$ ), however the highest mean value is seen in T1 ( $6.75 \pm 0.85$ ), while the lowest mean value was recorded in T3 ( $5.64 \pm 1.24$ ). Semen volumes collected of all the experimental groups presented were not significant ( $P > 0.05$ ). T1 (0.00 %) show the least volume of 0.05 ml. Libido response assessment test (LRT) was carried out to check for libido responses for each group using a matured teaser female (Doe). Time recording was achieved with aids of a stop watch. The libido responses (LR) were taken and recorded; some show response in less than ( $< 2$  seconds) others greater ( $> 1$  minute) and above (2 minutes) indicate or shows no libido response. All the experimental animals showed a spectrum of responses. T5 show a significant and higher libido response assessment test (LRT) while T3 show a high number of no response. The economic analysis of the feed cost shows that as the proportion of ALBM increases, the feed cost decreases. The feed cost ranging from 40.00 ₦/kg, 60.50 ₦/kg. In conclusion, the Fermented African Locust Bean Meal can be included in the male rabbit diet for the overall improvement in sperm motility and libido response assessment test (LRT). Therefore, Fermented African Locust Bean Meal is recommended up to (100 %) level of replacement for better epididymal sperm reserve of male rabbits.

**Keywords:** African Locust Bean, Libido Responses, Epididymal Sperm Reserve, Feeding, extraction, semen

### 1.0 INTRODUCTION

Rabbit production in Nigeria plays a significant role in the development and provision of food and family income (Salihu and Abdulrahman, 2020). Rabbit has been identified as an economic livestock that can adequately meet the animal protein requirement of Nigerians due to its ability to convert feeds that are not directly utilized by man. In most developing countries like Nigeria, there is insufficient intake of animal protein due to low animal productivity (Wafar *et al.*, 2019). Over the years, rabbit farming has grown from

raising a few rabbits for family consumption to large commercial operations with hundreds of rabbits. Expansion is much simpler than other livestock alternatives because a large range of existing facilities can be modified for rabbits and land requirements are negligible (Robert *et al.*, 2008). Rabbits are perhaps the most economical and profitable of all kinds of livestock as it can utilize inferior feeds and still provide quality meat and fur. Moreover, with a better-quality feeding program, there will be high increase in

production, thus allowing a higher profit margin for the producer (Ikhimalo *et al.*, 2006). The management of rabbit is done on a small-scale mostly by peasant farmers for many different purposes; they can play a very significant role in a small sustainable family operation. While the most common use in agricultural industries is for meat, rabbit also is raised for pets, by product for manure in gardens, show, and laboratory uses. Rabbit meat is high in protein 33 g (66 %) and low in fat, 3.5 % calories 173 and cholesterol 123 mg (41 %), when compared to most of the meat eaten (Victoria, 2020). Rabbits are prolific and will breed all year-round if well managed (Salihu and Abdulrahman, 2020). Feeding is key for reproduction, and certain ingredient has been known to influence it. Feed is the most important input in a profitable livestock production; it accounts for 70-80% of the total cost of production. High cost of feeding has continued to remain a major impending factor in intensive animal production. Therefore, it is imperative to explore the use of nonconventional feedstuffs that are cheap (Wafar *et al.*, 2019). Regarding the quantity of feed that should be administered to rabbit bucks, a restricted dietary protocol reduces *libido* and some seminal traits, however, the most important factor is not the amount of diet furnished but its chemical characteristics (Cesare, 2008). African Locust Bean. (*Parkia biglobosa*) is a perennial deciduous tree of the Fabaceae family. It is a leguminous plant which produces seed grain that is often cheap and readily available in Northern Nigeria; the seed is rich in protein and is used as a flavor intensifier for soups and stew (Orwa *et al.*, 2009). African locust bean tree has a very broad crown that may reach a height of 20 m. The species grows under a wide range of conditions, where annual rainfall ranges from 600 to 1500 mm and the dry season lasts 5–7 months. It occurs in natural and seminatural habitats such as savannahs and woodlands, sometimes on rocky slopes, stony ridges and sandstone hills. It is able to withstand drought because of its deep taproot (Modupe, 2014). *Parkia biglobosa* pulp is a good source of energy and vitamin C, while the fermented seeds contribute calcium, lipids and proteins to the diets of rabbits (Orwa *et al.*, 2009). Regular consumption makes it an important source of

nutrients (Boedecker *et al.*, 2014). It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Fermented seeds Dawadawa (Hausa), Netetu (Senegal) serve primarily as a condiment for seasoning sauces and soups (Foraminira, 2019). Roasted seeds are used as a coffee substitute known as ‘Sudan coffee’ or ‘café nègre’. Grinded seeds are mixed with *Moringa oleifera* leaves to prepare sauce, and are also used to make doughnuts. The mealy pulp from the fruits is eaten or mixed with water to make a sweet and refreshing drink rich in carbohydrates. Boiled pods are used to dye pottery black; the ash is applied as a mordant. The bark is rich in tannins and may be used for tanning hides, but the resulting leather is often of moderate quality especially with regard to colour, which is often reddish, uneven, and darkens when exposed to light (Modupe, 2014). The aim of this research is to evaluate the epididymal semen characteristics of male rabbit fed graded levels of African Locust Bean Meals.

## 2.0 MATERIALS AND METHODS

### 2.1 Experimental site

The experiment was carried out in a wellconstructed rabbit house of the college of Agriculture Bauchi, in Bauchi local Government. Geographically, Bauchi State occupies a total land area of 49,119 km<sup>2</sup> (18,965 sq mi) representing about 5.3% of Nigeria's total land mass with 20 local Government located between latitudes 9° 3' and 12° 3' north and longitudes 8° 50' and 11° east, north-eastern part of Nigeria.

**2.2 Experimental design and Sample Size** The rabbits were grouped into five treatments (5) and were randomly allotted into the experimental cages, as T1, T2, T3, T4, T5 using Completely Randomized Design (CRD) and replicated 4 times. There were 2 rabbit per unit of replications which make a total number of 40 experimental bucks.

**2.3 Sources of experimental animal** Rabbit (bucks) of mix breed were purchased from local rabbit farmers in Bauchi and neighboring LGAs of different colors, but of same age group and weight.

## 2.4 Sources of experimental diet

All experimental diets were available within the study area. African Locust Bean was purchased from a nearby market in Bauchi, Dass and Bogoro market and its environs. The seeds were then processed to obtain a Fermented Locust Beans “daddawa in hausa” dry and ground then added to other ingredients purchased from different sources that will be used to formulate the experimental diets.

## 2.5 Processing Techniques

The raw locust beans was carefully sorted and boiled for at least 24 hours with water being added frequently for it to be properly cooked, then mixed with wood ash and pounded and washed several times to remove the seed coats. The beans is then boiled again for another 3-4 hours until they become softer, and then spread in a large flat basket, covered with leaves and allowed to ferment for two days. On the third day, the locust bean mass is transferred to a deep bowl and allowed to ferment for another 24 hours. It is

then spread out again in a large flat basket and then allowed to dry properly under sun for several hours. Finally the processed locust bean grinds to permit proper mixing with other feed ingredients and also to maintain its preservative quality.

## 2.6 Method of compounding the experimental diet and replacement percentage

The following ingredients were used to formulate the experimental diet: Maize: T1 50.1 %, T2 42%, T3 46.88 %, T4 47.73 %, and T5 36.92 %. While Soya bean was: 9.84 %, 12.37 %, 3.11 %, 0.26 % and 3.0 % respectively. Ground nut husk was 20 % across the treatments. African Locust Bean was 0 %, 5 %, 10 %, 15 % and 20 % respectively. Wheat offal was 10 % across all the treatments, Fish meal 5.6 % in all the treatments, Bone Meal was 3% across the treatments, Premix, methionine, lysine and Salt were fixed at 0.5 %, 0.2 %, 0.2 % and 0.5 respectively. A conventional method (Pearson square method) was adapted to formulate the experimental diet.

**Table 1:** Composition and Calculated Analysis of Experimental Diet

INGREDIENTS	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Maize	50.16	42.64	46.88	44.73	36.92
Soya bean	9.84	12.37	3.11	0.26	3.0
Wheat offal	10	10	10	10	10
Groundnut husk	20	20	20	20	20
ALBM	0	5	10	15	20
Fish meal	5.6	5.6	5.6	5.6	5.6
Bone	3	3	3	3	3
Premix	0.5	0.5	0.5	0.5	0.5
Methionine	0.2	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5	0.5
<b>TOTAL</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Calculated analysis</b>					
ME (Kcal/kg)	3026.58	3025.77	3039.51	3045.74	3041.65
Crude protein (%)	16.02	17.62	16.18	16.41	18.14
Crude fibre (%)	5.93	6.77	7.36	8.30	9.41
Ether Extra EE (%)	4.48	4.61	4.58	4.51	5.18
Calcium Ca (%)	0.53	0.81	0.55	0.55	0.57
Phosphorus P (%)	0.44	0.44	0.39	0.34	0.40

ALBM- African locust bean meal, ME= Metabolizable energy.

Mineral/Vitamin Premix contained the following: Vitamin A 7,000IU, Vitamin D3 1,800IU, Vitamin B2 1,000mg Vitamin E 1,000IU, Vitamin K3 1,500mg, Vitamin B6 1,500mg, Vitamin B12 10mg Folic 500mg, Niacin 3,000mg, Pantothenate 3,000mg, Biotin 400mg, Choline 160g, Thyroxine 300mg, Copper 1,000mg, Iron 4g, Manganese 5.5g, Iodine 0.2mg, Zinc 5g, Cobalt 100mg, Seleniun 100mg. Antioxidant 1,250mg.

## 2.7 Data Collection

### 2.7.1 Gonadal and extra-gonadal Characteristics

After the trial research period experimental animals were taken to a post mortem room where all the samples were collected, two rabbits were randomly selected from each treatment and sacrificed. The scrotal sacs were incised to exteriorize the testes. After the eight-week feeding trial, all the experimental rabbits were slaughtered and their reproductive systems were carefully dissected, and excised; the testes and epididymides carefully collected, trimming off adhering tissues and weighed using a sensitive electronic balance. The testicular and epididymal morphometric characteristics such as length, diameter and volume of the testis, and epididymal length were also measured. The testis length, width and epididymal length were measured with the aid of a pair of vernier calipers, Paired and mean testicular and epididymal parameters were computed from data for left and right testes and epididymis. The castration procedure was performed using a standard method, the scrotal sac was shaved and disinfected with alcohol and a local anesthesia (lignocaine) was used to infiltrate the incision site to exposed the testicles immediately, the sperm in the total epididymal length was milked out.

### 2.7.2 Determination of Caudal Epididymal Sperm Characteristics

The cauda epididymides were placed in beakers containing physiological saline (maintained at 37° C) in a water bath and several lacerations were made on them to enable the spermatozoa swim out. Sperm motility was immediately determined by placing a drop of the suspension on a clean glass slide under the cover slip and viewed on a binocular microscope. Sperm motility was also assessed immediately by counting both motile and immotile spermatozoa per unit area at the magnification of  $\times 40$  as previously described by Zemjamis (1970). The count was expressed as million/ml of suspension.

### 2.7.3 Reaction Time (Libido Assessment)

A matured cyclic doe female rabbit (teaser) was introduced to the buck every 2 weeks interval to

monitor their sex drive. In this study, reaction time was considered as an indication for libido. The time in seconds it took for the rabbit bucks to sniff, groom and mount the female was recorded with a stop watch, as Described by (Ifeanyi *et al.*, 2009) and (Ebenebe *et al.*, 2017).

### 2.7.4 Seminogram

Parameter from Seminogram include: semen volume, semen viability and colour. The collected sperm are often measured immediately and a fraction is placed on a clean microscope slide then using a cover stick to cover it then examine under a microscope at X10 magnification to record sperm viability or motility at the same observing the physical colour of the semen.

## 2.8 Chemical Analysis

Sample of the five (5) Experimental Diet Drew African Locost bean meal and fermented African Locust bean meal more analyzed for their proximate composition using the methods of AOAC 2000.

## 2.9 Statistical Analysis

Data obtained from this study were expressed as mean ( $\pm$ SEM) and subjected to statistical package for social science (SPSS) version 25, 2017 was used to analyzed the data collected, significant differences between treatment means were considered at  $P < 0.05$ , using T-test and the means were separately using Turkey range test.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Proximate Composition of Experimental Diets

Dried African Locust Bean Meal and Fermented African Locust Bean Meal The Proximate Composition of the Experimental Diets, Dried African Locust Bean Meal and Fermented African Locust Bean Meal are presented in Table 2. The crude protein content of the experimental Diets, Dried African Locust Bean Meal and Fermented African Locust Bean Meal ranged from 16.02% to 18.14%. The values are adequate for male rabbits.( Onyimonyi and Onukwafor, 2003) The metabolizable energy ranged from 3025.77 to 3457.74 kcal/kg they are isocaloric and adequate for rabbit bucks Ochej

*et al.*, 2000; Ngodigha and Okejim 1999). The ether extracts levels of 4.48-5.18% are adequate for rabbits Ocheja *et al* 2008. The dry matter of the experimental diets are very high (90-94%)

and can providee adequate dry matter intake for the rabbits (Taiwo *et al.*, 2005). The crule protein content of the DALBM increased from 13.18 to 40.16 % in FALBM but the NFE decreased from 12.62% to 0.02%, this was a sharp decrease.

**Table 2:** Proximate Composition of Experimental Diets, Dried Locust Bean Meal and Fermented Locust Bean Meal

	Experimental Diets						
	T1	T2	T3	T4	T5	DALBM	FALBM
Crude protein	16.80	16.95	17.40	17.90	18.10	13.18	40.16
Crude fibre	6.0	6.20	6.60	6.50	6.80	18.50	21.70
Nitrogen free extracts	56.80	56.35	55.25	55.84	57.70	12.32	0.02
Ether extracts	5.20	5.25	5.10	5.30	5.40	43.80	29.70
Ash	6.20	6.30	6.35	6.40	6.60	4.15	5.50
Dry matter	91.00	90.05	92.00	93.00	94.00	92.58	83.25

DALBM- Dried African Locust Bean Meal

FALBM- Fermented African Locust Bean Meal

### 3.2 Testicular and epididymal morphometric responses of Bucks fed graded levels of Fermented Locust Bean meal.

Table 1, shows testicular and epididymal morphometric responses of buck rabbit fed graded levels of fermented locust bean meal. All parameters investigated under testicular and epididymal morphometrics responses of buck rabbit (initial weight, testicular weight right, testicular weight left, testicular circumference right, testicular circumference left, epididymis right, epididymis left, semen volume, sperm motility and semen colour) were similar except Final weight ( $P < 0.05$ ).

The initial and final weights, of the testicular circumference, testicular weight, epididymal length, semen volume, semen motility and color for different groups of experimental buck rabbit fed with different levels of fermented African locust beans meal (FLBM), the testicular weight result showed there was no significant difference at ( $p < 0.05$ ), however, on both testes mean value for T5 (100 %) Was the highest ( $1.88 \pm 0.13$ ) while the least was ( $1.25 \pm 0.32$ ) in T2 (25 %) similar to (Abu *et al.*, 2016). The testicular circumference

for paired testes, ranging from 6.00-8.43cm is similar to that reported by Abu *et al.* (2016). The epididymis characteristic (length) for paired epididymis showed no significant difference ( $p < 0.05$ ), However T1 ( $6.75 \pm 0.85$ ) was higher than the value reported by (Abu *et al.*, 2016), with the highest mean value while the least mean value is seen in T3 ( $5.64 \pm 1.24$ ) the highest length mean value observed in T1 possibly suggest being a control group without FLBM in the diet, this outcome seems to explain that there's no relationship or effect of the experimental diet (graded level of fermented African locust bean) on testicular weight, testicular circumference and epididymal length. Semen volumes of all the experimental groups presented no significant difference ( $p < 0.05$ ) T1 (0 %) control group showed the least value of 0.05ml lower than the value earlier reported by (Ebenebe *et al.*, 2017). T5 recorded 0.25ml (T5) below the value (0.58ml) as recorded by (Ebenebe *et al.*, 2017). T1 with decreased final weight of 1kg, this suggests that, decrease in weight below 1.4kg might affect the volume of semen for buck rabbit.

The semen motility expressed in percentage is also known as semen viability. The control group (T1) showed the lowest motility of 39.75 % while T5 show the highest motility of 62.50 % lower than the value reported (Abu *et al.*, 2016) when

*Tephrosia bracteolata* Leaf Meal. The high motility shown by T5 could be due to high level FLBM in the diets. This recorded value is similar to (Ebenebe *et al.*, 2017), when *Garcinia kola* was used.

**Table 3:** Testicular and epididymal morphometrics responses of Buck Rabbit fed graded levels of Fermented Locust Bean

Parameters (cm)	Treatment				
	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
IW	1.35±0.03	1.35±0.14	1.30±0.07	1.55±0.16	1.55±0.06
FW	1.0±0.11 <sup>b</sup>	1.45±0.13 <sup>ab</sup>	1.40±0.14 <sup>ab</sup>	1.53±0.10 <sup>a</sup>	1.40±0.09 <sup>ab</sup>
TWR	1.63±0.38	1.25±0.32	1.75±0.25	1.50±0.29	1.88±0.13
TWL	1.63±0.38	1.39±0.37	1.75±0.50	1.7±50.50	1.85±0.13
PTW	3.26	2.64	3.50	3.20	3.73
TCR	3.55±0.17	3.30±0.46	4.15±0.29	3.23±0.97	3.38±0.17
TCL	3.79±0.29	2.70±0.46	4.28±0.29	3.20±0.90	3.43±0.19
PTC	7.34	6.00	8.43	6.43	6.81
ER	6.75±0.85	6.28±0.24	5.64±1.24	6.33±1.19	6.05±1.08
EL	6.75±0.85	6.28±0.24	5.89±1.27	6.25±0.66	6.13±0.97
PEL	13.50	12.56	11.53	12.58	12.10
SV	0.05±0.03	0.15±0.05	0.13±0.05	0.23±0.11	0.25±0.10
SM	39.75±22.95	61.00±20.46	59.00±39.88	61.50±21.02	62.50±21.16
SC	milky-white	milky-white	milky-white	milky-white	milky-white

<sup>abc</sup> means on the same row with different superscripts are significantly different at (P<0.05), T1 - control, T2 – 25 % fermented locost bean meal, T3 – 50 % fermented locost bean meal, T4 – 75 % fermented locost bean meal, T5 –100 % fermented locost bean meal, RV – range value. IW- Initial weight, Final Weight, TWR- Testicular weight right, TWL- Testicular weight left, PTW-Paired testicular weight, TCR- Testicular circumference right, TCL- Testicular circumference left, PTC- Paired testicular circumference, ER- Epididymis right, EL- Epididymis left, PEL- Paired epididymal length SV- Semen volume, SM- Semen motility, SC- Semen colour

### 3.3 Libido response assessment Rabbit Bucks fed graded levels of Fermented African Locust Bean Meal (second per minute).

Table 2 shows relationship between diet and libido responses (L) in less than <2 seconds, greater than >2 seconds but less than <2minutes, while those responses above 2 minutes indicates no responses, Where T5 shows multiple or significant responses than all the group, and T3 with high number of no response

The rabbits bucks under experimentation are not difficult to measure because on introduction of teaser female, the male usually makes several attempts to mount after sniffing. The male responses are often recorded using a stopwatch in seconds as described by (Ifeyani *et al* 2009;

**Table 4** Buck libido responses assessment fed graded levels of Fermented African Locust Bean Meal (second per minute)

Treatment	L < 2s	2s >L < 2m	L > 2m (No response)
T <sub>1</sub>	3	7	2
T <sub>2</sub>	5	4	2
T <sub>3</sub>	1	3	6
T <sub>4</sub>	4	3	3
T <sub>5</sub>	8	3	1

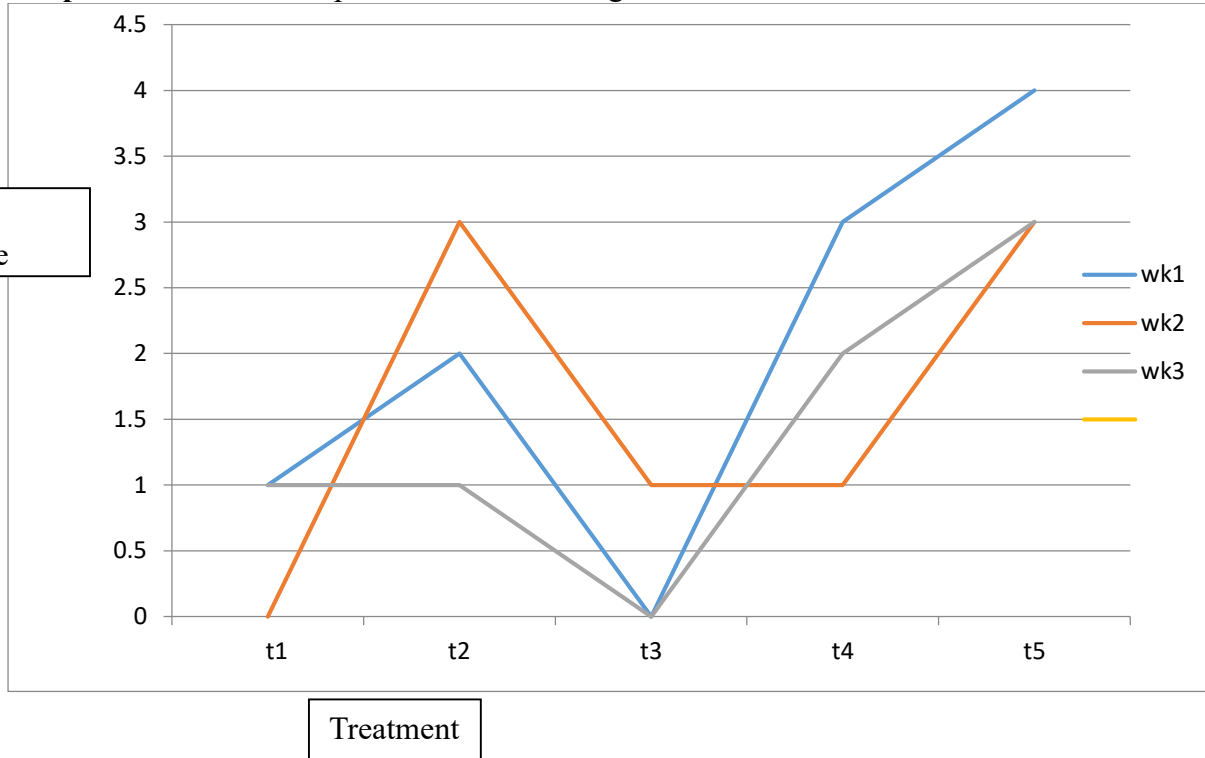
L- Libido responses, <>- Less than or greater than, s- second, m- minute

**Graph 1:** Shows libido responses of bucks fed graded levels of African Locust Bean Meal at  $\leq 2$ sec. It shows a pattern of responses within a given period of time, diet and libido response. Less than 2 second of libido response was higher

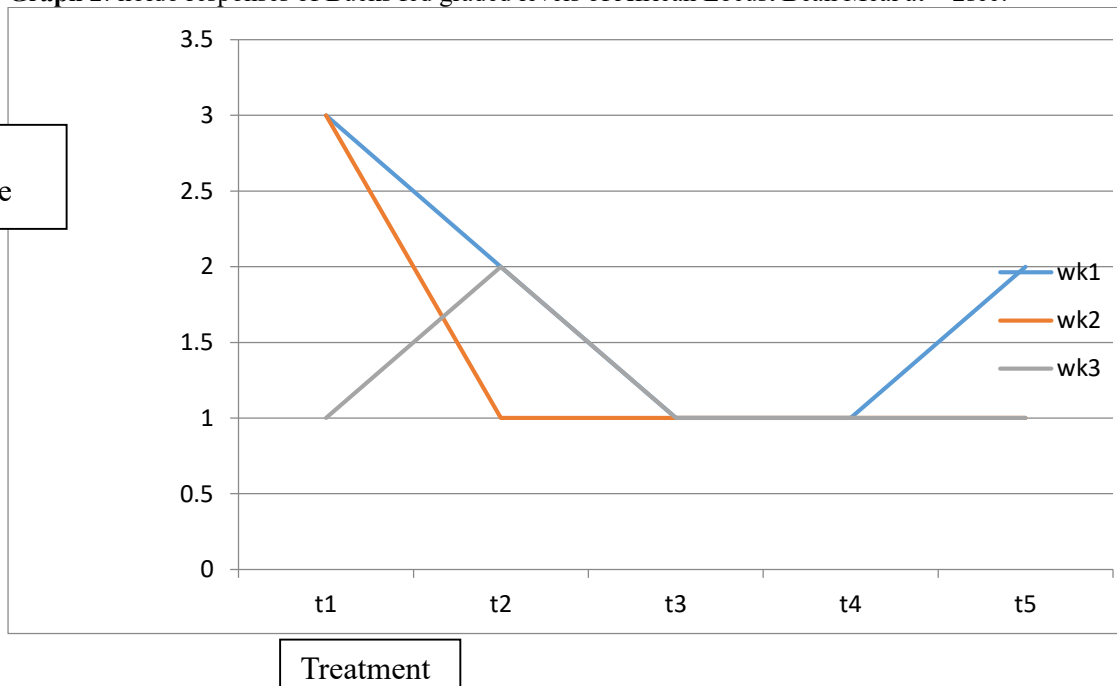
in bucks fed T5 than other bucks in the treatment groups.

**Graph 2:** The Libido responses of bucks fed graded levels of African Locust Bean Meal at  $> 2$ sec was lower in treatment (T3) compared to other treatment levels.

**Graph 1:**Show libido responses of Bucks Fed graded levels of African Locust Bean Meal at  $\leq 2$ sec



**Graph 2:** libido responses of Bucks fed graded levels of African Locust Bean Meal at  $> 2$ sec.



## 4.0 CONCLUSION AND RECOMMENDATIONS

### 4.1 Conclusion

Inclusion of fermented African locust bean meal was seen to produce significant effects on testicular, epididymal morphometrics and libido responses in buck rabbit. It was observed from the research that a little quantity of fermented African locust bean meal can be a maker for libido response. Thus, a little or higher limit produces varying libido responses.

### 4.2 Recommendations

i. The level of replacement of fermented African locust bean at 100 % is good for epididymal morphometrics and libido responses.

ii. Further research should be conducted to determine if there is seasonal effect of fermented African locust bean meal on reproductive performance.

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