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Effect of *Mucuna pruriens* (Velvet Bean) Seed Meal Diet at Varying Levels on Blood Profile and Reproductive Performance of Rabbit Bucks

S. D. Vermsy[1*] and T. Ahemen[1]

[1]Department of Animal Breeding and Physiology, College of Animal Science, University of Agriculture, P.M.B. 2373, Makurdi, Nigeria.

Authors' contributions

This work was carried out in collaboration with author TA. Author SDV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SDV and TA managed the analyses of the study. Author SDV managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted (August to October, 2018) to investigate the effect of varying levels of Mucuna pruriens seed meal diet on reproductive performance and blood profile of rabbit bucks. A total of 30 rabbit bucks weighing 1083 g to 1100 g were randomly allocated to five experimental diets replicated into six containing 0, 5, 10, 15 and 20% of *Mucuna pruriens* seed meal diet in a 2 month (8 week) trial. The phytochemical screening results shows no cardiac glycosides and alkaloids, in *Mucuna pruriens* seed screened, but a weak presence of resins, saponins, glycosides, steroids and terpens and antitraques, and moderate presence of flavonoids and tannins in *Mucuna pruriens* seed. The results revealed that the inclusion of *Mucuna pruriens* seed meal in the diet of rabbit bucks had significant ($P < 0.05$) effect on average daily feed intake with the highest value occurring at 0% (T_1) MSM level of inclusion and lowest at 10% (T_3), average final weight and average weight gain with the highest values at 20% (5) MSM level of inclusion and lowest at 0% (T_1) for AFW and AWG respectively. The feed conversion ratio was significantly ($P < 0.05$) different

**Corresponding author: E-mail: vermsolomon@gmail.com;*

between the treatment groups, and the values were generally similar in numerical comparison to those in the control group. A significant ($P < 0.05$) difference occurred in the relative weight of vital internal organs of the rabbit bucks between the treatment groups with respect to liver with highest relative weight at 0% (T_1) and lowest value in 15% (T_3) MSM level of inclusion, and the relative weight of the organs were generally similar in numerical comparison to those in the control group. The inclusion of *Mucuna pruriens* seed meal in the diets of the rabbit bucks also had no significant effect ($P > 0.05$) on relative weights of the reproductive organs except the paired testes weight which was significantly ($P < 0.05$) influenced by the effect of the MSM diet meanwhile testis volume, length and width did not show a significant ($P > 0.05$) difference between the groups but the mean epididymal (left) length significantly ($P < 0.05$) affected by the influenced of the MSM diet. The haematological parameters evaluated showed no significant ($P > 0.05$) difference on PCV, RBC, MCV, MCH, WBC and LDC (leukocytes differential count (N.L.M.E.B)). There were significant ($P < 0.05$) difference among treatments in Hb and MCHC. Rabbits fed 0 and 20% for Hb and 15% for MCHC MSM diets had significantly ($P < 0.05$) higher value than those of rabbits fed 20% MSM diet. The effect of MSM diet did not influenced ($P > 0.05$) the rabbits on total protein, albumin and glucose levels, and their values were comparable to those in the control, thus MSM diet influenced ($P < 0.05$) rabbit bucks on globulin, urea, creatinine, cholesterol, AST, ALT and ALP among the treatment groups. Significant ($P < 0.05$) differences in the mean value of testis density were observed in the left and paired testis density between the treatment groups and this could be attributed to increase sperm production. The MSM had positive effect on the physiological status (RR and HR) of the rabbit bucks and improved significantly ($P < 0.05$) most of the growth and reproductive traits studied. The lowest (17.44°C) Temperature humidity index during the study was observed in August (first week of experiment) and the highest (21.16°C) Temperature humidity index also was observed in August (third week of experiment), 2018. The study revealed that the rabbit bucks did not experience heat stress throughout the experimental period since the weekly THI means observed (17.44 to 21.16°C) were below (27.8°C) heat stress condition. The histological parameters showed normal structure of the seminiferous tubules and germ cells in their various stages of maturation arranged in a layered order. However, the seminiferous tubules were better organized with complete spermatogenesis, and more clearly defined in groups 2 (5%), 3 (10%) and 4 (15%) respectively and their epithelia were structurally intact and show normal germ cells compared to those in group 1 (control) and 5 (20%). It is concluded that the inclusion of *Mucuna pruriens* seed meal diet up to 20% in rabbit bucks diet would guarantee a good health and growth performance without any deleterious effect on germ cell differentiation, reproductive organ weights and physiological responses. From the findings, it appears Mucuna pruriens seed meal diet is a potential enhancer of male reproductive performance that can be recommended to rabbit farmers for improving reproductive performance, hence a boon to reproduction and production in rabbit farming industry.

Keywords: Mucuna pruriens (velvet bean); seed meal diet; blood profile; reproductive performance; rabbit bucks.

1. INTRODUCTION

Reproduction is of paramount importance in livestock farming, as it contributes to improved animal productivity as well as perpetuation of animal species [1]. Reproductive efficiency is of great economic importance in animal production which greatly determines the profitability of livestock enterprise [2,3]. Its dysfunction leads to a decrease in performances with very important economic losses. Extensive research has been conducted in the recent past with focus on replacement of conventional drugs with plant concoctions, which contain natural compounds and are considered as pure and ecologically friendly [4,5] for treatment of various ailments. Several plants are reported to contain aphrodisiac properties and/or fertility-enhancing compounds thus boosting sexual performance or libido [4,6,7]. Nutrition is one of the several factors affecting the physiology of farm animals, which is the most important aspect of animal production; because of feed provide necessary nutrients to animal [8].

Mucuna pruriens (L.) DC. is one such plant; it is also widely used in Africa, South America, and South Asia, the tropics and sub-tropics as pulse and forage [10]. It is a legume belonging to the family Fabaceae. It grows fast and has a long

growing season in frost-free environments. It is usually used as a cover crop and green manure because it protects the soil from erosion as well as improves soil fertility. It can establish very quickly with or without land preparation. Owing to its aggressive growth habit, it is able to outcompete common weeds and disease attacks [10]. *Mucuna pruriens* has widely been adopted in sub-Saharan Africa where it is used in conservation agriculture, mainly in areas where land scarcity is a major constraint to crop-livestock production [11,12].

In Nigeria, its roots, leaves, and seeds are commonly used for treatment of impotence, snake bite, diabetes, cancer, and Parkinson's disease [13]. Its seeds are rich in proteins reaching up to 26% crude protein content [11] and contain amino acids such as levo-3,4-dihydroxyphenylalanine (L-dopa), methionine, tyrosine, lysine, glycine, aspartic acid, glutamic acid, leucine, and serine along with globulins and albumins [14]. Several studies have reported *Mucuna pruriens* seeds to enhance reproductive performance in animals [15,16]. A study by Ahmad et al. [17] reported fertility enhancing potential of Mucuna seed meal in men. These performance and fertility enhancement properties are attributed to several bioactive compounds such as tryptamine, alkylamines, steroids, flavonoids, coumarins, cardenolides, and metals such as magnesium, copper, zinc, manganese, and iron, found in seeds [18]. Seeds also contain oleic acid, linoleic acid, and palmitic acid [19] which are reported to have aphrodisiac activities [14]. The availability and affordability of *Mucuna pruriens* extract by small-holder farmers' poses a challenge to its applicability. Supplementing raw MSM in animal feed such as rabbit diet could help to solve the above problems. However, little is known about the effect of that supplementation on rabbit buck fertility. This method of enhancing fertility in rabbit could be easily adopted by small-holder rabbit farmers of sub-tropical regions such as South Kivu, where *Mucuna pruriens* seeds are available throughout the year and rabbits are widely produced under traditional management. Indeed, rabbit is one of the mini-livestock used for malnutrition alleviation in South Kivu, but its production and fertility remains low compared to documented performances [20].

The main objective of this study was designed to determine the effect of *Mucuna pruriens* at varying levels on blood profile and reproductive performance of rabbit Bucks.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted at the National Veterinary Research Institute (NVRI) Rabbit unit, Vom Jos, Plateau State Nigeria. Vom is situated on the Jos Plateau 29 km South West Jos city. It is located on latitude 9°43'60''N and longitudes 8°46'60'' E at an altitude (elevation) of 1,222 meters above sea level, with mean annual rain fall of 1,400 mm (Microsoft Encarta, 2008). The area is defined by two seasons: rainy season (May to October) and dry season: (November to April), and temperature that ranges from 15 to 25°C from mid-November to late January. Night temperature drops as low as 11°C (52°F). The tropical grass land is Guinea Savannah (Jos – climate graph, 2013).

2.2 Source and Processing of *Mucuna pruriens* Seed

Seeds of *Mucuna pruriens* were obtained at the National Animal Production Research Institute (NAPRI), Shika Zaria Kaduna State. Weighed velvet bean seeds were toasted in a metal frying pan containing sand and heated by fire. Sand was used to achieve uniform heat in a frying pan. The seeds were constantly and continuously stirred to prevent charring until they become crispy with aroma of toasted beans. The toasted velvet bean were sieved out immediately, cooled, milled and taken to the laboratory to test for proximate composition.

2.3 Proximate composition of Toasted *Mucuna pruriens* Seed

The proximate composition of moisture, crude protein (CP), ether extract, crude fibre (CF) and ash were determined by following AOAC (Association of Official Analytical Chemist, 1992) methods. The nitrogen free extract (NFE) expressed as a percentage was obtained by subtracting the sum of the amounts of CP, EE, CF and ash from 100%. The metabolisable energy (ME, kcal/kg) was calculated using the method found in MAFF (1984).

2.4 Experimental Design

The design used in this study was a completely randomized design (CRD).

The statistical model used is shown below;

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where;

Y_{ij} = Individual observation,
μ = Population mean,
T_i = Fixed effect of diets (T = 1, 2, 3 and 4),
e_{ij} = Experimental independently distributed with mean zero and variance that of the population.

2.5 Experimental Diets

Five experimental diets coded T_1, T_2, T_3, T_4 and T_5 were formulated with a content of *Mucuna Pruriens* seed meal at 0, 5, 10, 15 and 20 % level of inclusion of *Mucuna pruriens* seed meal diet respectively. Diet T_1 with 0 % *Mucuna* seed meal served as the control. Other ingredients in the diets were, Maize, soybean meal, Brewers dried grain, rice offal, bone meal, premix, lysine, methionine and salt. The diet composition is presented in Table 4.

2.6 Experimental Animals and Management

A total of thirty (30) healthy rabbit bucks of mixed breeds, aged between 10 and 12 weeks with average weight of 995.75g were used for the study. Before the commencement of the experiment, animals were allowed to acclimatize for one week and were treated with Ivermectin against endo- and ecto-parasites. The rabbits were randomly assigned to the five (5) treatments containing 0 (control), 5, 10, 15 and 20% Toasted *Mucuna pruriens* seed meal diet respectively with six (6) rabbits per treatment. The rabbits were provided feed and water ad libitum twice daily at 8.00 and 16.00 h for 56 days of the experimental period. Each rabbit served as replicate in a Completely Randomized Design (CRD). The rabbits were weighed using a commercial grade compass scale inside an empty cartoon and placed individually at the beginning of the study and weekly thereafter. Parameters measured were daily feed intake and daily weight gain, while feed conversion ratio and protein efficiency ratio were calculated from weight gain, feed intake and protein intake values. The experimental animals were sacrificed at the end of the feeding trial that lasted for eight weeks.

2.7 Haematological Study

At the 8th week of the experiment, three rabbits from each treatment were selected for haematological studies. About 5mls of Blood samples were collected from the jugular veins of each slaughtered rabbit with sterile needles and syringes. The blood samples were collected into properly labeled sterilized bottles containing

Table 1. Composition of experimental diets with varying levels of toasted *Mucuna pruriens* seed meal (TMPSM) %

Ingredients (%)	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	T5 (20%)
Maize	44.75	43.00	41.00	39.00	37.00
Soybean	21.25	18.00	15.00	12.00	9.00
Mucuna (TMPSM)	-	5.00	10.00	15.00	20.00
Brewers dried grain	15.00	15.00	15.00	15.00	15.00
Rice offal	15.00	15.00	15.00	15.00	15.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Table salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated analysis					
ME (Kcal/kg)	2605.31	2652.40	2697.65	2742.89	2788.14
Crude protein (%)	17.00	16.90	16.91	16.92	16.93
Crude fibre (%)	10.77	11.07	11.39	11.71	12.03
Ether extract (%)	4.27	4.41	4.54	4.67	4.79
Lysine (%)	1.10	1.01	0.92	0.83	0.74
Methionine (%)	0.52	0.50	0.48	0.46	0.44
Calcium (%)	1.31	1.36	1.41	1.47	1.52
Phosphorus (%)	0.72	0.74	0.76	0.78	0.80

EDTA (ethylene diamine tetra-acetic acid) for haematological analysis. Packed cell volume (PCV), Haemoglobin concentration (Hb), Red blood cell (RBC), white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and Leukocytes differential count (LDC) were analysed.

2.8 Serum Biochemistry

Another 5 ml of blood sample was collected, after sectioning the jugular vein of rabbit, and put into tubes without anticoagulant, and allowed to clot, and centrifuged at 3000 rpm for 15 min for serum biochemical studies. Serum biochemical parameters determined were total protein, albumin, globulin, cholesterol, glucose, urea, creatinine alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) respectively. Evaluation was done as reported by Iwuji and Herbert [21].

2.9 Reproductive Tract Morphometry

At the end of the experiment, three rabbits were selected randomly after being starved for 24 hours. The animals were weighed individually to determine their live weight and thereafter slaughtered by stunning before cervical dislocation; the abdominal cavity was cut open through the ventral side to expose the organs. The testes of the rabbit were then removed and trimmed, freed of fats and connective tissues. The epididymis was separated from the testes weighed and recorded into left, right and paired epididymis. The right and left testes were weighed separately and paired testes were also weighed and recorded to the nearest 0.001 g using a very sensitive scale (Metler balance). Testis length, testis width and epididymal length were measured with the aid of a pair of vernier calipers.

Testis Volume: The volumes of the left and right testis were taken separately and also paired testes were measured using the Archimedes principle of water displacement with the use of a 25 ml measuring cylinder. The results were recorded in ml.

Testis Density: This was determined from a calculation using the formula:

Left Testis Density (LTD) = LTW / LTV

Right Testis Density (RTD) = RTW / RTV

Paired Testis Density (PTD) = LTW + RTW / LTV + RTV

NB: LTW; left testis weight, RTW; right testis weight, LTV; left testis volume and RTV; right testis volume.

2.10 Data Analysis

All data collected were subjected to one way analysis of variance (ANOVA) using SPSS version 17 of 2007. Treatment means were separated using Duncan Multiple range test (DMRT) of the same software.

3. RESULTS AND DISCUSSION

3.1 Effect of Experimental Diets on Haematological Indices of Rabbit Bucks

The haematological parameters of rabbit bucks fed varying levels of Mucuna pruriens seed meal diet. The results showed no significant ($P > 0.05$) effect on PCV, RBC, MCV, MCH, WBC and LDC (NLMEB) leukocytes differential counts between the control and the treatment groups. This is an indication that Mucuna seed meal is not toxic to the blood formation and immune system of the rabbits and hence has no deleterious effects on the rabbit bucks. The values fall within the normal physiological range for healthy rabbits as reported by Mitruka and Rwansley [22]. This is an indication that MSM is nutritionally adequate for rabbit bucks. The values ranged from (47% to 55%) and (2.25×10^{12} /l to 2.50×10^{12}/l) for PCV and RBC respectively. PCV range observed in this study were higher than (36.5% to 38.7%) reported by Ojebiyi et al.[23] for rabbits fed concentrates: *Aspilia africana* and *Tridax procumbens* and values (31% to 38%) reported by Shah et al. [24] and [25] on rabbits fed sesame seed meal. The values reported for these blood parameters were within the normal physiological range for rabbits [26,27,28,29,25]. All the PCV values were also within the normal range (25% to 45%) reported by Mitruka and Rawnsley [22], Ross et al. [30], Anon [31] and higher than (26.1% to 29.5%) reported by Ikhimioya et al. (2000), which implies that, irrespective of the processing effect, the diets were nutritionally adequate in providing a sound plane of nutrition. However, the observed rise in WBC in rabbits fed MSM supplemented diets, especially at 20% (T5 (55%)) in this study may be the product of immunostimulatory activities of MSM. The WBC counts possess a phagocytic functions and biomarkers for immune functions.

However, Hb and MCHC were significantly influenced by the treatment diets which ranged between (15.50 ± 0.50 g/dl to 18.50 ± 0.50 g/dl and 66.00 ± 2.00 g/dl to 90.50 ± 7.50 g/dl). The haemoglobin concentration (Hb) values obtained in this study was higher than the range of values (8.9 g/L to 15.5 g/L) reported by Hewitt et al. (1989) and Mitruka and Rwansley [22] for clinically healthy rabbits. The significant ($P < 0.05$) difference among treatment groups with respect to mean corpuscular haemoglobin concentration (MCHC) also implies that the animals were significantly influence by the dietary treatments. The significant differences noticed in MCHC and Hb in this study could be attributed to effect of processing on the test material in the experimental diets. The MCHC values in all the treatment groups in this study fall within the range (66.00 g/dl to 90.50 g/dl) lower than (311 g/dl to 370 g/dl) reported by Hewitt et al. (1989). The Hb values range from (15.50 g/dl to 18.50 g/dl) higher than the range (8.9 g/dl to 15.5 g/dl) for clinically healthy rabbits reported, was higher in broilers administered *Mucuna pruriens* seed meal (MSM) reported by Anon, [31] and Swenson [32]. This finding is in line with what has been earlier reported by Tuleun et al. [33] in broiler chickens and that of Adenkola et al. (2009) in rabbits that nutrient is an important factor in haemopoesis. The results of this study is in agreement with the results of Sese et al. [34] for growing rabbits fed Mucuna leaf meal diet, and this could be attributed to the blood boosting properties of Mucuna as reported by Ujowundu et al. [35].

The MCV, MCH, WBC and RBC observed had no significant ($P > 0.05$) effect among the various treatment groups. The result of WBC count showed no significant ($P > 0.05$) different among the treatment means (Table 3). In other words, processing the test material exerted no significant effect on the leukocytes. Champe et al. [36] observed neutrophils and microphages (monocytes) as components of WBC that are involved in both oxygen – independent and oxygen – dependent mechanism for combating viral, killing and engulfing bacteria. The Neutrophils and lymphocytes recorded highest and least values of (31.5% to 51.0%) and (44.0% to 68.0%) respectively and there was no similar variation among the groups. The results implies that, none of the predisposing methods predisposes rabbit bucks to infections as higher count than normal may mean that, the rabbits immune system may be combating some kind of infections, as reported by Frandson [37] and Adeyemo and Longe [38].

Monocytes, Eosinophils and Basophils count were found none in all the treatment groups except T_3 (10%) and T_5 (20%) which recorded Eosinophils (0.5% to 3.5%) and monocytes (0.5%) respectively. The absence of basophils in the blood of the rabbit bucks indicates that the inclusion of *Mucuna pruriens* seed meal in the

Table 2. Effect of experimental diets on haematological indices of rabbit bucks

	Experimental diets				
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**
PCV (%)	53.50 ± 3.50	52.50 ± 2.50	51.50 ± 0.50	47.00 ± 2.00	55.00 ± 1.00
RBC ($x10^{12}$/l)	2.50 ± 0.10	2.25 ± 0.50	2.50 ± 0.10	2.40 ± 0.10	2.30 ± 0.30
Hb (g/dl)	18.00 ± 1.00^{a}	17.50 ± 0.50^{ab}	$17.00\,0.00^{ab}$	15.50 ± 0.50^{b}	18.50 ± 0.50^{a}
MCV (fl)	75.00 ± 8.00	84.50 ± 5.50	78.00 ± 2.00	88.50 ± 7.50	76.00 ± 5.00
MCH (pg)	36.00 ± 1.00	38.50 ± 0.50	34.50 ± 1.50	33.00 ± 0.00	40.00 ± 5.00
MCHC (g/dl)	73.50 ± 5.50^{ab}	72.50 ± 6.50^{ab}	75.00 ± 1.00^{ab}	90.50 ± 7.50^{a}	66.00 ± 2.00^{b}
WBC ($x10^{9}$/l)	2.90 ± 0.50	2.15 ± 0.75	2.15 ± 0.15	3.00 ± 0.10	2.25 ± 0.55
N (%)	46.00 ± 12.00	51.00 ± 19.00	31.50 ± 13.50	32.50 ± 3.00	32.00 ± 2.00
L (%)	51.00 ± 19.00	44.00 ± 14.00	68.00 ± 14.00	63.50 ± 5.50	64.0 ± 5.00
M (%)	0.00 ± 00	0.00 ± 00	0.00 ± 00	0.00 ± 00	0.50 ± 0.05
E (%)	0.00 ± 00	0.00 ± 00	0.50 ± 0.50	0.00 ± 00	3.50 ± 2.50
B (%)	0.00 ± 00	0.00 ± 00	0.00 ± 00	0.00 ± 00	0.00 ± 00

[a,b] Means within each row with different superscripts are significantly different ($P < 0.05$). ns – not significantly different ($P > 0.05$). PCV – Pack Cell Volume; RBC – Red Blood Cell; Hb – Haemoglobin; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Haemoglobin; MCHC – Mean Corpuscular Haemoglobin Concentration; WBC – White Blood Cell; N – Neutrophils; L – Lymphocytes; M – Monocytes; E – Eosinophils; B – Basophils; T_1 – 0% Mucuna pruriens Seed Meal Diet; T_2 – 5% Mucuna pruriens Seed Meal Diet; T_3 – 10% Mucuna pruriens Seed Meal Diet; T_4 – 15% Mucna pruriens Sead Meal Diet; T_5 – 20% Mucuna pruriens Seed Meal Diet

diets of the animals did not cause any inflammation of organs, bronchoconstriction or anaphylaxis in the rabbit bucks (Forbes, 2008). Basophils contain the anticoagulant Heparin, which is normally releases in areas of inflammation to prevent clotting and stasis of blood and lymph [37]. Therefore, no inflammation was encountered in both the control and treatment groups. This showed that MSM had no adverse effect on blood and is an indication that the diet did not cause nutrient restriction.

3.2 Effect of Experimental Diets on Serum Biochemistry of Rabbit Bucks

The serum biochemistry indices of rabbit bucks fed toasted Mucuna seed meal diet. The result showed no significant ($P > 0.05$) difference for total protein, albumin and glucose. Total protein range (69.72 ± 9.91 g/l to 78.00 ± 7.64 g/l) did not conform to the reported values of (27.38 to 31.0 g/l) Steiner et al. [39] for broilers fed *Mucuna utilis* leaf meal. High total protein could be attributed to good protein reserve, thus reflecting the ability of the rabbits to store protein for tissue development. This is in agreement with Muhammad et al. [40] who attributed increased serum protein in broilers to dietary inclusion of velvet bean. The serum albumin (34.79 ± 7.86 g/l to 47.15 ± 9.46 g/l) obtained in this study disagrees with the reported range of (17.00 to 28.00 g/l) by Aderinola et al. [41] for broilers. Jiwuba et al. [42,43] attributed serum albumin to liver functioning. Hence the higher albumin reported in this study for the treatment groups may indicate a better liver functioning to the rabbits fed the respective diets. Ujowundu et al. [35] however reported that the leaf extract of *Mucuna utilis* are used to reduce high concentration of some antinutritional constituents in the body; thus a liver functioning property of *Mucuna utilis* leaves.

The serum globulin which revealed significant ($P < 0.05$) difference among the treatment groups with a range value of (22.58 ± 0.46 g/l to 39.09 ± 1.17 g/l) was high in rabbit bucks fed MSM compared with the range (9.30 g/l to 14.70 g/l) reported by Peter – Damian (2018) on dietary effect of velvet bean (*Mucuna utilis*) leaf meal on haematology and serum biochemistry of broiler finisher birds. This may suggest high immune response and sufficient antibody production as a result of diets containing MSM. The urea and creatinine levels were reduced by the administration of MSM. Both were statistically significant ($P < 0.05$). Thus, this is in agreement with the work of Adepoju and Odubena [44] who stated that the reduction in the creatinine and urea level make *Mucuna pruriens* a compromising or potential drug to improve kidney function. It could, therefore, be used in reducing high plasma creatinine and urea levels. The kidney is the major organ of excretion and its functional status is estimated by the Creatinine Clearance, CLcr, which inadvertently, is a measure of the Glomerular Filtration Rate (GFR). Creatine is formed by the metabolism of phosphorcreatinine, a high energy molecule which provides a rapid supply of ATP for the cellular functions. Phosphorcreatinine is converted spontaneously to the blood and excreted by the kidney as a metabolic waste. In experimental studies, creatinine measurement is used exclusively in the assessment of kidney function. The reduction in urea and creatinine thus make the Mucuna pruriens a potential source of bioactive components to improve kidney functions. The glucose range of value (2.08 ± 0.58 mmol/l to 2.89 ± 1.75 mmol/l) obtained in this study was lower and not within the range of (125 iu/l to 200 iu/l) reported by Anon [31]. It was not significant ($P > 0.05$) within the treatment groups. The *Mucuna pruriens* seed meal diet reduces the glucose load. The previous investigation suggested that the antidiebetic activity of *Mucuna pruriens* seed may be due to its dietary fibre content [45]. It is reported that cholesterol, urea and creatinine are responsible for increase in the blood glucose level.

Serum total cholesterol was significantly ($P < 0.05$) lower (48.72 ± 8.30 mg/dl to 95.52 ± 24.99 mg/dl) comparable with results obtained (136.3 ± 5.37 mg/dl to 162.0 ± 4.34 mg/dl) by Jayaweera and Samara - Singhe (2007) on the effect of feeding velvet bean (*Mucuna pruriens* L.) on the performance and lipid profile of broiler chickens. *Mucuna pruriens* significantly ($P < 0.05$) reduced the cholesterol level dose – dependent. It could therefore be used in reducing high plasma cholesterol levels (hypercholesterolaemia). The reduction of serum cholesterol levels in rabbits fed 5, 10, 15, and 20% diets in this study suggests that MSM supplementation interferes with the uptake and catabolism of cholesterol in rabbits. According to Lording and Friend [46], decrease uptake of cholesterol or increased loss or cholesterol catabolism is among the causes of hypocholesterolaemia. Saponins, one of the detected phytochemicals in MSM, were linked to the reduction of cholesterol uptake in the gut [47]. Oloruntola et al. [48,49] recorded reduction of serum cholesterol level in rabbits and broiler

chickens fed on diets containing 50 or 100 g/kg alchornea leaf meal. Similar cholesterol depressing effects due to feeding velvet bean in broilers have been observed by Carew et al. 1998a, [50,51]. This is also in agreement with results reported on rats by Pant et al. (1968) and lauk et al. (1989).

There were significant ($P < 0.05$) differences among the treatment groups. From the result, there was a dose dependent decrease ($P < 0.05$) in the level of the liver biomarkers (AST, ALT and ALP), by the administration of *Mucuna pruriens* when compared to the control particularly the ALP enzyme. This is in line with the work carried out by Chukwudi [52] who ascribed the decrease in the liver enzyme activities (AST < ALT and ALP) to the antioxidant property of the *Mucuna pruriens*. The antioxidant property might probably prevent the oxidative stress generated during liver metabolic process. AST, ALT and ALP are liver enzymes that have linkages between the liver and the blood. AST is an enzyme mostly found in the heart and liver and to some extent in muscles, when these organs are diseased or injured this enzyme is released in the blood circulation. The values recorded (29.40 ± 1.56 to 131.20 ± 6.68 u/l, 18.00 ± 2.00 to 29.00 ± 1.00 u/l and 15.78 ± 1.70 to 38.97 ± 10.54 u/l) for AST, ALT and ALP respectively in this study were lower than (214.330 ummol/l to 261.00 ummol/l and 101.33 ummol/l to 113.67 ummol/l) reported by Aliyu (2015) in broilers when administered MSM diet. The reduction of serum AST in this study suggests that MSM has protective and therapeutic properties, as abnormally rising AST concentration indicates liver and biliary system diseased, skeletal muscle diseased, myocardial/ injury disaeses, haemolytic disorder and haemolysis (Lording and Friend, 1991). This is further supported by the stability of the total protein; creatinine and bilirubin concentration in the rabbits fed the various experimental diets supplemented with varying levels of MSM. The presence of some phytochemicals at the therapeutic level [53,54] may be responsible for these observations in this study.

3.3 Effect of Experimental Diets on Relative Weight of Reproductive Organs of Rabbit Bucks

The results of reproductive organ weights of rabbit bucks fed MSM diet are presented in Table 5. There was a significant ($P < 0.05$) difference in paired testis weight among the rabbits fed 0, 5, 10, 15 and 20 % MSM diet on the weights among the treatment groups. It was observed that inclusion of varying levels of MSM diet had significant ($P < 0.05$) effect on reproductive organ weight of rabbit bucks. This result agrees with the report of Amao et al. [55] who observed significant effect of diets on testis weight of rabbits fed diets containing neem (*Azadirachta indica* A. Juss) ring meal in favour of bucks placed on control diet. The findings of this study disagrees with the report of Ahemen et al. [56] that no significant influence of diets recorded on testis of rabbits fed diets containing up to 15% *Gmelina arborea* leaf meal and Ewuola [57] who also found no significant influence of diets on the paired testes weight of rabbit bucks fed prebiotic and probiotic supplemented diets.

Table 3. Effect of experimental diets on serum biochemical indices of rabbit bucks

	Experimental diets				
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**
TP (g/l)	73.56 ± 2.82	71.82 ± 5.83	75.91 ± 0.95	78.00 ± 7.64	69.72 ± 9.91
ALB (g/l)	40.98 ± 3.90	35.97 ± 5.03	35.82 ± 3.80	34.79 ± 7.86	47.15 ± 9.46
GLB (g/l)`	32.68 ± 6.42^{ab}	35.86 ± 10.86^{ab}	39.09 ± 1.19^{a}	33.21 ± 0.57^{b}	22.58 ± 0.46^{c}
UREA (mmol/l)	7.79 ± 3.71^{a}	5.35 ± 0.20^{ab}	7.19 ± 1.96^{a}	3.89 ± 0.02^{b}	4.70 ± 0.47^{ab}
CREAT (µMol/l)	57.10 ± 14.20^{b}	91.80 ± 6.60^{a}	58.85 ± 4.95^{b}	39.20 ± 5.20^{b}	94.15 ± 1.35^{a}
GLU (mmol/l)	2.80 ± 0.66	2.64 ± 0.79	2.08 ± 0.58	2.89 ± 1.75	2.25 ± 0.73
CHOL (mg/dl)	67.54 ± 7.40^{a}	80.59 ± 14.92^{a}	43.72 ± 8.30^{b}	92.52 ± 24.99^{a}	65.86 ± 2.30^{a}
AST (µ/l)	114.73 ± 27.75^{b}	131.20 ± 6.68^{a}	40.53 ± 11.96^{d}	78.86 ± 6.34^{c}	29.40 ± 1.56^{e}
ALT (µ/l)	18.00 ± 2.00^{b}	20.50 ± 0.50^{b}	24.00 ± 2.00^{b}	22.00 ± 4.00^{b}	29.00 ± 1.00^{a}
ALP (µ/l)	38.97 ± 10.54^{a}	15.78 ± 1.70^{c}	22.09 ± 6.95^{b}	19.24 ± 2.28^{b}	21.79 ± 1.24^{b}

$^{a, b c d e}$ Means within each row with different superscripts are significantly different ($P < 0.05$). ns – not significantly different ($P > 0.05$). TP - Total Protein; ALB – Albumin; GLB - Globulin; CREAT - Creatinine; GLU - Glucose; CHOL - Cholesterol; AST – Aspartate Aminotransferase; ALT - Alanine Aminotransferase; ALP - Alkaline Phosphate; T_1 – 0% Mucuna pruriens Seed Meal Diet; T_2 – 5% Mucuna pruriens Seed Meal Diet; T_3 – 10% Mucuna pruriens Seed Meal Diet; T_4 – 15% Mucna pruriens Sead Meal Diet; T_5 – 20% Mucuna pruriens Seed Meal Diet

The left and right relative testis weights values (0.07 ± 0.01 g to 0.08 ± 0.02 g and 0.07 ± 0.00 g to 0.09 ± 0.01 g) obtained in this study were lower than the range of (1.73 g to 2.73 g and 1.60 g to 2.10 g) for left and right testis reported by Ahemen *et al*., [56]. The values were also lower than the range of (2.01 g to 2.44 g and 1.81 g to 2.23 g) for left and right testis reported by Ladipo *et al*., [58]. The range of values obtained for paired testes weight (0.14 ± 0.01 g to 0.17 ± 0.02 g) were lower than the paired testes weight (2.58 ± 0.40 g to 3.23 ± 0.19 g) reported by Ahemen *et al*., [56]. Results of the present study revealed that supplementation with *Mucuna pruriens* increase the relative weight of testis. These findings are similar to those reported by Chika *et al*. (2017) in rabbit bucks administered orally with 50 or 100 mg/kg body weight of *Securidaca longipedunculata (Polygalaceae)* root-bark methanol extract for 70 days. The increase in the mass of these organs could be attributed to the androgenic properties of the *Mucuna pruriens* seed meal such as flavonoids, phenols, and saponins [19,18], as also reported in *Securidaca longipedunculata* (Chika *et al*. 2017). Indeed, Gayrard [1] showed that the mass, size, and secretory function of the testis is regulated by androgens. Testosterone, an anabolic hormone, contributes to the synthesis of proteins and, therefore, muscle mass with consequent increase in volume of the testis. In mammals, testis and vas deferens elaborate and ensure the transport and survival of spermatozoa [1].

Increasing the weight of these organs would improve the motility, concentration, and decrease the anomalies of spermatozoa, which are correlated with a possible high fertility rate ([7]. The left, right and paired epididymal weight was not significantly (P > 0.05) different among the treatment. The mean epididymal weight for left, right and paired values ranged from (0.02 ± 0.00 g to 0.03 ± 0.01 g, 0.03 ± 0.00 g to 0.04 ± 0.01 g and 0.06 ± 0.00 g to 0.07 ± 0.02 g) respectively. The values were not significantly (P > 0.05) influenced by the effects of the diets.

3.4 Effect of Experimental Diets on Measurements of Reproductive Organs of Rabbit Bucks

The results of reproductive organ measurements of rabbits fed *Mucuna pruriens* seed meal diet are presented in Table 6. No significant effect was observed on the parameters measured except the left epididymal length. The knowledge of basic morphometric characteristics of the reproductive tract have been found to provide valuable information in the evaluation of breeding and fertility potential of the animals Ogbuewu et al., [59]. Gage and Freckleton (2003) described the mammalian testes as infallible predictors of spermatozoa production. The authors further asserted that knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. The range of values obtained for testis length (left: 2.45 ± 0.16 cm to 3.55 ± 0.15 cm; right 1.63 ± 0.31 cm to 2.90 ± 0.11 cm) were higher than the testis length (2.43 ± 0.0.07 cm to 2.80 ± 0.25 cm) reported by Ahemen *et al*., [56]. The values for testis length (left and right) and width (left: 0.76 ± 0.12 cm to 1.15 ± 0.15 cm; right: 0.63 ± 0.31 cm to 1.05 ± 0.05 cm) obtained in this study are similar to ranges of (2.26 cm to 4.40 cm and 0.94 cm to 1.10 cm) for testicular length and width reported by Ajayi et al. [60] in rabbits. Testis volume showed no significant (P < 0.05) different with the control having the highest value in right testis volume and paired testis volume (2.03 ± 0.03 g and 4.03 ± 0.03 g) respectively. According to Ezekwe [61] and Perry and Petterson (2001), testis size, length and width are good indicators of present and future sperm production. Inconsistent trend were recorded for left epididymal and right epididymal length. Rabbit bucks fed with *Mucuna pruriens* seed meal diet in T_5 (20%) had the highest value of (6.70 ± 0.05 cm and 6.20 ± 0.25 cm) for LEL and REL respectively.

3.5 Effect of Experimental Diets on Testis Density of Rabbit Bucks

Density of materials is the mass (g) of a unit volume of that substances or material. Density also shows the extent of compaction of materials [62]. The effect of dietary treatments on the weight of vital internal organs offered graded levels of *Mucuna pruriens* seed meal diet. Significant differences (P < 0.05) in the mean value of testis density were observed in left testis and paired testes density parameters in the treatment groups. These differences in testicular parameters could be due to breed differences or variation in age of rabbit bucks [7]. The ranges of values obtained for paired testes density (0.65 ± 0.07 g/ml to 1.16 ± 0.20 g/ml) were lower than the paired testes density (0.92 g/ml to 1.17 g/ml) obtained by Ogbuewu [59]. Following the trend of this result for testicular morphometry, it would be

agreed with Paufler et al. (1969) and Oyeyemi et al. [7] that the higher the testicular value (without any abnormality), the higher the capacity of cells during spermatogenesis. Skinner [63] in his findings also postulated that increase in testicular parameters is followed by a corresponding increase in the sperm production of related animal.

Table 4. Effect of experimental diets on relative weight of reproductive organs of rabbit bucks

	Experimental diets				
Traits	**TI**	**T2**	**T3**	**T4**	**T5**
LTW (g)	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
RTW (g)	0.09 ± 0.01	0.07 ± 0.02	0.07 ± 0.00	0.08 ± 0.01	0.08 ± 0.01
PTW (g)	0.15 ± 0.02^{b}	0.15 ± 0.04^{b}	0.14 ± 0.01^{b}	0.17 ± 0.02^{a}	0.17 ± 0.01^{a}
LEW (g)	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
REW (g)	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00
PEW (g)	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.00

^{a,b}Means within each row with different superscripts are significantly different (P < 0.05). ns – not significantly different (P > 0.05). LTW – Left Testis Weight; RTW – Right Testis Weight; PTW – Paired Testis Weight; LEW – Left Epididymal Weight; REW – Right Epididyma Weight; PEW – Paired Epididymal Weight; T_1 – 0% Mucuna pruriens Seed Meal Diet; T_2 – 5% Mucuna pruriens Seed Meal Diet; T_3 – 10% Mucuna pruriens Seed Meal Diet; T_4 – 15% Mucna pruriens Sead Meal Diet; T_5 – 20% Mucuna pruriens Seed Meal Diet

Table 5. Effect of experimental diets on measurements of reproductive organs of rabbit bucks

	Experimental diets				
Traits	**TI**	**T2**	**T3**	**T4**	**T5**
LTV (cm3)	2.00 ± 0.00	1.67 ± 0.33	1.47 ± 0.29	2.03 ± 0.03	0.63 ± 0.32
RTV (cm3)	2.03 ± 0.03	1.63 ± 0.47	1.53 ± 0.29	1.90 ± 0.05	1.67 ± 0.33
PTV (cm3)	4.03 ± 0.03	3.30 ± 0.80	3.00 ± 0.58	3.93 ± 0.07	3.30 ± 0.65
LTL (cm)	2.73 ± 0.19	3.03 ± 0.52	2.60 ± 0.10	3.10 ± 0.32	2.67 ± 0.09
LTW_d (cm)	0.97 ± 0.19	1.03 ± 0.15	0.77 ± 0.07	1.00 ± 0.17	0.90 ± 0.10
RTL (cm)	2.45 ± 0.05	2.37 ± 0.33	2.37 ± 6.82	2.60 ± 0.21	2.73 ± 0.09
RTW_d (g)	0.95 ± 0.05	0.90 ± 0.15	0.80 ± 0.06	1.00 ± 0.06	0.83 ± 0.12
LEL (cm)	6.03 ± 0.48^{ab}	5.73 ± 0.44^{ab}	5.23 ± 0.28^{b}	6.33 ± 0.37^{ab}	6.70 ± 0.06^{a}
REL(cm)	5.40 ± 0.30	5.63 ± 0.35	5.23 ± 0.43	6.03 ± 0.17	6.20 ± 0.25

^{a,b}Means within each row with different superscripts are significantly different (P < 0.05). ns – not significantly different (P > 0.05). LTV – Left Testis Volume; RTV – Right Testis Volume; PTV – Paired Testis Volume; LTL – Left Testis Length; LTW_d - Left Testis Width; RTL – Right Testis Length; RTW_d – Right Testis Width; LEL – Left Epididymal Length; REL – Right Epididymal Length; T_1 – 0% Mucuna pruriens Seed Meal Diet; T_2 – 5% Mucuna pruriens Seed Meal Diet; T_3 – 10% Mucuna pruriens Seed Meal Diet; T_4 – 15% Mucna pruriens Sead Meal Diet; T_5 – 20% Mucuna pruriens Seed Meal Diet

Table 6. Effect of experimental diets on testis density of rabbit bucks

	Experimental diets				
Traits	**T1**	**T2**	**T3**	**T4**	**T5**
LTD (g)	0.57 ± 0.10^{b}	0.94 ± 0.01^{ab}	0.92 ± 0.17^{ab}	0.74 ± 0.06^{b}	1.17 ± 0.18^{a}
RTD (g)	0.73 ± 0.06	0.84 ± 0.11	0.94 ± 0.05	0.83 ± 0.06	1.14 ± 0.22
PTD (g)	0.65 ± 0.07^{b}	0.89 ± 0.02^{ab}	0.92 ± 0.13^{ab}	0.77 ± 0.05^{ab}	1.16 ± 0.20^{a}

^{a,b} Means within each row with different superscripts are significantly different (P < 0.05). ns – not significantly different (P > 0.05). LTD – Left Testis Density; RTD - Right Testis Density; PTD – Paired Testis Density; T_1 – 0% Mucuna pruriens Seed Meal Diet; T_2 – 5% Mucuna pruriens Seed Meal Diet; T_3 – 10% Mucuna pruriens Seed Meal Diet; T_4 – 15% Mucna pruriens Sead Meal Diet; T_5 – 20% Mucuna pruriens Seed Meal Diet

3.6 Effect of Experimental Diets on Testicular Histology

The histological examination of the testes tissue sections showed that:

Plate 1: Of the treatment group which is the control diet showed seminiferous tubule atrophy with an increase in the seminiferous tubules space (white stars). The interstitial cells (black arrows) and the spermatids (white arrows) appeared diminished in most parts of the section and remained morphologically normal.

Plate 2: The group 2 tissue sections presented intact, distinct and densely populated seminiferous tubules clearly demarcated by a thick layer of basement membrane (black arrows) and interstitial cells of leydig. (White arrowheads = sertoli cells).

Plate 3: Test group 3 testes tissue sections showed an intact and distinct seminiferous tubules clearly demarcated by the basement membrane and interstitial cells of leydig (black arrowheads) each highly cellular indicating active spermatogenesis as cells of the spermatogenic series seen at different stages.

Plate 4: Test group 4 tissue sections showed normal tissue architecture. Basement membrane (white stars) slightly increased clearly demarcates the seminiferous tubules, highly cellular indicating active spermatogenesis and depicting presence of sertoli cells (black arrows), Primary spermatocytes (white arrows) and numerous spermatids (white arrowheads).

Plate 5: The testes tissue sections showed normal tissue architecture with decreased basement membrane and interstitial cells (black arrows) evident by the presence of white empty spaces between seminiferous tubules. The seminiferous tubules are highly cellular with cells of the spermatogenic series at different stages of development.

Plates 1 – 5 showed a cross section of the testis for the rabbits fed with diets containing varying inclusion levels of *Mucuna pruriens* seed meal diets. The plates showed normal histological structure of the seminiferous tubules and germ cells in their various stages of maturation arranged in a layered order. The seminiferous tubules were better organized and more clearly define in groups 2, 3 and 4 respectively compared to group 1 (control) and 5. The lumen of the seminiferous tubules in the treatment groups had higher density of sperm cells in a dose dependent manner. The histological findings in the average dose in groups 2, 3 and 4 is reflective of the assertion by Damela et al. 2015 and [14] that Mucuna pruriens seed extracts enhance reproductive performance in animals.

Testicular Histology

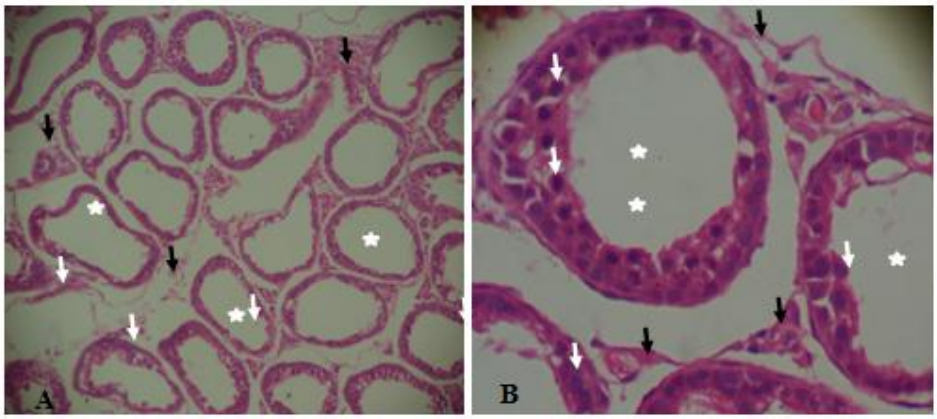

Plate 1. (T_1): A section of the testis of rabbit buck fed 0 % *Mucuna pruriens* seed meal diet for eight weeks, showing seminiferous tubule atrophy evident by the increase in the seminiferous tubule space (white stars). The interstitial cells (black arrows) are severely decreased and appear severely diminished in most parts of the section. The Spermatids (white arrows) though diminished, appear morphologically normal. No. H&E A: X100 B: X400

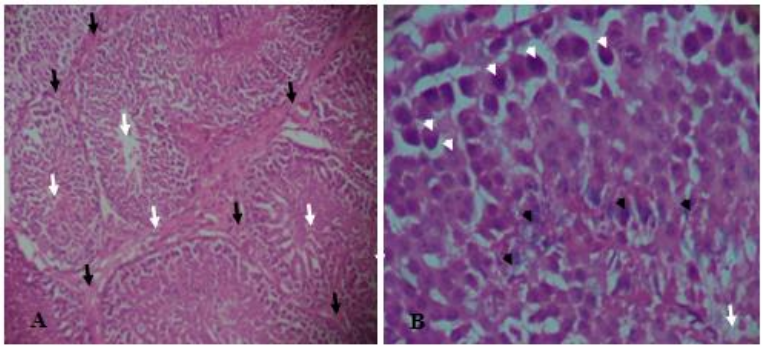

Plate 2. (T_2): A section of the testis of rabbit buck fed 5 % *Mucuna pruriens* seed meal diet for eight weeks showing normal morphology. The seminiferous tubules are clearly demarcated by a thick layer of basement membrane (black arrows) and interstitial cells (of Leydig).The seminiferous tubules are composed of cells at different stages of spermatogenesis. Black arrowheads= spermatids, White arrowheads= sertoli cells. H&E A: X100 B: X400

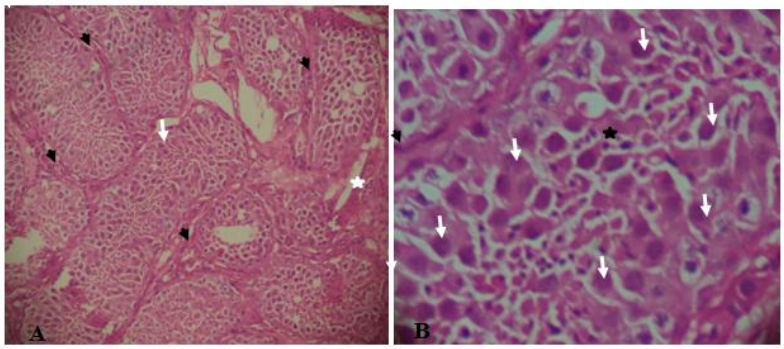

Plate 3. (T_3): A section of the testis of rabbit buck fed 10 % *Mucuna pruriens* seed meal diet for eight weeks, showing normal histology. The seminiferous tubules are clearly demarcated by the basement membrane and interstitial cells of Leydig (black arrowheads) and each is highly cellular indicating active spermatogenesis as cells of the spermatogenic series are seen at different stages. White arrows= sertoli cells. H&E A: X100 B: X400

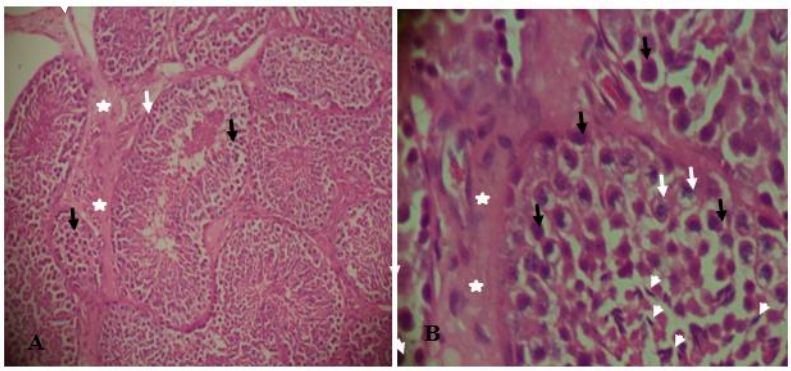

Plate 4. (T_4): A section of the testis of rabbit buck fed 15 % *Mucuna pruriens* seed meal diet for eight weeks, showing normal histology, showing normal tissue architecture. The Basement membrane (white stars) is slightly increased and clearly demarcates the seminiferous tubules. The seminiferous tubules are highly cellular indicating active spermatogenesis. Black arrows= sertoli cells, white arrows=Primary spermatocytes, white arrowheads= spermatids. H&E A: X100 B: X400

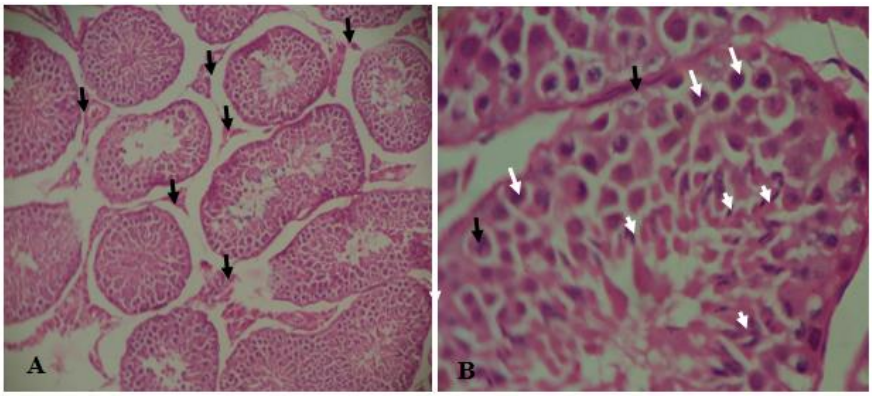

Plate 5. (T_5): A section of the testis of rabbit buck fed 15% *Mucuna pruriens* seed meal diet for eight weeks, showing normal tissue architecture with decreased basement membrane and interstitial cells (black arrows) evident by the presence of wide empty spaces between the seminiferous tubules. The seminiferous tubules are highly cellular with cells of the spermatogenic series at different stages of development. White arrows= sertoli cells, white arrowheads= spermatids. H&E A: X100 B: X400

4. CONCLUSION

- Results from this study showed that *Mucuna pruriens* seed meal improved the reproductive performance of rabbit bucks, thus improving rabbit production at 20%.
- From the findings, it appears Mucuna pruriens seed meal diet is a potential enhancer of male reproductive performance that can be recommended to rabbit farmers for improving reproductive performance, hence a boon to reproduction and production in rabbit farming industry.
- Incorporation of MSM up to 20% in the diets of rabbits had no deleterious effects on haematolgy and serum biochemical indices.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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