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Haematology and Hormonal Profiles of Adult Rabbits Fed Groundnut, Moringa and Lablab Forage Meal Based Diets

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Target Audience: Researchers, Rabbit farmers, Physiologists

Abstract

A study was carried out to evaluate haematology and hormonal profiles of rabbits fed groundnut, Moringa and lablab forage meal-based diets. A total of 48 adult rabbits (24 does and 24 bucks) 7 to 8 months old (crossbreds between imported Hyla rabbits and local adapted rabbits) with average initial weight of 3.50 kg were used. They were randomly allocated to four treatments in a completely randomized design in a 4x2 factorial arrangement. The lasted for 12 weeks with a one-week adjustment period. The factors considered were for heamatology were three types of forage (Arachis hypogea, Moringa oleifera and Lablab purpureus forage meals and a control/concentrate only) and sex (male and female). For hormonal profiles, the factors considered were three types of forage (Arachis hypogea, Moringa oleifera and Lablab purpureus forage meals and a control/concentrate only) and pregnancy status (before mating (open) and 3 weeks after mating (pregnant)) for rabbit does and for male rabbits (21 and 25 weeks of age). Haematological parameters such as white and red blood cells, haemoglobin concentration, PCV, were measured. Hormonal parameters such as progesterone, oestradiol and testosterone concentrations were measured. The result indicated that forage type had significant effect on WBC, lymphocytes, RBC, and oestradiol concentration. Rabbits fed Moringa oleifera forage meal diet had higher mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Sex had significant (P<0.05) effect on most heamatological parameters except WBC, MCHb conc and platelets. There was significant (P<0.05) interaction between forage type and sex on lymphocyte and mean corpuscular volume as well as between forage type and pregnancy status on progesterone concentration. Testosterone profile was depressed by lablab forage meal diet. Moringa and groundnut

Keywords: Rabbits, haematology, hormones, forage meal diets, groundnut, Moringa, Lablab.

Description of Problem

In recent years, the production of rabbits and other livestock species have been faced with the challenge of feed availability and rising cost of conventional feedstuff such as maize, groundnut cake, soyabean cake to mention a few. These conventional feedstuffs are also highly competed for by man, hence the need to source for cheaper and readily available feedstuffs to mitigate these challenges. Some of such feedstuffs consumed by rabbits are forages including *Arachis hypogea (groundnut), Moringa oleifera* (Moringa) and L*ablab purpureus* L. Sweet *(Lablab).* These are non-conventional feedstuff which could serve as cheap sources of fibre, protein and other nutrients for rabbits. Groundnut foliage has potential to be a valuable feed supplement for rabbit as it is rich in crude protein and has high organic matter digestibility (1). Recently there has been interest in the utilization of *Moringa oleifera* commonly called (drumstick tree or red horse tree), a potential inexpensive protein source for livestock feeding (2). Lablab is one of the most popular leguminous forage species in the world (3). Lablab is a dual-purpose legume, which means it can be traditionally grown as a pulse crop for human consumption, used as a fodder for livestock or as green manure (4). However, they have to be supplemented with additional feed sources such as concentrates for optimal utilization by rabbits.

Groundnut, lablab and Moringa leave meals at different studies enhanced reproductive function by increasing the secretion or availability of ovarian hormones such as progesterone and oestrogens in female rats (5), ewes (6) and rabbits (7). The main purpose of this study is to evaluate the influence of these forages (*Arachis hypogea, Moringa oleifera* and L*ablab purpureus*) on haematology and hormonal profiles of adult rabbits.

Materials and Methods

Location of experimental site

The experiment was conducted at the Rabbitry Unit, Swine and Rabbit Research Programme, National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. Shika is located in the Northern Guinea Savannah on latitude 11°8'N and longitude 07°4' E with altitude of about 663.77 mm above sea level (8). The average annual precipitation is 1,100 mm, which spreads from late April or early May to mid-October with a peak between June and September. Average temperature varies from 14°C during the early dry season (November–January) and 39.3°C during the late dry season (February to April) while the mean relative humidity during dry and wet season is 21% and 72% respectively.

Experimental Animals and management

A total of 48 adult rabbits (24 does and 24 bucks), age 7 to 8 months old with average initial weight of 3.50 kg (crossbreds between imported Hyla rabbits and local adapted rabbits) used, were sourced from the Rabbit Research Unit, Swine and Rabbit Research Program, National Animal Production Research Institute, Shika-Zaria. The experiments lasted for 12 weeks with a one-week adjustment period. The rabbits were housed in individually 120 x 60 x 50 cm metal cages with a dimension of and raised about 25 cm from the ground. The cages were placed in a well-ventilated house with wide open windows covered with wire mesh and screened using netting. The floor of the house was thoroughly washed and disinfected before the onset of the studies. The rabbits were prophylactically treated with subcutaneous injection of Ivomectine® 10 mg/ml at the rate of 0.2 ml/rabbit against both ecto- and endoparasites. The animals were treated against coccidiosis with Amprolium® according to their body weight and manufacturers prescription, anti-stress (Vitalyte)® and anti-biotic (Terramycin long acting (LA)® plus).

The rabbits were randomly allocated to four treatments made up of *Arachis hypogea, Moringa oleifera* and *Lablab purpureus* forage meals and a control (concentrate only) in a completely randomized design. A weighed amount of the experimental diets and clean water were fed *ad libitum* to the animals in the morning at 8:00am daily in flat bottom earthen pots with rims to prevent feed wastage.

Sourcing and processing of feedstuff

Moringa was purchased from Giwa market, Giwa Local Government area, Kaduna state. Groundnut haulm was obtained from the Rabbit Research Unit, Swine and Rabbit Research Program, NAPRI Shika Zaria plot while the lablab forage was obtained after seed harvesting from the Feed and Nutrition Research Program (FNRP), NAPRI, Shika-Zaria demonstration plots. The forages were air dried in a well-ventilated room (shade) to maintain its greenish colour and quality. The forages were milled at a feed mill and bagged before use.

Mating of the does

Hand-mating method was adopted. The does were taken to the bucks at 8:00 am and 4:00 pm to ensure good courtship and adequate service and returned to their cages after mating. The rabbits were mated at a ratio of one buck to two does. After mating, the does were checked for pregnancy by abdominal palpation after two weeks of mating. Does that were not pregnant were re-mated immediately.

Experimental design, data collection and procedures

The experimental design used for this study was completely randomized design with three forages (groundnut, Moringa and lablab forage meals incorporated in the control concentrate at a replacement of 10%) and a control (concentrate only) and two sex (male and female) in a 4x2 factorial arrangement. For hormonal profiles, the factors considered were three types of forage (*Arachis hypogea, Moringa oleifera* and *Lablab purpureus* forage meals and a control/concentrate only) and pregnancy status (before mating (open) and 3 weeks after mating (pregnant)) for rabbit does and for male rabbits (21 and 25 weeks of age). The concentrate (Table 1) and forage meals were manually mixed to form complete diets. Haematological (white blood cells, lymphocytes, red blood cells among others) and hormonal concentrations (progesterone, oestradiol and testosterone concentrations) were monitored.

Measurement of Haematological Parameters: Five (5) ml of blood was collected from the marginal ear vein of adult male and female rabbits into labelled bijou bottles containing an anticoagulant ethylene diamine tetra-acetic acid (EDTA) to

Table 1: Feed ingredient and nutrient composition of the concentrate diet fed to rabbits

	Concentrate diet(%)
Maize	39.24
Groundnut cake	42.26
Maize offal	15.00
Bone meal	3.00
Salt	0.25
Premix	0.25
Nutrient composition:	
Dry matter	92.90
Crude protein (%)	16.00
Crude fibre (%)	21.14
Ether extract (%)	4.22
Ash (%)	6.28
Nitrogen free extract (%)	52.36

*Vitamin/mineral premix content per kilogramme ration: Vit. A 1251 IU, Vit. D3 2750 IU, Vit. E 151 IU, Vit. K 0.002g, Vit. B_2 0.006g, Nicotinic acid 0.035, Calcium D-Pantothenate 0.01mg, Vit. B_6 0.0035g, Vit. B_{12} 0.02g, Folic acid 0.001g, Biotin 0.0005g, Vit. C 0.025g, Cholin chloride 0.39g, Zinc bacitracin 0.02g, Methionine 0.2g, Avatec (Lasolocid) 0.09g, Manganese 0.1g, Iron 0.05g, Zinc 0.04g, Copper 0.002g, Iodine 0.00153g, Cobalt 0.000225g, Selenium 0.0001g.

determine white blood cell (WBC), lymphocyte, red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCHb), mean corpuscular haemoglobin concentration (MCHb conc) and platelets.

Hormonal assay: Blood samples (5 ml) were collected from the marginal ear vein of the does, before mating and three weeks after mating, and used for hormonal profiling (oestradiol and progesterone concentration), while for the bucks, blood was collected at 21 and 25 weeks of age for testosterone concentration measurement. The blood samples were placed into sterile sample bottles containing oxalate fluoride to allow the serum to separate from the blood before the harvest of the serum in plain serum bottles. The serum samples were stored at -20ºC until assayed. The hormonal profile (oestradiol, progesterone and testosterone concentration) was determined by enzyme linked immunosorbent assay (ELISA) technique using the methods described by Olayemi (9). The hormonal evaluation was done in the Animal Reproduction Laboratory of NAPRI, Shika.

Statistical analysis

Data collected were subjected to two-way analysis of variance (ANOVA) using the SAS 9.0 (10). Significant differences in means between treatments were separated using Pairwise difference (10).

Haematological parameters were analysed using repeated measures. Shapiro-Wilks Test was used to test the assumptions of normality for the levels of the independent variables. Model statements is given below as:

$Y_{ij} = \mu + T_i + L_j + (TL)_{ij} + \varepsilon_{ij}$

Where:

Yij = Observation of i^{th} forage type and j^{th} mating date/age of rabbit

μ=overall mean

T_i = the effect of $_i^{th}$ of forage type

L_j = the effect of $_j^{th}$ mating date/age of rabbit

$(TL)_{ij}$ = the effect of interactions of i^{th} forage type and $_j^{th}$ mating date/age of rabbit

ε_{ij} = random error with mean 0 and variance σ^2

Model statement for repeated measures

$Y_{ijk} = \mu + \tau_i + \delta_{ij} + t_k + (\tau * t)_{ik} + \varepsilon_{ijk}$

μ = the overall mean

τ_i = the effect of i^{th} forage type

t_k = the effect of k^{th} sex

$(\tau * t)_{ik}$ = the effect of interaction between i^{th} forage type and k^{th} sex

δ_{ij} = random error with mean 0 and variance σ^2_δ, the variance between animals (subjects) within treatment and it is equal to the covariance between repeated measurements within animals

ε_{ijk} = random error with the mean 0 and variance σ^2, the variance between measurements within animals.

Results and Discussion

Haematological profiles

The effects of forage type and sex on haematological profile of adult rabbits is presented in Table 2. The results indicate that white blood cells, lymphocytes and red blood cells were significantly (P<0.05) affected by forage type. While sex had significant (P<0.05) effect on most heamatological parameters except WBC, MCHb conc and platelets. Rabbits fed concentrate diet and Moringa forage meal diet had similar (P>0.05) white blood cells as those fed lablab forage meal diet but significantly (P<0.05) higher than those fed groundnut forage meal diet. However, rabbits fed concentrate diet had significantly (P<0.05) higher lymphocytes than those fed groundnut and lablab forage meal diets though similar to rabbits fed Moringa forage meal diet. While the forage meal diets had lymphocytes within the normal range, rabbits on the concentrate diet had lymphocytes of 53.37% which is higher than the normal range of 15-50% (11). Rabbits fed concentrate diet, Moringa and lablab forage meal diets had significantly (P<0.05) higher red blood cells than those fed groundnut forage meal diet, which were also higher than the normal range of 3.50-5.50 $x10^{12}/l$ (11). Male rabbits had significantly (P<0.05) higher lymphocytes, RBC, Hb and PCV than female rabbits, while female rabbits had significantly (P<0.05) higher MCV and MCHb than male rabbits. PCV of rabbits on groundnut forage meal diet (27.50%) and female rabbits (29.69%) are lower than the normal range of 35-55% reported for rabbits (11). Also, rabbits on Moringa (34.31%) and lablab (33.35%) had lower PCV than normal. MCV for all the rabbits of 61.47-67.48 fl is below normal range of 75-100 fl (11).

The interaction between forage type and sex on haematological profile of adult rabbits fed forage meal diets is presented in Table 3. There was significant (P<0.05) interaction

Table 2: Forage type and sex effects on haematological profile of adult rabbits

	Forage type					Sex		
Parameter	Concentrate diet	Groundnut	Moringa	Lablab	P value	Male	Female	P value
WBC (10^9/l)	8.01±0.85^a	4.51±0.85^b	8.04±0.89^a	6.88±0.99^{ab}	0.018	7.64±0.59	6.08±0.68	0.0875
Lymph (%)	53.37±3.35^a	48.89±3.35^c	49.24±3.35^{ab}	45.29±3.91^{bc}	0.0344	50.42±2.31^a	47.90±2.67^b	0.0123
RBC (10^{12}/l)	5.57±0.34^a	4.21±0.34^b	5.28±0.35^a	5.23±0.39^a	0.0490	5.62±0.23^a	4.52±0.27^b	0.0029
Hb (g/dl)	11.20±0.69	8.79±0.69	10.93±0.72	10.78±0.80	0.0734	11.39±0.48^a	9.46±0.55^b	0.0122
PCV (%)	35.25±2.16	27.50±2.16	34.31±2.25	33.35±2.52	0.0694	35.51±1.47^a	29.69±1.72^b	0.0153
MCV (fl)	63.39±0.72	65.51±0.72	65.21±0.75	64.19±0.84	0.1759	63.41±0.5^b	65.75±0.58^a	0.0043
MCHb (pg/cell)	20.15±0.27	20.99±0.27	20.71±0.28	20.78±0.31	0.1431	20.31±0.19^b	21.00±0.21^a	0.0297
MCHb conc. (g/dl)	31.80±0.31	32.04±0.31	31.78±0.32	32.37±0.36	0.5890	32.03±0.21	31.96±0.24	0.8383
Platelets (10^9/l)	146.65±38.74	125.27±38.74	179.70±40.47	274.75±45.24	0.0901	206.10±26.77	157.08±30.91	0.2391

[abc]Means with different superscripts in the same row are significantly (P<0.05) different; WBC=White blood cells, Lymph=Lymphoc yte count, RBC=Red blood cells, Hb=Haemoglobin, MCV=Mean corpuscular volume, MCHb=Mean corpuscular haemoglobin, MCHb conc.=Mean corpuscular haemoglobin concentration

Table 3: Forage type and sex interaction on haematological profile of adult rabbits

Forage type	Concentrate diet		Groundnut		Moringa		Lablab		
Sex	Male	Female	Male	Female	Male	Female	Male	Female	P value
WBC (10^9/l)	8.63±1.15	7.38±1.25	4.55±1.15	4.46±1.25	10.12±1.25	5.96±1.25	7.27±1.15	6.50±1.62	0.392
Lymph (%)	47.55±4.51^{ab}	59.18±4.94^a	51.93±4.51^a	45.84±4.94^{ab}	56.72±4.94^b	41.76±4.94^b	45.48±4.51^b	45.1±6.38^{ab}	0.054
RBC (10^{12}/l)	5.83±0.45	5.30±0.50	4.93±0.45	3.48±0.50	6.28±0.50	4.28±0.50	5.45±0.45	5.00±0.64	0.366
Hb (g/dl)	11.47±0.93	10.94±1.02	10.45±0.93	7.12±1.02	12.68±1.02	9.18±1.02	10.95±0.93	10.60±1.31	0.259
PCV (%)	35.82±2.91	34.68±3.17	32.55±2.91	22.44±3.19	39.62±3.19	29.00±3.19	34.07±2.91	32.63±4.11	0.275
MCV (fl)	61.47±0.97^b	65.32±1.07^{ab}	66.3±0.97^a	64.72±1.07^{ab}	62.94±1.07^{bc}	67.48±1.07^a	62.92±0.97^b	65.47±1.38^{ab}	0.026
MCHb (pg/cell)	19.70±0.36	20.60±0.40	21.20±0.36	20.78±0.40	20.14±0.40	21.28±0.40	20.22±0.36	21.33±0.51	0.163
MCHb conc. (g/dl)	32.03±0.41	31.56±0.45	31.97±0.41	32.12±0.45	32.02±0.45	31.54±0.45	32.1±0.41	32.63±0.59	0.647
Platelets (10^9/l)	173.5±52.24	119.8±57.23	157.33±52.24	93.2±57.23	266.4±57.23	93±57.23	227.17±52.24	322.33±73.88	0.200

[ab]Means with different superscripts in the same row are significantly (P<0.05) different; WBC=White blood cells, Lymph=Lymphocyte count, RBC=Red blood cells, Hb=Haemoglobin, MCV=Mean corpuscular volume, MCHb=Mean corpuscular haemoglobin, MCHb conc.=Mean corpuscular haemoglobin concentration

between forage type and sex on lymphocytes and mean corpuscular volume. Female and male rabbits fed concentrate diet, groundnut and lablab forage meal diets had similar lymphocytes and mean corpuscular volume while male rabbits fed Moringa forage meal had significantly (P<0.05) higher lymphocytes and mean corpuscular volume than female rabbits. Female rabbits (59.18%) on concentrate diet, male rabbits on groundnut (51.98%) and Moringa (56.72%) forage meal diets had higher lymphocytes than the normal range while the other groups were within the normal range.

Significantly lower white blood cells and lymphocytes observed in rabbits fed groundnut forage meal diet compared to rabbits fed concentrate diet and Moringa forage meal diet suggests that the animals' immune system was boosted to aid the rabbits likely deal with environmental stress associated with high ambient temperatures effect of the tropics on the rabbits. Heat stress has an immune depressing effect on animals because of the compromised state of the internal organs associated with heat build-up in the rabbit system. (12) suggested that increase in lymphocyte concentration is usually a sign of viral infection. This does not appear to be the case in this study as the rabbits did not succumb to any disease conditions. Increased white blood cell count might be related to the production of more immune cells that play an important role in defending the biological system against different diseases (13). It was reported that a high white blood cell count could be caused by infection, immune system disorders, stress among others (14). White blood cell counts observed in this study are within the normal range of 3.5-10.0 x 10^9/l reported for rabbits (11). Earlier study reported high number of leucocytes in blood may be due to anaemia, bone marrow tumour, infectious diseases, inflammatory disease, severe physical stress, tissue damage among others (15). The function of the RBC is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues (16). Significantly lower red blood cell count obtained in the rabbits fed groundnut forage meal diet disagrees with the findings of (17) who reported significantly higher red blood cell count in broiler chickens fed groundnut haulms. The low RBC count and PCV recorded for rabbits on groundnut forage meal diet and female rabbits as well as low MCV and MCHb obtained in male rabbits in this study might likely mean that rabbits on groundnut forage and both sexes of rabbits were susceptible to anaemia-related disease conditions. MCV, MCH and MCHC blood indices are important for diagnosis of anaemia in most animals and also serve a useful index of the capacity of the bone marrow to produce red blood cells (18). This suggests that the rabbits might have low retained blood protein.

Retained proteins are reported to furnish the body with amino acids that are used for the synthesis of body proteins including blood proteins (19). (20) reported that PCV is the quickest indirect way of assessing values of red blood cells in circulation and is often used as a simple screening test for anaemia.

Hormonal profile

The effects of forage types and pregnancy status on hormonal profile of adult rabbits are presented in Table 4. The results indicate that only oestradiol concentration was significantly ($P<0.05$) affected by forage type while progesterone concentration was significantly affected by the pregnancy status of rabbits. Rabbits fed concentrate diet and groundnut forage meal diet had similar ($P>0.05$) oestradiol concentration as those on lablab forage meal diet but significantly ($P<0.05$) higher than those fed Moringa forage meal diet. This finding supports the report of (21) who reported significantly lower oestradiol concentration for female rabbits fed *Moringa oleifera* leaf powder, and hence suggests that Moringa *oleifera* might have a negative impact on the hypothalamic-pituitary-gonadal axis in the rabbits. Similar progesterone concentration observed in this study disagrees with (7) who reported significant ($P<0.05$) increase in serum progesterone levels in rabbits fed groundnut forage meal diet than those fed lablab forage meal diet.

Progesterone concentration was significantly ($P<0.05$) higher in female rabbits in the third week of pregnancy than in open does before mating as expected. This result is in agreement with the findings of (22) who reported higher progesterone after breeding in Hyla and crossbred rabbit does than before breeding. Greater concentration of

Table 4: Effects of forage type and weeks on hormonal profile of adult rabbits

Hormone	Forage type				P value	Pregnancy status		P value
	Concentrate diet	Groundnut	Moringa	Lablab		Before mating (open)	3rd week after mating (pregnant)	
Progesterone concentration (ng/ml)	2.86±0.54	2.79±0.51	4.72±0.57	3.29±0.57	0.068	0.73±0.36[b]	6.11±0.41[a]	0.001
Oestradiol concentration (pg/ml)	23.63±6.24[a]	33.33±5.95[a]	6.60±6.65[b]	13.30±6.65[ab]	0.026	15.40±4.20	23.02±4.79	0.240
	Forage type					**Weeks**		
Hormone	Concentrate diet	Groundnut	Moringa	Lablab	P value	21 weeks old	25 weeks old	P value
Testosterone concentration (ng/ml)	1.74±0.89	3.40±0.89	3.99±0.89	0.90±0.89	0.088	2.66±0.63	2.35±0.63	0.731

[abc]: Means with different superscripts in the same row are significantly (P<0.05) different

progesterone was observed during pregnancy than during non-pregnant diestrus period in domestic mammals (23). In pregnant rabbits, progesterone level must be sufficient to ensure a uterine environment favourable for the establishment and maintenance of pregnancy as its low level may be detrimental to foetal growth and survival (24) .Lablab was reported to contain protein and essential amino acids as well as it helps in the synthesis of progesterone and its transmitters in New Zealand White rabbit does (25). The high content of these nutrients could help boost the progesterone level in rabbits. Testosterone profile however was not affected (P>0.05) by forage type or age of rabbit in this study. Though lablab forage meal appeared to depress testosterone concentration. This result is at variance with the report of (7) who reported non-significant (P>0.05) difference in serum concentration of testosterone in rabbits fed groundnut and lablab forage meal diets.

The interaction between forage type and pregnancy status on hormonal profile of adult rabbits fed forage meal diets is presented in Table 5. The results indicate that there was significant (P<0.05) interaction between forage type and pregnancy status of rabbits on progesterone concentration. Rabbits fed Moringa and lablab forage meal diets had significantly (P<0.05) higher progesterone concentration in the third week of pregnancy than those fed concentrate diet and groundnut forage meal diet.

The effect of forage type and age of rabbit bucks on testosterone concentration of rabbits is presented in Figure 1. Testosterone concentration was higher on 21 weeks than 25 weeks of age on concentrate diet, there was a slight reduction on 25 weeks in rabbits fed groundnut forage meal and an increase in rabbits fed *Moringa* forage meal diet. While there was no change in testosterone concentration is bucks fed lablab forage meal diet which was also lowest at 21and 25 weeks than the other groups. *Moringa oleifera* has been reported to contain over 90 nutrients such as zinc, vitamins C and D and 46 antioxidants (26). This result shows that lablab forage might have low effect on spermiogenesis as a result of poor

Table 5: Forage type and Week interaction on hormonal profile of adult rabbits

Forage type	Concentrate diet		Groundnut		Moringa		Lablab		P value
Week	Before mating (open)	3rd week after mating (pregnant)	Before mating (open)	3rd week after mating (pregnant)	Before mating (open)	3rd week after mating (pregnant)	Before mating (open)	3rd week after mating (pregnant)	
Progesterone concentration (ng/ml)	$0.39{\pm}0.72^c$	$5.33{\pm}0.79^b$	$1.48{\pm}0.72^c$	$4.11{\pm}0.72^b$	$0.53{\pm}0.72^c$	$8.91{\pm}0.89^a$	$0.50{\pm}0.72^c$	$6.08{\pm}0.89^a$	0.0071
Oestradiol concentration (pg/ml)	22.16±8.41	25.10±9.21	27.17±8.41	39.50±8.41	3.44±8.41	9.75±10.30	8.84±8.41	17.75±10.30	0.9562

abc: Means with different superscripts in the same row are significantly ($P<0.05$) different

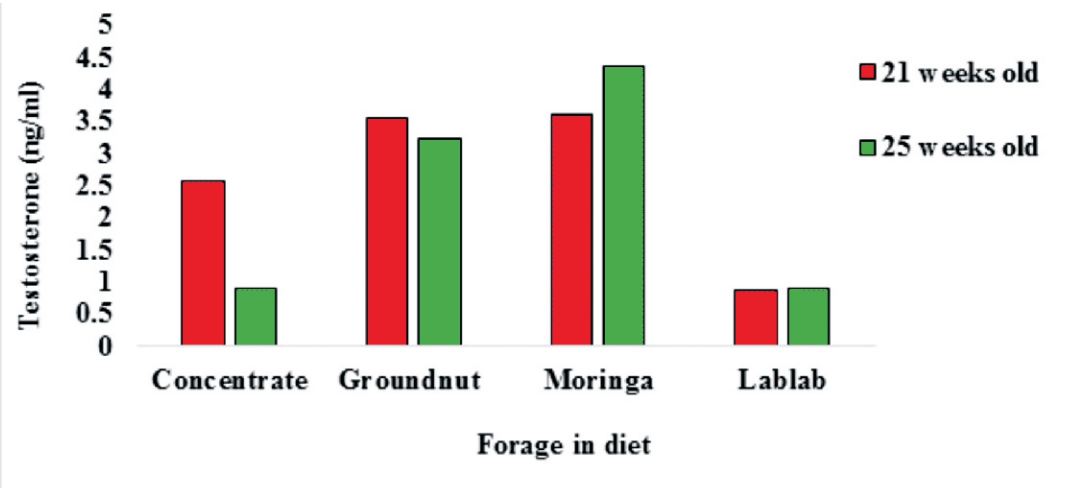

Figure 1: Forage type effect on testosterone concentration of adult rabbit bucks

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