National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email: japr@napri-ng.org Website: www.naprijapr.org

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EFFECT OF FORAGE TYPE, PALM OIL SUPPLEMENTATION AND SEX ON SERUM PROGESTERONE AND TESTOSTERONE PROFILE OF WEANER RABBITS

Iyeghe-Erakpotobor, G. T,*Okunlola, B.O., and Barje, P. P.
National Animal Production Research Institute, Ahmadu Bello University, PMB 1096, Shika-Zaria, Nigeria.
** Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Samaru-Zaria, Nigeria.*
Corresponding author email: g.t.iyeghe-erakpotobor@napri-ng.org; gtierak96@gmail.com

ABSTRACT

A study was conducted using a total of 96 mixed breed rabbits aged between 5-7 weeks were used to evaluate the effect of forage type, forage level, palm oil (PO) supplementation and sex on serum progesterone and testosterone profile of weaned rabbits. The rabbits were randomly allotted to the treatment groups in a Completely Randomized Design with 2x2x2x2 factorial arrangement. The factors considered were forage type (groundnut, lablab), level(20, 40%) palm oil supplementation (without, with) and sex (male, female). Water and feed were supplied *ad libitum*. The study lasted 98 days duration.Blood samples were collected starting from the twelfth week of age and subsequently at two weeks interval up to the eighteenth week to monitor progesterone and testosterone profiles of weaned rabbits. Progesterone and testosterone concentration increased ($P<0.05$) from the twelfth to eighteenth week of agein male and female rabbits. However, concentration of progesterone was significantly ($P<0.05$) higher in females than males of the same age. Serum testosterone concentration was not affected ($P>0.05$) by forage type. However, male and female rabbits fed groundnut forage meal had significantly ($P<0.05$) higher serum progesterone than those fed lablab forage meal. There were no significant ($P>0.05$) differences in testosterone profile of male and progesterone concentration in female rabbits fed diets with 20% or 40% forage meal however, significantly ($P<0.001$) higher progesterone concentration was observed in male rabbits fed 40% forage level than 20%. There were no significant differences ($P>0.05$) in serum testosterone in male and progesterone concentrationin female rabbits fed diets with or without PO. Progesterone levels were highly significant ($P<0.01$) in male rabbits fed diets with palm oil than those fed diets without PO.It is concluded that testosterone and progesterone profiles of weaned rabbits were influenced by feeding groundnut or lablab forage meals at 20 or 40% levels supplemented with palm oil.Based on testosterone and progesterone concentrations, male and female rabbits attained puberty by 14 weeks of age. Rabbits on 20% forage meal or lablab attained puberty later at 16 weeks while those on 40% forage meal or groundnut forage attained puberty earlier at 14 weeks of age.

Keywords: Progesterone, testosterone, forage meal, groundnut, lablab, palm oil, puberty, rabbits

INTRODUCTION

Progesterone is a steroid hormone secreted mainly by cells of the *corpus luteum*, placenta and adrenal gland; serum progesterone has also been used to check the effectiveness of ovulation induction (Muzvondiwa *et al*., 2011). Testosterone is needed to initiate spermatogenesis at puberty and for the maintenance of this process in the adult; it is also required for the completion of meiosis and differentiation of spermatids (Castro *et al*., 2002). Androgens rose sharply in male hamsters after day 30 to peak levels on day 50. Progesterone in males also remained low until

after day 30. Serum progesterone and oestradiol in females showedsome fluctuations between 10–30 days of age, which presumably represent maturational changes in the ovary. (Vomachka and Greenwald, 2013).Younglai *et al*. (1989) reported that progesterone concentration <2 ng/ml indicates absence of functional corpora lutea which also indicates an absence of ovulation. El-Tayeb *et al*. (1991) reported that some heifers showed visible signs of oestrus before progesterone was detected in their peripheral blood.

Nutrition is one of the environmental factors that influence hormonal profile and consequently, reproductive and productive performance of animals (Meshreky and Metry, 2000; Chiericato *et al.,* 2001; Meshreky and Shaheed, 2003,Meshreky *et al*., 2007).Nutritional factors influence hypothalamic-pituitary function and therefore gonadotrophin profiles, directly through effects of nutrients or metabolic hormones such as insulin acting on target organs or through changes in sensitivity of these organs to oestradiol, progesterone and other hormonal feedback (Rhind 1992, Muzvondiwa *et al*., 2011) mechnism. Diet had a significant effect on plasma progesterone concentration (Muzvondiwa *et al*., 2011).Sexual maturity was detected by the first presence of progesterone in the peripheral blood of the heifers fed green *Sorghum bicolor* alone or supplemented with low or high level of concentrate (El-Tayeb *et al*., 1991). High fiber diet loweredmetabolic clearance rate of progesterone compared with high cornstarch diet by lowering the half-life of progesterone (Lemley *et al*., 2010). This study was conducted to study progesterone and testosterone profiles of growing rabbits fed two forage types, forage levels and palm oil supplementation on attainment of puberty.

MATERIALS AND METHODS

The experiment was conducted at the Rabbit Unit of the Teaching and Research farm, Department of Animal Science, Ahmadu Bello University, Zaria. Zaria is within the Northern Guinea savannah zone. Zaria is located between Latitude 11° and12°N, Longitude 7°33ˈE and altitude of 646m above sea level(Meteorological Unit, IAR 2011).

A total of ninety-six crossbred rabbits, aged 5-7 weeks of average weight of 789.71 grams used in this study were purchased from the open market in Zaria. The rabbits were randomly allocated to four factors in a 2×2×2×2 factorial arrangement in a Completely Randomized Design (CRD). The factors considered were; forage type (groundnut and lablab), level of inclusion of forage meal (20 and 40%), palm oil supplementation (with, without), and sex effect (male and female).

The rabbits were housed individually in metal cages, measuring 40x60 cm in dimensionlocated in a well-ventilated house with large windows. Each hutch was equipped with flat bottom earthen pots to prevent feed wastage. The rabbits were treated with an anti-stress (Vitalyte; Phoenix pharmaceuticals U.S.A), antibiotics (Tetracin; Vetinida Pharmaceutical Limited India), coccidiostat (Amprolium; Lonye Technology Limited Guangzhou, China) and Ivomec manufactured by Savorite Pharmaceutical Limited Baroda (Guj), India against external and internal parasites during the one week adjustment period. The rabbits were fed 100 g of feed daily and given clean water *ad libitum.*

Lablab was obtained from the Forage Production Unit of the National Animal Production Research Institute (NAPRI). Groundnut haulm was purchased from the open market in Zaria. The concentrate was compounded with maize 39.5%, maize offal 15%, groundnut cake 42.26%, bone meal 3%, salt 0.25% and biomix premix 0.25%. The forages (lablab and groundnut haulms) were air-dried separately for seven days, chopped, milled, and incorporated into the experimental diets.

Effect of forage type, palm oil supplementation and sex

Blood samples were collected starting from the twelfth week of age and subsequently at two weeks interval up to the 18 weeks of age to monitor testosterone (males) and progesterone (males and females) profileswhich was used to monitor the likely age at attainment of puberty in the rabbits. The blood samples were collected through the ear vein; into plain tubes and centrifuged at 4000rpm for 15mins to harvest the serum. Serum samples were stored at -20ºC until assayed. Samples were analyzed for concentration of progesterone (Eggleston *et al*., 1990) and testosterone (Olayemi, 2007) using Enzyme-Linked Immunosorbent Assay test kits obtained from Accu-Bind Diagnostics, USA.

Data collected were log transformed and subjected to Analysis of Variance (ANOVA) test using the General Linear Model (GLM) procedure while Pairwise Difference Procedure was used to separate significant differences among means (SAS, 2001).

RESULTS AND DISCUSSION

There was a significant increase ($P<0.05$) in serum concentration of testosterone in males and progesterone in both male and female rabbits from 12 to 18 weeks of age **(**Table 1). The results show a high surge ($P<0.05$) in serum concentration of testosterone and progesterone levels between the 12^{th} and 14^{th} weeks and a slower increase subsequently. This indicates that the rabbits attained puberty by 14 weeks of age. This agrees with the report of Yamini *et al*. (2008) that puberty occurred in rabbits at 12-14 weeks of age. Progesterone concentration <2 ng/ml indicates absence of functional corpora lutea which indicates an absence of ovulation (Younglai *et al*., 1989).

Shahnaz *et al*. (2000) observed that during the pre-pubertal phase; progesterone and estradiol concentrations remained at the basal levels of 0.1-0.6 ng/ml, however a few days before the onset of puberty some fluctuations were observed.Plasma progesterone levels were 1.5 ng/ml (prior to mating), and 3.3 ng/ml at the end of gestation(Baldwin and Stabenfeldt, 1974). Serum progesterone level of 2.17±0.54 ng/ml obtained in this study is higher than progesterone concentration of 1.48±0.14 at 100 days (14 weeks) of age obtained by Younglai *et al*. (1989).This might be as a result of differences in the type of diets fed. However level of progesterone were significantly higher ($P<0.05$) in females than males of the same age in this study. This is not surprising since progesterone is produced more in female animals than males.

Table 1: Effect of age on testosterone and progesterone profiles of growing rabbits

	Age of rabbits (weeks)				
Hormone (ng/ml)	12	14	16	18	P value
Testosterone (M)	0.81 ± 0.48^{c}	2.50 ± 0.48^{b}	3.35 ± 0.48^{b}	5.67 ± 0.48^{a}	0.024
Progesterone (F)	0.59 ± 0.54^{d}	2.17 ± 0.54^{c}	3.83 ± 0.54^{b}	6.27 ± 0.54^{a}	0.035
Progesterone (M)	0.11 ± 0.12^{c}	0.24 ± 0.12^{c}	0.75 ± 0.12^{b}	1.50 ± 0.12^{a}	0.032

[abc]Means with different superscripts within the same row are significantly different. ng/ml: nanogram per milliliter, F: Female, M: Male

The effect of forage type on hormone profiles of rabbits is presented in Table 2. There was no significant ($P>0.05$) difference in serum concentration of testosterone in rabbits fed groundnut and lablab forage meals. However, there was significant ($P<0.05$) increase in serum progesterone levels in female ($P<0.05$) and male ($P<0.001$) rabbits fed groundnut forage meal than those fed lablab forage meal. The reason for this effect is not clear but it might probably have to do with the differences in forage type. It has been established that groundnut forage has

oestrogenicproperties; however its effect on progesterone or testosterone concentrations has not been established. There is a dearth of literature on the effect of forage types on testosterone and progesterone concentrationin animals.

Forage level did not significantly($P>0.05$) affect testosterone concentration in male rabbits and progesterone concentration in female rabbits fed diets with 20% or 40% forage meal (Table 2). Surprisingly, significantly ($P<0.001$) higher progesterone level was however, observed in male rabbits fed diets with 40% forage level than those fed 20%. The reason for this result might have to do with increased clearance rate of progesterone on high concentrate. Cows supplemented with 2 kg/d of finely ground corn had higher serum concentration of progesterone than those supplemented with 6 kg/d (Pescara *et al.,* 2010). Grazing pubertal heifers offered energy-based supplements three times weekly had decreased progesterone concentrations on days that supplements were offered, and forage fed cows similarly supplemented had decreased progesterone concentrations 4h after supplements had been offered (Cooke *et al*., 2007 cited in Pescara *et al*., 2010). Folman *et al*. (1973) reported a progesterone concentration of 5 ng/ml or more appeared earlier in cows maintained on a high level of nutrition than in cows on a standard level of nutrition. Though first ovulation occurred at about the same time, first oestrus was 10 days earlier in cows on a high level of nutrition.

Table 2: Effect of forage type and level on hormone profile of rabbits

Hormone (ng/ml)	Forage type			Forage level		
	Groundnut	Lablab	P value	20	40	P value
Testosterone (M)	3.02±0.46	3.13±0.46	0.87	3.15±0.46	3.02±0.46	0.801
Progesterone (F)	3.90±0.43[a]	2.53±0.43[b]	0.03	2.88±0.44	3.55±0.11	0.029
Progesterone (M)	0.92±0.11[a]	0.39±0.11[b]	0.0007	0.44±0.11[b]	0.87±0.11[a]	0.007

[ab]Means with different superscripts within the same row are significantly different. ng/ml: nanogram per milliliter, F: Female, M: Male.

Palm oil supplementation did not significantly ($P>0.05$) affect serum testosterone concentration in males and progesterone concentration in female rabbits (Table 3). Progesterone levels were however, highly significantly ($P<0.01$) affected in male rabbit diets supplemented with palm oil than those not supplemented with palm oil. In contrast to results obtained in this study, Clinton *et al*. (1997) and Dorgan *et al*. (1997) reported increased plasma androgen concentration on high fat diet. Likewise, reduction in fat intake caused a decrease in plasma testosterone concentration (Hanis *et al*., 1990; Perheentup *et al*., 1995).Gronadzka-Ostrowska *et al*. (2002) observed that testosterone production and uptake by target organs in male rats were significantly influenced by dietary fat type and feeding period, but not fat level. Testosterone level increased in quails fed 3% palm oil (Fitriyah *et al*., 2008).

Table 3: Effect of palm oil (PO) supplementation on testosterone and progesterone profile of rabbits

	Palm oil supplementation		
Hormone (ng/ml)	Without PO	With PO	P value
Testosterone (M)	3.46±0.46	2.71±0.46	0.25
Progesterone (F)	2.98±0.44	3.46±0.44	0.44
Progesterone (M)	0.46±0.11[b]	0.85±0.11[a]	0.01

[ab]Means with different superscripts within the same row are significantly different. ng/ml: nanogram per milliliter, F: Female, M: Male. PO: palm oil.

Interaction between forage type and age on hormone profile of rabbits is shown in Table 4. The result showed that serum testosterone and progesterone concentration of rabbits increased significantly ($P<0.05$) as the animal aged. At 18 weeks, rabbits fed lablab forage had significantly ($P<0.05$) higher testosterone and lower progesterone concentration in male and female rabbits fed groundnut forage meal. The general trend observed in this result indicated that as the animal aged, the serum testosterone in male rabbits increased at a faster rate in rabbits fed lablab forage meal than groundnut forage indicating that male rabbits fed lablab would have attained puberty earlier at 14 weeks than those on groundnut forage that would have attained puberty at 16 weeks of age. Adeyemi *et al*. (2014) did not observe significant difference in the testosterone profile of rabbits fed *Moringa oleifera* leaf meal. The range of testosterone (0.78-6.45 ng/ml) obtained in this study is similar to the range of 0.5-10 ng/ml reported by Moor and Younglai (1975).

Serum progesterone of female rabbits fed groundnut increased as the animals aged but the increase in the progesterone concentration of animals fed lablab was lower than those fed groundnut forage. Progesterone concentration in female and male rabbits increased at a faster rate in rabbits fed groundnut forage meal than on lablab forage meal. Based on the progesterone concentration observed in this study, it would appear that female rabbits fed groundnut forage would have attained puberty at 14 weeks of age while rabbits on lablab forage would have attained puberty at 16 weeks of age. Progesterone is required for ovulation responsible for changes associated with luteal phase of oestrous cycle, essential for establishment and maintenance of early pregnancy (Hu *et al*., 2010). Plasma levels of progesterone are less than 1 ng/ml at oestrus and 5 to 9 ng/ml at peak of the luteal phase (Folman *et al.,* 1973).

The interaction between forage level and age on hormone profile of rabbits is presented in Table 5. A trend was established in serum progesterone and testosterone levels with increased level of forage and as the rabbits aged. Rabbits fed 20 and 40% forage had significantly ($P<0.05$) higher serum testosterone concentration as they aged being similar for 20 and 40% at similar ages. The serum progesterone concentration of female rabbits followed similar trend with testosterone, increasing in concentration as the rabbit aged up to 18 weeks. Based on progesterone concentration of the female rabbits, it appears that rabbits on 20% forage meal attained progesterone concentration of 2.94±0.77 ng/ml at 16 weeks of age while those on 40%forage meal attained progesterone concentration of 2.78±0.77 ng/ml at 14 weeks of age. This indicates that rabbits on 20% forage meal attained puberty later (at 16 weeks) than those on 40% forage

meal that attained puberty earlier (at 14 weeks of age). Patterson *et al*. (1992) reported lower planes of nutrition delayed the onset of puberty by inhibiting maturation of the endocrine system. In contrast to this study, El-Tayeb *et al*. (1991) reported that heifers fed high level of concentrate reached puberty at a significantly ($P<0.05$) younger age than those fed low level of concentrate.

Serum progesterone concentration of male rabbits fed 40% forage at 16 and 18 weeks were significantly ($P<0.05$) higher than those fed 20% forage at 16 and 18 weeks ofage. This could be attributed to better utilization of the legume forages and subsequently its effect on hormone concentration. Environmental conditions, such as nutrition or environmental temperature can influence the hormonal profile of rabbits consequently the reproductive and productive performance of animals (Meshreky and Metry, 2000;Chiericato *et al*., 2001; Meshreky and Shaheed, 2003; Meshreky *et al*., 2007).

Table 4: Interaction between forage type and age on testosterone and progesterone profile of rabbits

Forage types	Groundnut				Lablab				
Age (weeks)	12	14	16	18	12	14	16	18	Pvalue
Hormones(ng/ml)									
Testosterone (M)	0.78±0.62^{de}	1.80±0.62^{cde}	4.65±0.62^{b}	4.89±0.62^{b}	0.84±0.62^{d}	3.20±0.62^{c}	2.05±0.62^{cd}	6.45±0.62^{a}	0.03
Progesterone (F)	1.03±0.76^{cd}	2.55±0.76^{c}	4.56±0.76^{b}	7.45±0.76^{a}	0.16±0.76^{e}	1.79±0.76^{cd}	3.10±0.76^{bc}	5.09±0.76^{b}	0.02
Progesterone (M)	0.14±0.17^{c}	0.36±0.17^{c}	1.11±0.17^{b}	2.05±0.17^{a}	0.08±0.17^{c}	0.13±0.17^{c}	0.39±0.17^{c}	0.95±0.17^{b}	0.001

^{abc}Means with different superscripts within the same row are significantly (P<0.05) different. F:Female, M:Male

Table 5: Interaction between forage level and age on testosterone and progesterone profile of rabbits

Forage level	20				40				
Age (weeks)	12	14	16	18	12	14	16	18	P value
Hormones (ng/ml)									
Testosterone (M)	1.05±0.69^{cd}	2.84±0.69^{b}	3.00±0.69^{b}	5.70±0.69^{a}	0.56±0.69^{d}	2.16±0.69^{bc}	3.70±0.69^{b}	5.64±0.69^{a}	0.03
Progesterone (F)	0.21±0.77^{de}	1.56±0.77^{d}	2.94±0.77^{d}	6.82±0.77^{a}	0.98±0.77^{de}	2.78±0.77^{d}	4.72±0.77^{bc}	5.71±0.77^{ab}	0.03
Progesterone (M)	0.10±0.17^{e}	0.31±0.17^{d}	0.49±0.17^{d}	0.85±0.17^{bc}	0.13±0.17^{de}	0.18±0.17^{d}	1.01±0.17^{b}	2.15±0.17^{a}	0.04

^{abc}Means with different superscripts within the same row are significantly (P<0.05) different. ng/ml: nanogram per milliliter, G/haulm: groundnut haulm, F:Female, M:Male

CONCLUSION

Forage type influenced progesterone concentration in male and female rabbits. Forage level and palm oil supplementation influenced progesterone concentration only in male rabbits. Testosterone level was only influenced by age. Groundnut forage meal influenced higher progesterone concentration in male and female rabbits while forage level and palm oil supplementation increased progesterone concentration only in male rabbits. Based on testosterone and progesterone concentrations, male and female rabbits attained puberty between 14 and 16 weeks of age. Rabbits on 20% forage meal or lablab forage attained puberty later at 16 weeks than those on 40% forage meal or groundnut forage that attained puberty earlier at 14 weeks of age.

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