

INFLUENCE OF FORAGE LEGUMES (*CENTROSEMA PUBESCENS* AND *CALOPOGONIUM PHASELOIDES*) ON SEMEN CHARACTERISTICS OF RABBITS.

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ABSTRACT

A study to determine the influence of forage legumes on the reproductive characteristics of the buck was conducted using twelve-mixed breed rabbits aged 8 to 12 months. The rabbits were divided into two groups of 6 bucks each. The group serving as control (CTL) was placed on diets containing grass-legume forage and concentrate. The trial group (EXP) was placed on legume forage and concentrate. The forages fed to CTL were *Panicum maximum*, *Centrosema pubescens* and *Calopogonium phaseloides* while those fed to EXP were *Centrosema pubescens* and *Calopogonium phaseloides*. The results of the study showed that sperm concentration of $297.22 \pm 36.87 \times 10^6/\text{ml}$ recorded by EXP was significantly ($P < 0.05$) higher than the $193.89 \pm 22.52 \times 10^6/\text{ml}$ recorded for CTL. There were no significant differences ($P > 0.05$) between Exp and CTL in semen volume, sperm motility, live and dead sperm cells and reaction time. However, sperm motility and ratio of live and dead sperm cells were numerically higher for the treatment group. Right epididymal length was significantly longer ($P < 0.05$) in the EXP (1.70 ± 0.00 cm) than the CTL (1.25 ± 0.05 cm) whereas other testicular dimensions showed no significant differences ($P > 0.05$). Numerical values for EXP were higher than those for CTL with respect to reproductive tract weight (EXP- 14.45 ± 1.55 g and CTL- 11.55 ± 1.15 g) as well as paired testis weight (EXP- 4.58 ± 0.23 g and CTL- 3.48 ± 0.48 g). Feeding these forage legumes to mature bucks increased their sperm concentration and enhanced sperm motility as well as percent live sperm cells which are indices of potential fertility in any male animal.

Key words: Rabbits, Buck, Forage Legumes, Semen, Sperm cells

INTRODUCTION

Rabbit production has enormous potential in alleviating the problems of animal protein supply in a developing economy. (Biobaku and Dosumu, 2003; Nwangwu and Ezekwe, 2006). The most advantageous attributes of the rabbit are that it has high reproductive potential and fast growth rate (Ojewola *et al.*, 2006). This is due to its short gestation length, early maturity, high prolificacy and ability to re-breed shortly after parturition (Odubote and Akinokun, 1991). Most of the investigations carried out on the reproductive performance of rabbits have been directed mainly on the doe. For the realization of the full reproductive potentials of rabbits, virile bucks of proven reproductive capacity are required. However, there is paucity of information on the reproductive performance and semen characteristics of the bucks.

There is a direct relationship between nutrition and the outstanding reproductive qualities of the rabbit as observed by (Egbunike and Ladokun 1998). Nutrition affects the secretory functions of the accessory sex glands that is, the products that make up the seminal plasma (Herbert *et al.*, 2005). These authors further observed that nutrition plays a vital role in enhancing the reproductive capacity of an animal and the overall increase of animal products such as meat, milk and egg. Protein plays an important role in spermatogenesis. The task of alleviating animal protein need and supply through rabbit production can only be sustained if there is improved and increased semen production and quality of bucks used in breeding. Therefore, forage legume feeding has been advocated and is being adopted by small to medium scale livestock farmers in the tropics to boost nutritional regimes of their animals (Preston and Leng, 1989; Herbert *et al.*, 2005).

Legumes are high in protein and crude fibre as is the case with *Centrosema pubescens* (21.38% CP and 35.27% CF) among other legumes (Aduku, (2005). Legumes have high digestibility, high protein level and

also contain five times the calcium, 30 to 50% more phosphorus, and twice the magnesium of grasses (Evers, 2000). He also stated that milk production, growth and reproduction are always higher on a pasture with legumes than one without legumes given the same amount of available forage. Iyeghe-Erakpotobor and Muhammad (2006) observed that rabbits accepted legume forages better than grasses. However, Egbunike and Ladokun (1998) noted that although the protein requirements of rabbits have been placed between 18 and 22% in a tropical environment, there has not been any planned investigation on the effects of dietary protein on development and puberty in the buck. Thus legumes have performed well as nutrient source but not much is known of their influence on semen characteristics in the rabbit.

The objective of this study is to evaluate the effects of specific forage legumes (*Centrosema pubescens* and *Calopogonium phaseloides*) on semen characteristics and testicular dimensions of the buck.

MATERIALS AND METHODS

Experimental location

The experiment was carried out in the Rabbitry Unit of the Teaching and Research Farm as well as the laboratory of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike. Umudike is geographically located in Abia State on latitude of 05° 29' North, longitude 07° 33' East and at an altitude of 122 m above sea level. It lies in the humid rain forest zone of West Africa, which is characterized by long period of rainy season (March to October) and short period of dry season (November to February). Average rainfall is 2169.8mm in 149 to 155 rain days. Average temperature is 26°C with maximum of 32°C and minimum of 22°C. Relative humidity ranges from 50 to 95%. All meteorological data were obtained from the Meteorological Station at the National Root Crop Research Institute Umudike, Abia State, Nigeria.

Management of experimental animals

Twelve mixed breed adult rabbit bucks aged 8 to 12 months were used for this experiment. They were purchased from States Ministry of Agriculture Uyo in Akwa Ibom. On arrival at the University Farm, the rabbits were quarantined for two weeks during which they were vaccinated against mange and dewormed to ensure healthy conditions before commencement of the trial. The experimental animals were housed singly per pen. The hutch was made of wood and wire mesh. The environment was swept and kept clean daily with the feeders and drinkers cleaned thoroughly. The feeders and drinkers provided were fastened to the side of the individual compartments using wire to prevent wastage of feed and water spillage. The animals were fed concentrate diets containing approximately 15.60% crude protein. Forage and water were supplied *ad libitum*.

Experimental procedures

The bucks were divided into two groups (A and B) of 6 animals per group. The control group (CTL) was fed grass and legume forages with concentrate. The forages fed consisted mainly of *Panicum maximum*, *Centrosema pubescens* and *Calopogonium phaseloides*. The treatment group (EXP) was fed legume forage with concentrate only. The forages consisted mainly of fresh *Centrosema pubescens* and *Calopogonium phaseloides*. All the groups were fed for 6 weeks before commencement of semen collection to ensure that the test ingredient was fully utilized for spermatogenesis.

Semen Collection and evaluation

Semen was collected from the bucks using the artificial vagina (AV) designed and constructed by Herbert and Adejumo (1995). Semen was collected from the bucks twice a week for 3 weeks. Collection was in the morning between 0800 and 0900 hr. Semen evaluation commenced immediately after collection. The AV was warmed by immersion in warm water for 5 to 10 minutes in order to make it warm thus simulating the vagina temperature of the doe. Then it was blotted dry with absorbent tissue paper and smeared with glycerol, a viscous fluid, which served as a lubricant synonymous to vagina fluid to aid intromission and minimize friction.

Semen characteristics of rabbits fed forages

During semen collection, a teaser doe was introduced into the buck's hutch. On introduction, the buck attempted to make false thrust and as it made further thrust, the warmed AV was quickly introduced and subsequent ejaculation took place immediately. During the course of semen collection, a water bath at 38 to 40° C was improvised to maintain the semen collected at the normal body temperature.

Volume: After each collection, the semen volume was read off the calibrated collection tube.

Motility: Motility was evaluated within seconds of collection. In determining the motility of the semen, a droplet of the semen was placed on a slide, observed under a microscope and scored for mass progressive motility.

Sperm concentration:

The sperm concentration was determined by viewing and counting sperm cells under a microscope with the aid of a Neubauer haemocytometer. Prior to counting, the sperm cells were made immotile to prevent movement by adding semen to a diluent adapted from Herbert and Adejumo (1995). A drop of the diluted semen was placed on the haemocytometer and left to settle before it was viewed under the microscope and counted.

Live/Dead Proportion:

The live/dead proportion (membrane integrity and morphology) of the sperm cells was determined by staining the cells with *Eosin*, *Nigrosin* stain. The stained slide was then examined under the microscope using a 40 x 100 objective lens. The stain produced a dark background on which the sperm cells stood out. Normal live sperm eluded the stain and appeared white in colour whereas the "dead sperm" took up or absorbed the stain and appeared violet in colour.

Testicular Parameters:

At the end of semen collection, the rabbits from each of the groups were slaughtered for the evaluation of testicular parameters. Testicular parameters evaluated were; paired testis weights, individual testis weights, length of vas deferens, length of testis, length of epididymis, weight of epididymis, circumference of the testis and weight of reproductive tract. The whole reproductive tract was excised completely with the fat and adhering muscles carefully and properly trimmed off. The various weights were obtained by using a sensitive electronic scale (Ames brand). The various lengths were obtained by using a thread, the lengths of which were read off the metric rule.

Data analysis:

Data generated were analysed using the student's T test. All statistical analyses were according to the methods of Steel and Torrie (1980).

RESULTS AND DISCUSSION

The result of the semen characteristics and the testicular dimensions are presented in Tables 1 and 2, respectively.

Table 1: Seminal characteristics of the experimental rabbits 1

Parameters	CTL (Mean ± SE)	Exp (Mean ± SE)
Semen volume (ml)	0.38±0.03	0.31 ± 0.04
Progressive motility (%)	52.69± 4.09	62.36±5.24
Sperm concentration (x10 ⁶ /ml)	193.89± 22.57 ^b	297.22 ±36.87 ^a
Live/dead ratio (%)	60.33 ±3.50	66.33±2.14
Reaction time (seconds)	14.75 ±2.22	14.67±1.42

^{a,b}, Means in same row with different superscripts are significantly different ($P < 0.05$).

CTL= control; EXP= Experiment.

Table 2: Testicular dimensions of the experimental rabbits

Parameters	CTL (Mean \pm SE)	Exp (Mean \pm SE)
Left Testis Weight (LTW g)	1.30 \pm 0.20	2.40 \pm 0.25
Left Vas deference Length (LVL cm)	10.40 \pm 0.70	8.10 \pm 0.90
Left Testis Length (LTL cm)	3.50 \pm 0.01	3.50 \pm 0.00
Left Epididymis Length (LEL cm)	1.25 \pm 0.15	1.50 \pm 0.00
Left Epididymis Weight (LEW g)	0.30 \pm 0.00	0.35 \pm 0.05
Left Testis Circumference (LTC cm)	7.40 \pm 0.40	7.55 \pm 0.55
Right Testis Weight (RTW g)	2.18 \pm 0.33	2.18 \pm 0.03
Right Vas deference Length (RVL cm)	9.85 \pm 0.35	8.30 \pm 0.70
Right Testis Length (RTL cm)	3.75 \pm 0.25	4.00 \pm 0.00
Right Epididymis Length (REL cm)	1.25 \pm 0.05 ^b	1.70 \pm 0.00 ^a
Right Epididymis Weight (REW g)	0.30 \pm 0.00	0.30 \pm 0.05
Right Testis Circumference (RTC cm)	7.60 \pm 0.40	8.25 \pm 0.45

^{a,b} means in same row with different superscripts are significantly different ($P < 0.05$).

There were no significant differences ($P > 0.05$) between the treatment groups and the control in semen volume, sperm motility, live, dead and reaction time. With the exception of reaction time, the values obtained for these parameters in the treatment group (EXP) were numerically higher than those of the control group (CTL). The values obtained for reaction time were 14.75 \pm 2.22 seconds for the control and 14.67 \pm 1.42 for the treatment groups. Semen volumes of the two groups although not significantly different (Table 1), fall within the range reported for rabbits by several authors. Lebas (1986) and (Herbert and Adejumo, 1995) stated that volume of semen ejaculated by rabbits is about 0.3 to 0.6ml. Kobal and Kosec (1997) recorded semen volume of 0.71 and 0.27 to 0.93 ml, respectively, in white New Zealand rabbits. Fielding (1991) on the other hand, recorded 0.6 ml.

The sperm motility obtained in this study was in agreement with Herbert *et al* (2005) who recorded sperm motility of 67.5 \pm 0.5 %, 62.1 \pm 0.1% and 60.5 \pm 0.5% and Ezeobi (2000) who also recorded sperm motility of 62.5 \pm 1.26%, 61.89 \pm 0.63% and 58.13 \pm 1.57%. Motility obtained in this study could have been affected by periods of sexual inactivity as observed by Rouge (2002) and Tomar *et al.* (1968) who stated that second ejaculate is usually better in initial motility if the interval of semen collection is more than once a week. The bucks were housed separately, far from the reach of the females and were also not exposed to any female during the periods of investigation/experimentation thus reducing libido which is known to be enhanced by the presence of female. This could have led to a drop in motility. The non-significant differences between the treatment and control groups in live/dead proportion indicated that the forages fed to the bucks did not stimulate production of excessive levels of dead sperm cells in the semen.

The non-significant difference in the reaction time of the two groups could be related to the experience of the operator, the receptability of the teaser which is influenced by her physiological state, and also the sexual behaviour exhibited by the buck. Some of the bucks on introduction of the teaser doe mounted instantly and attempted false thrust while some preferred nudging, smelling, biting and nipping the hindquarters of the teaser before mounting. In relation to sperm concentration (193.89 \times 10⁶ /ml and 297.22 \times 10⁶/ml) obtained in this study fall within the range 150 to 500 \times 10⁶/ml recorded by Lebas (1986). From the results of this study, it is indicative that legumes played a significant role in spermatogenesis through the synthesis of protein within the body of the animals. This protein in turn is directly involved in spermatogenesis with the resultant higher concentration of sperm cells in the EXP group. The significant greater length of the right epididymis and weight of reproductive tract of the treatment group may be attributed to the effects of the legumes. This in turn may have also contributed to the greater sperm output of this group.

Semen characteristics of rabbits fed forages

The testicular dimensions obtained for the different groups did not differ significantly ($P>0.05$) except, the right epididymal length where the treatment group (EXP) had a significantly higher ($P<0.05$) value of 1.70 ± 0.00 cm than the control group (CTL) which had the value of 1.25 ± 0.05 cm. However the EXP group exhibited higher numerical values than the control with respect to reproductive tract weight 14.45 ± 1.55 g and 11.55 ± 1.15 g for treatment and control groups, respectively. Similar trends were also observed for paired testes weight 4.58 ± 0.23 g and 3.48 ± 0.48 g of the treatment and control groups, respectively.

CONCLUSION

Generally, it was observed that the bucks exhibited the normal state of physiology of reproduction. The results obtained showed the treatment group having higher responses in the sperm concentration, motility, live/dead sperm and the right epididymal length of the rabbit bucks than the control. Small scale rabbit farmers and breeders can use the forage legumes to improve the reproductive efficiency of their breeding stock.

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REFERENCES

- Aduku, A. O. (2005). Tropical Feedstuff Analysis Table. Ahmadu Bello University, Samaru Zaria, Nigeria. Pp.1.
- Biobaku, W. O. and Dosumu, E. O. (2003). Effect of a supplementary diet based on maize and rice bran with 3 different improved forages on feed intake, digestibility and growth in rabbits. *Nig. J. Anim. Production* 22: 17-18.
- Egbunike, G. N. and Ladokun, A. O. (1998). Testis function in pubertal rabbits as affected by three dietary proteins. 3rd ASAN conference Ikeja, Nigeria pp.210-212.
- Evers, G. (2000). Utilization of forage legumes. <http://overton.tamu.edu/clover/cool/utilization.htm>
- Ezeobi, N.A (2000). Induction of spermatogenesis in matured bucks using clomipene citrate. Unpublished B.Sc. Project thesis, Federal University of Technology, Owerri, Nigeria.
- Fielding, D (1991). Rabbit: The Tropical Agriculturist. CTA. Macmillan Education Ltd. London.
- Herbert, U and Adejumo, D. O. (1995). Construction and evaluation of an artificial vagina for collecting rabbit semen. *Delta Agric* 2: 99-108.
- Herbert, U. Ozoje, M. O. and Adejumo, D. O. (2005). Effect of Leucaena and Gliricidia leaf meals on the seminal characteristics, testis weights and seminiferous tubules diameters in rabbits. *Anim. Res.* 54: 173-178.
- Iyeghe-Erakpotobor, G.T. and Muhammad, I.R. (2006). Intake and weight change of rabbits fed grass, legume and legume-grass combination. *Proc. 31st Ann. Conf. Nig. Soc. For Anim. Prod. (NSAP)*. Bayero Univ. Kano. Pp.296-300.

- Kobal, S, and Kosec, M. (1997). Macroscopic and microscopic semen properties in white New Zealand rabbits. Proceedings of the 6th Zavrnik memorial meeting 9th Lepica, 155-159.
- Lebas, F. (1986). The Rabbit: Husbandry, Health and Production. FAO, Animal production and health series No. 21, Food and Agricultural Organization; Rome. Italy.
- Nwangwu, A. C. and Ezekwe, A. G (2006). Effect of feeding concentrate and forage on the reproductive performance of does and growth of their kids. Proc. 31st Ann. Conf. Nig. Soc. for Animal Prod. (NSAP). 123-125.
- Odubote, I.K and Akinokun, J.O (1991) Reproductive and body weight performance of the New Zealand White rabbits in the humid tropics of Nigeria. Nig. J. Anim. Prod. 18: 61-65.
- Ojewola, G.S, Abasiokong, S.F., Uko, M.U. and Oguike, M.A (2006) Influence of Lysine Supplementation on the reproductive performance of rabbits in a tropical environment. Journal of Animal and Veterinary Advances. 5 (2): 1-17.
- Preston, T.R. and Leng, R.A (1989). The green house effect and its implication for world Agriculture: the need for environment friendly development, Livest. Res. Rural Dev 1:23-30.
- Rouge, M. (2002) Collection and evaluation of semen. Rbowen@lamar.colostate.edu.
- Steel, R.G.D and Torrie, J. H. (1980). Principles and Procedures of Statistics. A Biometrical approach (2nd edition), McGraw-hill book Inc., New York.
- Tomar, N.S., Sharima S.C and Desail, R.N (1968). Repeatability estimates of semen qualities. *Indian Vet. J.* 42: 360-364.