



Research Article

Available online at www.journal-advances-developmental-research.com

Journal of Advances in Developmental Research

ISSN: 0976-4704 (Print), e-ISSN: 0976-4844 (Online)

J.Adv.Dev.Res. Volume 2, No.2, December 2011

Effect of Ethiopian Pepper (*Xylopiya aethiopic*) on Testis and Semen Quality of Rabbit Bucks

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Abstract

Eighteen healthy Dutch breed rabbit bucks aged 6 months with an average weight of 2.11 kg were used in a CRD experiment with 6 bucks per treatments represented as T₁ (control), T₂ and T₃. Bucks in T₁ were fed concentrate without *Xylopiya aethiopic* while T₂ and T₃ were fed concentrate in which dried *Xylopiya aethiopic* was incorporated at 1 % and 2 % inclusion levels, respectively. The concentrate in all the groups were supplemented with mixed grass and legumes. Semen was collected twice a week for four consecutive weeks. The results of the study showed that the groups (T₂ and T₃) fed diets containing *X. aethiopic* had significantly (P < 0.05) higher sperm concentration (289.56 and 320.33 × 10⁹ml⁻¹), sperm motility (63.54 and 72.55 %), total ejaculate ml⁻¹ (109.44 and 113.44) and live sperm cells (75.68 and 78.53 %), respectively, than those of T₁ (157.89 × 10⁹ml⁻¹), 58.67 %, 69.67 ejaculate ml⁻¹ and 66.38 %). The percent dead sperm cells (33.62 %) of the control (T₁) was significantly (P < 0.05) higher than those of T₂ and T₃ (24.32 and 19.65 %), respectively. The Epididymis length and weight of the left and right segments of the testis of the groups T₂ and T₃ fed the test diets were significantly (P < 0.05) higher than the control (T₁). There were no significant (P > 0.05) differences in the semen volume, testicular and vas deferens length between the treatments. The significantly higher sperm concentration, sperm motility and live sperm cells showed that *Xylopiya aethiopic* had positive effects on the reproductive functions of rabbit buck.

Key words: sperm quality, reproductive functions, protein, Uda, Atta

Introduction

The shortage of animal protein facing most developing countries cannot be solved by large animals but by intensifying the production of highly reproductive animals in the livestock unit¹. The increase in demand for animal protein necessitates the exploitation of the potentials of small livestock species and stimulates their introduction into animal research and economic development programmes especially of developing nations². Rabbits are among the micro-livestock species that can utilize up to 30% of crude fibre diets compared to chicken that utilizes less than 10% crude fibre diets. The ability of the rabbit to turn forages into high quality protein and yet remain within the

investment range of the poorest families is an unequalled advantage amongst domesticated animals³. The testis produces gametes – spermatozoa – suspended in the seminal plasma secreted by the accessory glands. The spermatozoa and the seminal plasma constitute the semen which is the tool for reproduction. The seminal plasma contains a variety of substances including various electrolytes, fructose, citric acid and sorbitol. The fertility in male animals is measured as the number of services resulting to conception and this largely depends on the quality and concentration of semen of the male. The number of litter doe⁻¹ also depends on the quality and concentration of semen from the male during natural mating or artificial insemination. Seminal plasma is an essential

component in most natural mating processes because it serves as a carrier and protector of spermatozoa⁴. Testicular characteristics of some species of farm animals vary according to system of management⁵. These are also subject to nutrition and nutrients available to the animal.

Ethiopian pepper (*Xylopien aehiopica*) commonly known as Negro pepper fruits and locally called “Uda’ and “Atta” by the Igbo’s and Ibibio’s of Nigeria, is a tropical evergreen tree native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, but largely located in west central and southern Africa. These trees are widely distributed in the humid forest zones of West Africa especially along rivers in the drier area of the region⁶. This evergreen tree can grow up to 15- 30 m in height, with a straight stem and a slightly stripped or smooth bark. The fruits are rather small and look like twisted bean-pods. When dry, the fruits turn dark brown, and cylindrical measuring 2.5 to 5 cm long and 4 to 6 mm thick. The dried fruit of Ethiopian pepper are used as a spice, condiment and in traditional medicine. Spices and condiments are products of plants which are mostly used for seasoning and flavouring and thus enhancing the taste of food, beverages and drugs^{7,8}. Plants used as spices and condiments are usually aromatic and pungent⁹. The local women folk in the eastern part of Nigeria hold the belief that soup made from its fruits enhances lactation and fertility in women.

Improvement in the reproductive performance of breeding bucks will lead to increase in the overall reproductive economy of rabbits on the long term. It has been shown that some male farm animals sometimes have low level of reproductive performance. This according to ¹⁰ is due to the poor quality of semen and concentration of sperm cells produced per ejaculate. Presently, there are very few researches in bucks to assess the effects of spices on their reproductive functions. The aim of the study was to determine the effect of Ethiopian pepper (*Xylopien aethiopica*) on semen characteristics and testicular dimensions of the rabbit bucks.

Experimental

Experimental location

The experiment was carried out in the Rabbitry Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Geographically, Umudike is located on Latitude 05°

29’ North and longitude 07° 33’ East of the equator. It is situated within the humid rain forest zone of West Africa characterized by long duration of rainy season (March – October) and short period of dry season (November – February). The average rainfall is 2169.8mm and average ambient temperature is 26° C with maximum and minimum of 32° C and 22° C respectively. Relative humidity is between 50 to 90%.

Experimental animals and management

Eighteen healthy Dutch breed rabbit bucks aged 6 months with an average weight of 2.11 kg were sourced from a Rabbit Farm situated in Ikot Ekpene, Akwa Ibom State, Nigeria. The animals were quarantined for a period of two weeks during which they were vaccinated against ecto and endo-parasites. The animals were provided with commercial concentrate diet and mixed forages during the quarantine period in order to allow the animals acclimatize to their new environment before introduction of experimental diets. Clean drinking water was provided *ad libitum* throughout the experiment. Daily hygienic routine management was also carried out throughout the experimental period.

Experimental procedures

Experimental design

The experiment was a CRD with 3 treatments represented as T₁ (control), T₂ and T₃ with 6 rabbits randomly assigned to each treatment. The treatments T₁, T₂ and T₃ consisted of 0, 1 and 2 % graded levels of dried, ground *Xylopien aethiopica* (Ethiopian pepper), respectively, incorporated in the concentrate diets. The diet of the experimental animals and the composition of the test ingredient are presented in Tables 1 and 2, respectively. The bucks were fed for 49 days after which semen was collected between 0800 to 0900 hours local time twice a week for 4 consecutive weeks.

Table 1. Percentage composition of the experimental diets fed to the bucks

Ingredients (%)	T ₁	T ₂	T ₃
Maize Offals	44.33	44.33	44.33
PKC	44.32	43.32	42.32
Soy meal	8.35	8.35	8.35
<i>Xylopien aethiopica</i>	-	1	2
Bone meal	2.50	2.50	2.50
Premix	0.25	0.25	0.25
Salt	0.25	0.25	0.25
CP	16.16	16.06	16.08
MEK (cal kg ⁻¹)	2699.31	2698.31	2697.32

Table 2. Composition of Ethiopian pepper (*Xylopiya aethiopica*)

Proximate composition	%
Moisture	8.30
Crude protein	7.44
Ash	3.30
Oil (Ether extract)	2.40
Crude fibre	2.30
Mineral and Vitamin compositions	
Magnesium	0.44
Calcium	0.09
Sodium (ppm)	10.5
Potassium (ppm)	5.23
Iron (mg/100g)	1.82
Phosphorus (mg/100g)	7.73
Vitamin C (IU)	8.80
Vitamin E (IU)	6.70

Semen collection and analyses

Collection of semen was done using an Artificial Vagina (AV), described and constructed by¹¹. Prior to collection, the (AV) was dipped into water at the temperature of 40°C for at least 15 minutes to simulate the vagina temperature of the doe. The AV was then lubricated with glycerol to make it resembles vaginal fluid in reducing friction, thereby enhancing intromission. Following brief exposure of the female to the male (in the male's cage), the male was allowed to mount the teaser doe and the already prepared AV was introduced tactfully, then semen was collected from the buck. Thereafter, semen samples were analyzed as soon as they were collected.

Testicular Measurements

Four rabbit bucks from each of the groups were scarified for evaluation of testicular parameters. The following testicular dimensions were measured: paired testis weight, total weight of reproductive tract, epididymis using a sensitive electronic weighing scale. Length of testes, vas deferens and circumference of testes were measured by using thread, and then dimensions were read from a meter rule.

Statistical analyses

The data obtained were subjected to statistical evaluation using the Analysis of Variance (ANOVA) as described by¹³. Significant means were separated using Least Significant Difference (LSD).

Results and discussion

The results of the semen quality characteristics are presented in Table 3. There were no significant differences ($P > 0.05$) in the semen volume of the different treatments. The groups T_2 and T_3 fed diet containing *X. aethiopica* had significantly ($P < 0.05$) higher sperm concentration ($\times 10^9 \text{ml}^{-1}$), sperm motility (%), total ejaculate ml^{-1} and live sperm cells than the control (T_1). On the other hand, the percent dead sperm cells of T_1 (control) was significantly ($P < 0.05$) higher than the dead sperm cells of T_2 and T_3 . The semen volume of the bucks recorded in the study (Table 3) falls within the range 0.3 to 0.6 ml for rabbit as reported by¹⁴. On the other hand, the semen volume recorded in the present study was lower than 0.61ml and 0.71ml reported by^{15,12}. The sperm concentration ranging from 157.89 to 320.33 ($\times 10^9 \text{ml}^{-1}$) obtained in the study were in agreement with concentration of rabbit sperm cells of 150 to 500 ($\times 10^9 \text{ml}^{-1}$) reported by¹⁴. The higher sperm concentration of T_2 and T_3 compared to T_1 could be attributed to the test ingredients since that was the only varying factor in the feed. Also, the overall higher performance of T_3 group which was fed the diet containing higher level of the test ingredient could be attributed to the additional mineral elements contributed to their diet by *X aethiopica*. *Xylopiya aethiopica* was found to be rich in sodium, potassium, phosphorus, calcium, magnesium and vitamins C and E. It has been reported that sodium and potassium ions maintain equilibrium in different fluids¹⁶. Sodium plays vital roles in cell hydration and helps maintenance of acid-alkaline equilibrium. The additional minerals from the test ingredients may have contributed to the metabolic regulation of

Table 3. Effect of Ethiopian pepper on semen characteristics

Parameters	T_1	T_2	T_3	SEM
Volume of semen (mL)	0.44	0.38	0.35	0.02
Sperm concentration ($\times 10^9 \text{mL}^{-1}$)	157.89 ^c	289.56 ^b	320.33 ^a	4.12
Total sperm ejaculate (mL)	69.67 ^c	109.44 ^b	113.44 ^a	4.88
Sperm motility (%)	58.67 ^c	63.54 ^b	72.55 ^a	1.97
Live sperm cells (%)	66.38 ^b	75.68 ^{ab}	78.35 ^a	2.66
Dead sperm cells (%)	33.62 ^a	24.32 ^{ab}	19.65 ^b	1.67

a, b, c Means on the same row with different superscript are significantly different ($P < 0.05$); SEM= standard error of means

sperm cells, enhanced enzyme activity of the semen and increased spermatogenic activities. Possibly this could result to high testosterone levels. Testosterone is known to be critically involved in the development of sperm cells¹⁷ along with the gonadotropins. The high progressive motility of the T₂ and T₃ compared favourably with 70 % reported by^{12, 18}. The increased sperm concentration, live sperm cells and motility indicated that *X. aethiopica* could improve and enhance the fertilizing capacity of semen. These qualities were often used as a measure of semen quality, testicular function and / or male fertility. Low sperm concentration, low motility and high percentage abnormal spermatozoa level each has been associated with reduced fertility^{19,20}. The increased live sperm cells in the treated groups in this study compared with the control group indicated that *X. aethiopica* had positive impact on the live of the spermatozoa.

Testicular parameters

The results of the testicular dimensions are presented in Table 4. The epididymis lengths and weights of the left and right segments of the testis of the groups fed diets containing *X. aethiopica* were significantly ($P < 0.05$) higher than the control (T₁). Increased epididymal weight of the groups fed diets containing *X. aethiopica* suggested that they stored more sperm cells than the control. The rest of the testicular parameters showed no significant differences ($P > 0.05$) among the different treatments. The paired testis weight and whole reproductive tract of T₁ (2.15 and 10.40 g), T₂ (3.10 and 13.56 g) and T₃ (3.28 and 14.05 g) respectively showed no significant differences ($P > 0.05$). The

testis weight recorded in this study was lower than 6.7 g reported by²⁰. The differences in the testis could be due to breed differences or variation in size of the bucks.

Conclusion

The observed increase in sperm count, motility, live sperm cells and lower dead cells of the treated groups showed that *Xylopi aethiopica* had positive effects on the reproductive functions of rabbit buck. Therefore, supplementing the diets of rabbit especially bucks with Ethiopian pepper will improve sperm concentration, sperm motility, live sperm cells thereby enhancing higher chances of fertilizing the doe's ova.

Acknowledgements

The authors are grateful to the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Nigeria for providing the facilities for the study.

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Table 4. Testicular characteristics of the bucks in different treatments

Parameters	T ₁	T ₂	T ₃	SEM
Left testis weight (g)	1.10	1.80	1.88	0.16
Left vas deferens length (cm)	10.25	9.44	9.48	0.18
Left epididymis weight (g)	0.40 ^c	0.55 ^b	0.58 ^a	0.04
Left epididymis length (cm)	5.20 ^c	5.53 ^b	5.85 ^a	0.12
Left testis length (cm)	3.34	3.50	3.67	0.06
Left testis circumference (cm)	4.50	4.10	4.35	0.08
Right testis weight (g)	1.05	1.30	1.40	0.07
Right vas deferens length (cm)	10.10	8.89	9.50	0.22
Right epididymis weight (g)	0.45 ^b	0.58 ^{ab}	0.60 ^a	0.03
Right epididymis length (cm)	5.20 ^c	5.53 ^b	5.80 ^a	0.12
Right testis length (cm)	3.35	3.45	3.62	0.05
Right testis circumference (cm)	4.30	4.20	4.35	0.03

^{a, b, c} Means on the same row with different superscript are significantly different ($P < 0.05$); SEM= standard error of means

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