Evaluation of reproductive hormones, testicular and sperm characteristics of rabbit bucks fed dietary baobab (*Adansonia digitata* L.) leaf meal

***[1]Amao O. A., [1]Akinbowale, O. F., [1]Ayoola, S. J. and [2]Hammed, O. O.**

[1]Department of Animal Nutrition and Biotechnology,
[2]Department of Animal Production and Health,
Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Oyo State. Nigeria
***Corresponding author:** oaamao@lautech.edu.ng; olajideamao@yahoo.com

Abstract

This experiment evaluated the reproductive hormones, testicular and sperm characteristics of rabbit bucks fed dietary Baobab Leaf Meal (BLM). Thirty six (36) mixed breed (6 weeks old) rabbit bucks were involved in the study. The bucks were balanced for weight and allocated to six dietary treatments T1, T2, T3, T4, T5 and T6. Treatment T1 (Control) did not contain BLM, while T2, T3, T4, T5 and T6 contained 0.25, 0.75, 1.25, 1.75 and 2.25% BLM, respectively. The bucks were fed experimental diets for 12 weeks. Data were obtained for testicular characteristics (Testis weight, Testis length, Testis width and Testis volume), reproductive hormones (Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone) and extra-gonadal sperm characteristics (Sperm count, Sperm motility, Normal sperm, Live sperm, Round and Elongated spermatids). Results showed that Left testis weight, right testis length, left testis width, right testis width and mean testis width were significantly ($p<0.05$) affected by the treatment. The BLM had no significant ($p>0.05$) effect on the reproductive hormones of the rabbit bucks. Except for elongated spermatids, other sperm characteristics were significantly ($p<0.05$) influenced by the treatment. The mean values for sperm count revealed that T1, T2, T3, T4 and T5 were similar but significantly ($p<0.05$) higher than T6. Normal sperm and live sperm followed a similar trend. Highest values for these parameters were obtained at T4 (1.25% BLM). The mean values for sperm motility showed that T1, T2, T3 and T4 were similar but significantly higher than T5 and T6. It was concluded that inclusion of 1.25% Baobab leaf meal in the diets of rabbit bucks supported reproductive hormones, testicular morphometrics and sperm characteristics in rabbit bucks.

Keywords: Rabbit bucks, Baobab Leaf Meal, Sperm characteristics, Testosterone.

Évaluation des hormones reproductrices, des caractéristiques testiculaires et du sperme des lapins mâles nourris avec un repas de feuilles de baobab (*Adansonia digitata* L.)

Résumé

Cette expérience a évalué les hormones reproductrices, les caractéristiques testiculaires et du sperme des lapins mâles nourris avec un repas de feuilles de baobab (RFB). Trente-six (36) lapins mâles de race mixte âgés de 6 semaines ont participé à l'étude. Les lapins ont été équilibrés en fonction de leur poids et répartis en six traitements alimentaires : T1, T2, T3, T4, T5 et T6. Le traitement T1 (Contrôle) ne contenait pas de BLM, tandis que les traitements T2, T3, T4, T5 et T6 contenaient respectivement 0,25, 0,75, 1,25, 1,75 et 2,25 % de RFB. Les lapins ont été nourris avec les régimes expérimentaux pendant 12 semaines. Les données ont été recueillies sur les caractéristiques testiculaires (poids des testicules, longueur des testicules, largeur des testicules et volume des testicules), les hormones reproductrices (hormone folliculo-stimulante (HFS), hormone lutéinisante (LH) et testostérone) et les caractéristiques du sperme extra-gonadique (nombre de spermatozoïdes, mobilité des spermatozoïdes, spermatozoïdes normaux, spermatozoïdes vivants, spermatides ronds et allongés). Les résultats ont montré que le poids du testicule gauche, la longueur du testicule droit, la largeur du testicule gauche, la largeur du testicule droit et la

largeur moyenne des testicules étaient significativement (p < 0,05) influencés par le traitement. Le RFB n'a pas eu d'effet significatif (p > 0,05) sur les hormones reproductrices des lapins mâles. À l'exception des spermatides allongés, les autres caractéristiques du sperme ont été significativement (p < 0,05) influencées par le traitement. Les valeurs moyennes du nombre de spermatozoïdes ont révélé que T1, T2, T3, T4 et T5 étaient similaires mais significativement (p < 0,05) plus élevées que T6. Les spermatozoïdes normaux et les spermatozoïdes vivants ont suivi une tendance similaire. Les valeurs les plus élevées pour ces paramètres ont été obtenues avec T4 (1,25 % de RFB). Les valeurs moyennes pour la motilité des spermatozoïdes ont montré que T1, T2, T3 et T4 étaient similaires mais significativement plus élevées que T5 et T6. Il a été conclu que l'inclusion de 1,25 % de repas de feuilles de baobab dans les régimes alimentaires des lapins mâles soutenait les hormones reproductrices, les morphométriques testiculaires et les caractéristiques du sperme des lapins mâles.

Mots-clés : Lapins mâles, repas de feuilles de baobab, caractéristiques du sperme, testostérone

Introduction

Human population growth in developed countries is stabilizing while that of developing countries including Nigeria is still increasing rapidly. Economic indices indicate that as the population continues to increase, more people are to be fed (Mailafia *et al*., 2010). In order to maximize food production to meet protein requirement in Nigeria, viable options need to be explored and evaluated (Owen *et al.,* 2009). Among such alternatives is the use of livestock species that are yet to be fully explored to bridge the gap between the demand and supply of animal protein by the ever increasing populace in Nigeria. Rabbit, a fast-growing livestock possesses a number of features that could be of advantage in small holder subsistence-type integrated farming in developing countries.

Rabbit production is a veritable way of alleviating animal protein deficiency in Nigeria (Ajala and Balogun, 2004). Rabbits are herbivores, meaning they primarily eat plant-based food. Their diets consist of grass, hay, leafy greens and other vegetables. They have a unique digestive system that allows them to extract maximum nutrients from fibrous plant materials. Rabbit has short gestation length, low cost of production, small bodied size, high prolificacy, rapid growth rate, high adaptability over an extensive range of eco-friendly environments and capability to utilize by-products from agriculture and forages. Their management requires less land space and they can be kept in the backyard of a farmer's house (Ogunwande, 2023). However, to maximize all these potentials, reproduction must be efficient.

Efficiency of reproduction largely determines the success and profitability of any livestock farm. This in turn depends on a number of factors including nutrition which constitutes the largest proportion of the cost of production in livestock industry. Availability of forage, especially during the dry season, and competition between man and animals for cereals and grains used for concentrate feeds are a major challenge facing the animal industry. This situation is further exacerbated by the exorbitant cost of feed ingredients for the production of concentrate feeds for animals, with consequent relatively smaller weight gain and reduced productivity during the dry season.

Ume *et al*. (2017) reported that forage unavailab ility sometimes is the limiting factor in rabbit production, especially conventional forage such as groundnut hay in which there is competition between the rabbit and ruminant animals. This calls for a search for less utilized but readily available unconventional feed resources such as forest trees that abound in most African countries. One of such forest trees is Baobab (*Adansonia digitata* L.) tree. Baobab is a wild African tree with multiple uses (Rahul *et al*., 2015). It can

survive the harsh conditions of the dry season as it stores large quantity of water in its thick bark and deep roots (Gwana *et al*., 2016). In fact it is widely spread in the drier parts of African continent. Various parts of the plant (leaves, bark and seeds) have been used as panacea to treat diseases like malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery and toothache (Nguta *et al*., 2010).

Specifically, baobab leaves have been used in various forms as an antipyretic or febrifuge against fevers (Adetola *et al*., 2023), anti-stress, tonic, immune-stimulant (De Caluwe *et al*., 2010), and antioxidant, being rich in vitamin C (Wudil *et al*., 2020) that could help prevent oxidative stress related diseases (Blomhoff *et al*., 2010; Brady, 2011). The antioxidant property helps in eliminating free radicals that are capable destroying cell membranes of vital structures in the body including reproductive cells. This study therefore, evaluated the reproductive hormones, testicular and sperm characteristics of buck rabbits fed dietary Baobab leaf meal.

Materials and methods

Experimental site

The experiment was conducted at the Rabbit Research and Production Unit of the Teaching and Research Farm, Lakode Akintola University of Technology, Ogbomoso, Oyo State Nigeria. Ogbomoso is located in the derived savanna zone of Nigeria. It lies on longitude 4^0 15′ East of the Greenwich Meridian and Latitude 8^0 15′ North of the Equator. The altitude is between 300m and 600m above sea level while the mean temperature and annual rainfall are 27^0C and 1,247mm, respectively (Ayinla and Odetoye, 2015).

Collection and processing of test ingredient

Fresh Baobab leaves *(Adansonia digitata* L.) were harvested from baobab trees within Mayin village Surulere Local Government Area, in Ogbomoso, Oyo State, Nigeria. The harvested baobab leaves were air-dried under shade until constant weight was obtained. During this period the leaves were turned thrice a day for uniform drying. The leaves were milled into powder to obtain Baobab Leaf Meal (BLM). Samples were subjected to proximate analysis.

Experimental diets and design

Thirty-six (36) weaned mixed breed rabbit bucks, 6 weeks old, were balanced for weight and allocated for six treatment diets containing 0, 2.5, 7.5, 12.5, 17.5 and 22.5g/kg diet of BLM respectively, translating to 0, 0.25, 0.75, 1.25, 1.75 and 2.25 % BLM in diets respectively. Animals were allowed to acclimatize for two weeks during which they were fed with the control diet containing 16% crude protein (CP) and 2400kcalKg^{-1}ME; and treated prophylactically against endo and ecto-parasites. After the adaptation period, the animals were subjected to 12 weeks feeding trial. The rabbits were housed individually in wooden cages designed for good ventilation and easy collection of faeces. Six bucks were allotted to each treatment in a Completely Randomized Design with each rabbit serving as a replicate. Table 1 shows the gross composition of experimental diets.

Table 1: Gross composition of experimental diets

Ingredients (%)	T1 (0% BLM)	T2 (0.25% BLM)	T3 (0.75% BLM)	T4 (1.25% BLM)	T5 (1.75% BLM)	T6 (2.25% BLM)
Maize	32.60	32.36	31.98	31.43	30.95	30.57
SBM	16.40	16.39	16.27	16.32	16.30	16.17
BLM	-	0.25	0.75	1.25	1.75	2.25
PKC	15.00	15.00	15.00	15.00	15.00	15.00
Rice Husk	30.00	30.00	30.00	30.00	30.00	30.00
Fish Meal(65%)	3.00	3.00	3.00	3.00	3.00	3.00
Oyster Shell	2.00	2.00	2.00	2.00	2.00	2.00
Bone Meal	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Premix*	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrients						
Crude Protein (%)	16.00	16.00	16.00	16.00	16.00	16.00
Crude Fibre (%)	12.55	12.58	12.61	12.66	12.69	12.78
**ME (kcal/kg)	2400.00	2390.57	2383.78	2375.74	2368.22	2375.47

***Vitamin Premix**: Supply per Kg diet: 2 000 000 iu D3; 8.0g vit. E; 4g Vit. $_{b1}$; 1.0g Vit $_{b2;}$ 0.6gVit.; 0.4mg Vit. $_{b12;}$ 24.0mg Niacin; 0.2g Folic acid; 8.0g Biotin; 48.0g Choline; 320.0g BHT; 16.0g Manganase; 8.0g Iron; 7.2g Zinc; 0.32 Copper; 0.25 Iodine; 36.0mg Cobalt; 16.0mg selenium. ME= Metabolizable Energy, SBM= Soybean Meal, BML= Baobab leaf meal, PKC Paml kernel cake.**Calculated using Pauzenga (1985).

Data collection

At the end of the feeding trial, 3 animals per treatment were sacrificed and their testes were carefully extracted and trimmed of adhering tissues. Testicular characteristics; testis weight, testis length, testis width and testis volume were measured. The testes were weighed using a sensitive electronic balance. Testis length and testis width were measured with the aid of a pair of Vernier calipers, while the testis volume was measured by water displacement according to Archimedes principle (Amao *et al*., 2012). Paired and mean testicular parameters were computed from the left and right testicular parameters.

Sperm analysis

Gonadal sperm morphology was determined according to the method of Amao *et al*. (2012). A smear of the sperm was made by cutting the left testis along the equatorial region and rubbing the cut surface on a clean glass slide. Two drops of eosin-nigrosin dye that had been thoroughly mixed were added. A smear was made on another slide and viewed under a light microscope to identify normal and abnormal cells from several fields on the slide. The normal cells were then expressed as the percentage of number of cells counted on each field of the slide. Only the mature sperm cells were observed for normality and abnormality. Dead cells were also identified and recorded. Sperm count was determined haemocytometrically by homogenization technique as described by Adejumo (2006) and Amao *et al.* (2012). The tunica albuginea was carefully removed from the testis and the testicular parenchyma was weighed. A portion of the parenchyma tissue was taken and homogenized by maceration with a pair of sharp scissors for 5 minutes in a beaker containing 10 ml of physiological saline solution. The homogenate was filtered through a double layer cheese cloth and the filtrate diluted to ratio 1:20 with de-ionized water. Some drops of the homogenate were introduced into an improved Neubauer haemocytometer counting chamber. All the elongated spermatids and mature sperm cells in the four diagonal and the centre squares of the haemocytometer were counted in each diluted homogenate. Motility was determined by the method of Ewuola and Egbunike (2010). A drop of the homogenate was placed on a sterile slide, covered with a cover slip and observed under the microscope at X 400 Magnification and scored between 0 and 100%.

Hormonal assay

Blood samples (5ml) were collected through the ear vein of three randomly selected bucks from each treatment into plain plastic bottles devoid of anticoagulant. The blood samples were allowed to clot and then centrifuged at 3000 rpm for 15 minutes. The serum obtained was stored in the refrigerator until analyzed. The concentration of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone in the blood serum were determined using commercially available ELISA kits (Diagnostic Procedure Corp., Los Angeles, CA, USA) according to the manufacturer's instructions.

Statistical analysis

Data collected were subjected to One-way analysis of variance (ANOVA), using SAS, (2002) analytical software. Significant means were separated by Duncan's Multiple Range Test option of the same statistical software.

Results

The results of this study are presented in Tables 2 to 5. The proximate composition of Baobab leaf meal is presented in Table 2. The crude protein, crude fibre, ether extract, dry matter and ash were 14.69, 11.20, 6.00, 93.74 and 6.00%, respectively.

Table 2: Proximate composition of Baobab Leaf Meal (BLM)

Nutrients (%)	Composition (%)
Crude Protein (CP)	14.69
Crude Fibre (CF)	11.20
Ether Extract (EE)	6.00
Dry matter	93.74
Ash	6.00

Reproductive hormones

Table 3 shows the reproductive hormones of rabbit bucks fed graded levels of baobab

leaf meal. The result shows that BLM had no significant ($p>0.05$) effect on luteinizing hormone (LH) and follicle stimulating hormone (FSH) while testosterone was significantly ($p<0.05$) influenced. Rabbit bucks fed diet containing 1.25 and 1.75% BLM (T4 and T5) had significantly higher testosterone concentration than bucks in other treatments.

Table 3: Reproductive hormones of rabbit bucks fed dietary Baobab leaf meal (BLM).

Parameters	T1 (Control)	T2 (0.25% BLM)	T3 (0.75% BLM)	T4 (1.25% BLM	T5 (1.75% BLM)	T6 (2.25% BLM)	SEM
LH (mIU/ml)	0.27	0.32	0.42	0.31	0.29	0.29	0.03
FSH (mIU/ml)	0.77	0.87	0.69	0.71	0.82	0.64	0.91
Testosterone (ng/ml)	0.46^{b}	0.55^{b}	0.64^{b}	2.37^{a}	1.39^{a}	0.58^{b}	0.28

a, b: Means within the same row with different superscripts differ significantly ($p<0.05$)
LH = Luteinizing hormone, FSH = Follicle stimulating hormone

Testicular characteristics

Table 4 shows the testicular characteristics of rabbit bucks fed graded levels of BLM. The result indicates that the left testis weight, right testis length and right testis width were significantly ($p<0.05$) influenced by the treatment. Rabbit bucks fed 1.25% of BLM had significantly ($p<0.05$) higher left testis weight, right testis length, left testis width, right testis width and mean testis width than those fed 1.75% BLM but similar to those of other treatments. Other testicular parameters were not significantly ($p>0.05$) affected by the BLM.

Table 4: Effect of Baobab Leaf Meal (BLM) on Testicular Characteristics of rabbit bucks

Parameter	T1(Control)	T2 (0.25% BLM)	T3 (0.75% BLM)	T4 (1.25% BLM)	T5 (1.75% BLM)	T6 (2.25% BLM)	SEM
Left Testis Weight(g)	2.04^{ab}	1.77^{ab}	1.89^{ab}	2.49^{a}	1.50^{b}	1.73^{ab}	0.12
Right Testis Weight(g)	1.87	1.68	1.89	2.11	1.55	1.71	0.11
Paired Testes Weight(g)	3.91	3.44	3.78	3.60	3.05	3.44	0.21
Left Testis Length (cm)	2.13	1.87	1.97	2.03	1.97	1.90	0.06
Right Testis Length (cm	1.93^{ab}	2.67 ^{a}	2.63^{a}	2.77^{a}	1.77^{b}	2.07^{ab}	0.13
Mean Testes Length (cm)	2.03	2.32	2.30	2.35	1.87	1.98	0.08
Left testis Width (cm)	1.10^{ab}	1.03^{b}	1.10^{ab}	1.17 ^{a}	1.00^{b}	1.07^{ab}	0.02
Right Testis Width (cm**)**	1.07^{ab}	0.87^{b}	1.07^{ab}	1.17^{a}	0.97^{ab}	1.07^{ab}	0.03
Mean Testes Width (cm)	1.09^{ab}	0.95^{ab}	1.09^{ab}	1.17^{a}	0.99^{ab}	1.07^{ab}	0.03
Left Testis Volume (ml)	2.33	1.67	1.67	2.33	1.67	1.83	0.13
Right Testis Volume (ml)	1.83	1.83	2.33	2.33	2.00	2.00	0.10
Paired Testes Volume (ml)	4.17	3.50	4.00	4.67	3.67	3.83	0.18

a, b: Means within the same row with different superscripts differ significantly (p<0.05)

Sperm characteristics

The results of sperm characteristics are presented in Table 5. The results indicate that all parameters analyzed were significantly (P<0.05) influenced except the elongated spermatids. The sperm count and normal sperm of rabbit bucks fed T1, T2, T3, T4 and T5 diets were similar but higher (p<0.05) than those fed T6 diet. Mean values for sperm motility revealed that T1, T2, T3 and T4 were similar but significantly (p<0.05) higher than T5 and T6 with T4 recording highest value. Rabbit bucks on T1, T2, T3, T4 and T5 had similar values for live sperm which were significantly (p<0.05) higher than T6. Bucks that were fed 0.75, 1.25 and 1.75% BLM had similar round spermatids with the control but significantly (p<0.05) higher than those fed 0, 0.25 and 2.25% BLM.

Table 5: Sperm characteristics of rabbit bucks fed dietary Baobab leaf meal (BLM)

Parameter	T1 (Control)	T2 (0.25% BLM)	T3 (0.75% BLM)	T4 (1.25% BLM)	T 5(1.75% BLM)	T6 (2.25% BLM)	SEM
Sperm Count (x10^9)	50.93^{ab}	52.46^{a}	53.00^{a}	55.53^{a}	49.26^{ab}	44.00^{b}	1.23
Sperm Motility (%)	78.98^{ab}	75.20^{abc}	74.89^{abc}	82.36^{a}	69.78^{bc}	63.68^{c}	9.91
Normal sperm (%)	82.89^{a}	79.13^{ab}	74.69^{ab}	85.86^{a}	72.76^{ab}	67.55^{b}	2.12
Live sperm (%)	86.53^{ab}	81.33^{ab}	78.21^{ab}	89.10^{a}	76.74^{ab}	74.81^{b}	1.89
Round Spermatids (x10^6)	86.00^{a}	78.00^{b}	93.00^{a}	91.66^{a}	88.33^{a}	68.33^{c}	2.92
Elongated Spermatids (x 10^6)	71.66	73.66	88.83	85.33	83.66	63.66	3.40

a, b, c : Means within the same row with different superscripts differ significantly ($p < 0.05$). SEM: Standard Error of Mean

Discussion

The observation from this study that BLM level had no significant effect on LH and FSH of rabbit bucks suggests the potential of BLM to maintain the LH and FSH in the bucks. The observed significant increase in testosterone level at T4 and T5 indicates the ability of BLM to enhance testosterone production in rabbit bucks. This observation is in agreement with the report of Oyewopo *et al*. (2015) who reported that *Adansonia digitata* leaf extract significantly reversed the lowering effect of carbon tetrachloride on testosterone and luteinizing hormone in male Wistar rats. Data from this study also corroborate the report of Anoh *et al*. (2018) that baobab fruit pulp meal supplementation increased the testosterone level in heat-stressed rabbits. Testosterone has been recognized as the major hormone of the testis whose function is to stimulate sperm production (Abdel Khalek *et al*., 2005).

The right testis length, left, right and mean testis width as well as testis weight were significantly influenced by the dietary treatment. The higher values of the length and width of testis of the rabbit bucks observed in T4 is in agreement with the report of Ajayi *et al.* (2009) who observed a significant increase in testicular length of rabbits when they included blood-wild sunflower leaf meal mixture in diet. In adult males, testicular volume is measured in relation to sperm volume and spermatogenic activity, whereas in young males, its measurement is mainly important in assessing the onset or development of puberty (Abu *et al*., 2016). According to Perry and Peterson (2001), size, length and width of testis are good indicators of present and future sperm production.

Findings from this study show that sperm characteristics of rabbit bucks fed with BLM were significantly enhanced up to 1.25% inclusion level. This could be associated with the anti-stress and antioxidant properties of BLM being rich in vitamin C (Wudil *et al*., 2020). Probably the antimicrobial, anti-stress and antioxidant properties of the BLM were deployed to eliminate any possible microbial infection and free radicals that might want to attack the reproductive cells as reported by De Caluwe *et al*. (2010); Blomhoff *et al*. (2010) and Brady (2011). This result is in agreement with the findings of Amao and Farayola, (2022) who observed a significant increase in the life sperm of rabbit bucks treated with Baoabab Fruit Pulp (BFP)

solution up to 100g/L. Good semen samples have a minimum of 75% life sperm (Ajala *et al*., 2001). Therefore the percentage life sperm cells obtained in this study can be termed suitable for efficient reproduction. This shows that BLM had no adverse effect on sperm production and therefore may serve as an efficient spermatogenesis booster.

The sperm motility of rabbit bucks fed 1.25% baobab leaf meal diet in the present study was higher ($P<0.05$) than 1.75% and 2.25% BLM diets. The report of Amao and Farayola (2022) showed that both 50 and 100 g/L baobab fruit pulp solution administered for either 7 or 14 days improved sperm count, motile sperm, normal sperm and live sperm in rabbit bucks. The findings from this study is also in tandem with the report of Anoh *et al*. (2017) who reported a significant increase in the sperm motility of rabbit bucks fed baobab fruit pulp meal up to 4.5%. This is also similar to the findings of Youssef *et al.* (2003) who observed that Vitamin C and E improved rabbit male fertility by increasing sperm concentration and motility while decreasing abnormal and dead sperm after 12 weeks. The BLM has been reported to contain appreciable amount of vitamin C (Wudil *et al*., 2020).

The observation that sperm count was highest in rabbit bucks that were fed 1.25% BLM suggests that this level could be the optimum inclusion level for enhanced spermatogenesis in rabbit bucks. This observation also supports the findings of Castellini (2008) who reported that baobab fruit pulp has the potential to enhance the reproductive and physiological parameters.

In general, findings from this study indicate that inclusion of BLM up to 1.25% could promote sperm count, motility and morphology of rabbit bucks. This potential may be attributed to the presence of vitamins and minerals as well as antioxidant property of baobab leaves which have been reported to enhance male reproductive potentials.

Conclusion

In conclusion, this study revealed that Baobab leaf meal could be included in diets of rabbit bucks up to 1.25% to enhance sperm quality and testosterone production without adverse effects on testicular parameters.

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