https://dx.doi.org/10.4314/gjas.v58i1.4

Testicular and epididymal anatomy and spermatozoa reserves of rabbit bucks fed *Moringa oleifera* leaf meal-based diets supplemented with mixtures of garlic, ginger or black pepper

A.A. MOHAMMED*, G.T. IYEGHE-ERAKPOTOBOR, D. ZAHRADDEEN, P.P. BARJE & F.U. SAMUEL

(A.A.M.: Department of Animal Science, Usmanu Danfodiyo University, Sokoto, Nigeria; G.T.I-E.: Swine and Rabbit Research Unit, National Animal Production Research Institute, Ahmadu Bello University, Shika-Zaria, Nigeria; D.Z.: Department of Animal Science, Ahmadu Bello University, Zaria., Nigeria; P.P.B.: Dairy Research Unit, National Animal Production Research Institute, Ahmadu Bello University, Shika-Zaria, Nigeria; F.U.S.: Biotechnology Research Unit, National Animal Production Research Institute, Ahmadu Bello University, Shika-Zaria, Nigeria)

**Corresponding author's email: aliyuabdullahimohammed@gmail.com*

ABSTRACT

Twenty-five mature male crossbred (Chinchilla × California× New Zealand White × Dutch) bucks (aged between six and seven months) of average weight (1915±125 g) were used. The bucks were fed either of five diets with *Moringa oleifera* leaf meal (MOLM) as a complement and mixtures of either garlic, ginger or black pepper (spices) as supplements; diet one having no MOLM or spices; diet two having 6% MOLM; diet three having 6% MOLM with mixtures of 3.5 g garlic (GR) and 3.5g ginger (GG) per kg diet; diet four having 6% MOLM with mixtures of 3.5 g garlic (GR) and 3.5 g black pepper (BP) per kg diet; diet five having 6% MOLM with mixtures of 3.5 g ginger (GG) and 3.5 g black pepper (BP) per kg diet in a CRD for three months. Data was analyzed using the GLM procedure while mean separation was done using pairwise difference method of SAS. Significantly ($p < 0.05$) lowest testes weight was obtained for MOLM + GR + BP fed bucks. Total testes volume was significantly ($p < 0.05$) lowest for MOLM + GR + GG fed bucks. Total caudal epididymidis sperm reserves were significantly ($p < 0.01$) highest for MOLM fed bucks. Daily sperm production and daily sperm production per gramme parenchyma were not ($p > 0.05$) affected by MOLM or addition of the spices to the diets. It is concluded that testes volume was increased by 6% MOLM + 3.5 g GR + 3.5 g BP. Also, MOLM fed at 6% dietary level increased caudal epididymides sperm reserves.

Keywords: Spermatozoa reserves; rabbit bucks; moringa; garlic; ginger; black pepper

Original scientific paper. Received 28 Mar 2022; revised 07 Jun 2023

Introduction

Micro-livestock are important because the developing world's animal production is only a fraction of what it should be (BOSTID, 2022). Rabbit production has contributed to food security and income of some developing countries (Casindra, 2015). Through implementation of research and development, successful rabbit projects have been recorded in some parts of Africa, for example Benin Republic (Lukefahr, 2000), Egypt and Ghana (Deliwe *et al*., 2016). Rabbits offer an avenue for rapid transformation in animal protein (Zahraddeen *et al*., 2006). The production of rabbits in Nigeria is low and it is imperative to understand and improve the reproductive performance of rabbits in Nigeria (Zahraddeen *et al*., 2007).

Knowledge of physiology, nutrition and breeding is of vital importance for a successful rabbit production enterprise (Mohammed *et al*., 2017). There are countless feed additives that are added to feed in various forms however the results obtained from them on animal performance are controversial (Foldesiova *et al*., 2013). Many of these products are fed to rabbits usually without recourse to the health and physiological implications on the animals (Aro *et al.,* 2013). Worldwide interest in herbal products has grown significantly over time, as such researchers and feed companies have increased their efforts to develop safer and more natural feed additives, to improve the productivity of rabbits (Zotte *et al*., 2016). This has resulted in the use of herbs, spices and their extracts (botanicals) such as moringa, ginger, black pepper, garlic, cloves and cinnamon to mention a few.

New initiatives in the livestock and pharmaceutical industries are seeking to promote the use of alternative materials that combine the effects of nutritional and medicinal properties, simultaneously (Esiegwu *et al*., 2014). There is an increasing interest in the use of natural feed additives from whole or extracts of some herbs and edible plants as safe supplements instead of chemically produced compounds. Natural antioxidants are recognized to be better than synthetic antioxidants due to their lower cytotoxicity and tissue residue (Gupta & Sharma, 2006; Sen *et al*., 2010).

Moringa is a potent antioxidant and immune stimulant (Olugbemi *et al*., 2010; Ogbunugafor *et al*., 2011; Ahmad, 2021), and fed as an alternative feed ingredient (Etalem *et al*., 2013). Addition of 6% *Moringa oleifera* leaf meal (MOLM) to rabbit buck diets increased caudal epididymal sperm reserves while 6% MOLM plus 7 g Black pepper per kg diet enhanced daily sperm production per gramme parenchyma (Mohammed, 2019). The compatibility status of garlic, ginger, black pepper and their mixtures with other feed ingredients in the diet may affect their effectiveness (Yang *et al*., 2009).

Determination of gonadal and extragonadal sperm reserves can assist in determining how often a male animal can be used for mating without having any depression in herd fertility (Orlu & Egbunike, 2009). Moreover, knowledge of the basic morphometric characteristics of the reproductive organs is of utmost importance for assessment and prediction of fertility (Gage & Freckleton, 2003). This study was therefore designed to evaluate gross testicular and epididymal anatomy and spermatozoa reserves of rabbit bucks fed *Moringa oleifera* diet with mixtures of garlic, ginger and black pepper as additives.

Materials and Methods

Experimental site

The study was carried out at the Rabbitry Unit of the National Animal Production Research

Institute (NAPRI), Shika, Zaria. NAPRI is located in the Northern Guinea Savanna ecological zone (10°11′N, 7°8′E, 650 m above sea level). The area receives an annual rainfall of 1100 mm, spread from April to October (Wet Season). The mean minimum and maximum temperatures are 20°C and 34°C (Iyeghe-Erakpotobor *et al*., 2006). The mean relative humidity during the rainy season (May–October) is 72%, and during the dusty Harmattan period (December to February) it drops to 21% (Tanko *et al*., 2012).

Source and processing of dietary test ingredients

Moringa oleifera leaf, garlic and black pepper were obtained fresh from Sokoto central market. Ginger was obtained from Sabongari market, Zaria metropolis, Kaduna State. The garlic (GR), ginger (GG) and black pepper (BP) were chopped into pieces with the aid of a sharp knife. Thereafter, the chopped garlic, ginger, black pepper and the *Moringa oleifera* leaf were spread separately on clean fabric mats in the shade. They were constantly stirred during drying until they became crispy to touch (considered dry). The dried test ingredients were then separately milled using a milling machine (SIEBTECHNIK TEMA Laboratory disc mill). Each of the milled test ingredient was then packaged in air tight polythene bags and kept at room temperature until when needed for feed formulation.

Phytochemical analyses

The methods used for the determination of alkaloids, flavonoids, phenols, saponins and tannins were those of Harborne (1973), Kumaran & Karunakaran (2006), Hagerman *et al*. (2000), Obdoni & Ochuko (2001), and Van-Burden & Robinson (1981) respectively. Table 1 shows the phytochemical compositions of the test ingredients.

TABLE 1
Phytochemical components of MOLM, GR, GG and BP (mg/g)

Parameters	MOLM	Garlic	Ginger	Black Pepper
Alkaloids	4.17	1.52	11.10	6.26
Flavonoids	4.72	0.78	4.55	2.64
Phenols	1.34	2.60	0.77	4.50
Saponins	6.03	10.37	2.50	9.03
Tannins	15.23	2.97	9.40	2.49

Experimental diets

Five diets were formulated with diet one (positive control) having no *Moringa oleifera* leaf meal (MOLM) or spices; diet two having 6% MOLM as a complement of the diet; diet three having 6% MOLM as a complement of the diet supplemented with mixtures of 3.5 g garlic (GR) per kg diet and 3.5 g ginger (GG) per kg diet; diet four having 6% MOLM as a complement of the diet supplemented with mixtures of 3.5 g garlic (GR) per kg diet and 3.5 g black pepper (BP) per kg diet; diet five having 6% MOLM as a complement of the diet supplemented with mixtures of 3.5 g ginger (GG) per kg diet and 3.5 g black pepper (BP) per kg diet. The gross composition and calculated analyses of the experimental diets is shown in Table 2.

TABLE 2

Feed ingredient(s) composition and calculated nutrient analyses for mixed spices supplementation

Ingredients (%)	**Control**	**MOLM**	**MOLM+GR+GG**	**MOLM+GR+BP**	**MOLM+GG +BP**
Maize	48.00	50.50	50.50	50.50	50.50
Wheat Offals	30.20	22.20	22.20	22.20	22.20
Moringa	0.00	6.00	6.00	6.00	6.00
Soya cake	18.00	17.50	17.50	17.50	17.5
Salt	0.25	0.25	0.25	0.25	0.25
Bone meal	2.80	2.80	2.80	2.80	2.80
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
*Mineral&Vitamin Premix	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100** *+350gGR +350g GG*	**100** *+350g GR+350g BP*	**100** *+350g GG+350g BP*
Calculated Analyses					
ME (Kcal/Kg)	2649	2705	2705	2705	2705
CP(%)	18.35	18.46	18.46	18.46	18.46
Ether Extract (%)	3.61	4.10	4.10	4.10	4.10
Crude Fibre (%)	4.70	4.58	4.58	4.58	4.58
Calcium (%)	1.08	1.15	1.15	1.15	1.15
Available P (%)	0.66	0.67	0.67	0.67	0.67
Methionine (%)	0.52	0.52	0.52	0.52	0.52
Lysine (%)	1.13	1.13	1.13	1.13	1.13

*Bio-mix Broiler starter premix supplied per kg of diet: vitamin A: 5,000 I.U.; Vit D3: 1,000 I.U.; Vit E: 20 mg; Vit K3: 1 mg; Vit B1: 0.2 mg; Vit B2: 2.4 mg; Vit. B6: 2.4 mg; Niacin: 16 mg; Calcium Pantothenate: 4 mg; Biotin: 0.032; Vit B12: 0.01 mg; Folic acid: 0.4 mg; Choline Chloride: 120 mg; Manganese: 40 mg; Iron: 5 mg; Zinc: 18 mg; Cobalt: 0.1 mg; Iodine: 0.62 mg; Selenium: 0.04 mg. MOLM = *Moringa oleifera* leaf meal; GR = Garlic; GG = Ginger; BP = Black Pepper; P = Phosphorous

Proximate analyses

The proximate components of the experimental diets were determined using the methods of AOAC (2007). Estimate metabolisable energy of the diets was calculated using Pauzenga (1985) method:

ME (Kcal/kg DM) = (37 x % CP) + (81.8 x % fat) + (35.5 x % NFE)

The proximate analyses is shown in Table 3.

TABLE 3

Analyzed proximate composition of mixed aromatic plant parts diets

Parameter	**Control**	**MOLM**	**MOLM+GR+GG**	**MOLM+GR+BP**	**MOLM+G-G+BP**
Dry matter (%)	93	91	92	92	92
Crude protein (%)	19.60	20.30	20.30	20.30	20.30
Ether extract (%)	4.40	6.00	6.00	6.00	6.00
Crude fibre (%)	7.30	7.50	7.50	7.50	7.50
Ash (%)	16.60	16.80	16.80	16.90	17.00
Nitrogen free extract (%)	43.30	40.40	41.40	41.30	41.20
*Metabolisable energy (Kcal/kg)	2622	2676	2712	2708	2705

*Calculated using Pauzenga, (1985) method. MOLM = *Moringa oleifera* leaf meal; GR = Garlic; GG = Ginger; BP = Black Pepper

Experimental animals

Twenty-five mature male crossbred (Chinchilla × California × New Zealand White × Dutch) rabbit bucks (aged between six and seven months) of average weight (1915±125 g) were used in a completely randomized design. Each buck was housed in an individual metal cage of 0.6 m x 0.5 m x 0.4 m. There were five treatments with five replicates per treatment. Each replicate had one buck.

Determination of testicular and epididymal parameters

Two bucks per treatment were slaughtered at the end of three months feeding period. Immediately after slaughter the animals were taken to the fertility unit laboratory and dissected to obtain the testes and epididymidis. Each epididymis was carefully removed from the testis with scalpel blade and the length and weight of the head, body and tail portion were determined using a measuring tape and digital weighing balance respectively (Olukole *et al*., 2010). The weight of each testis and epididymis was obtained using a sensitive electronic weighing scale. The volume of each testis was obtained using the Archimedes principle of water displacement. Percent testes weight was calculated as paired testes weight divided by live body weight multiplied by 100. Testes density was calculated as testes weight/ testes volume (g/ml). Percent epididymidis weight was calculated as paired epididymis weight all over live body weight multiplied by 100 (Eme & Egbunike, 2010).

Determination of gonadal and extra gonadal sperm reserves and sperm production

Gonadal and extra gonadal sperm reserves were determined as described by Rekwot *et al*. (1994). Two bucks from each treatment were slaughtered and the testes removed, the length, weight and volume of each testis were determined using a measuring tape, digital weight balance and water displacement method respectively and the values recorded. The *tunica albuginea* was carefully removed with a scalpel blade from each testis. The testicular spermatozoa number was determined by homogenization (Igboeli & Rakha, 1971; Egbunike *et al*., 1976).

Each testis was homogenized in 25 ml of physiological saline solution using a scissors to mince. Antibiotics (Streptomycin sulphate 1 mg/ml and Penicilin g 100 iu/ml) were added to the solution. The homogenate volume was measured after rinsing the scissors with 10 ml of physiologic saline solution and adding the effluent. The homogenate (2.5 ml) was transferred into a conical flask and further diluted with 40 ml of saline solution. The diluted testicular homogenate sample was stored overnight at 5˚C and filtered through gauze and the filtrate volume measured. Spermatozoa/spermatids concentration was determined using Neubauer haemocytometer (model PPHB 205 STANDARD) according to the method of Kwari & Waziri (2001).

The portions of each epididymis were minced separately in 20 ml of normal saline with a sharp scissors and stored for 24 hours at 5˚C. The products were then filtered through gauze and the volume measured. Then 1 ml of epididymal filtrate was placed in a test tube and further diluted with 2 ml of normal saline and the concentration of the sperm reserves was determined using Neubauer haemocytometer under a light microscope (Kwari & Waziri, 2001). The daily sperm production was obtained using the formular: Daily sperm production = Testes sperm count (gonadal sperm reserve) / Time divisor. The value of the time divisor for

rabbits was 3.43 (Amann, 1970).

Experimental model and data analyses

A completely randomized design was used, with the following statistical model:

$$X_{ij} = \mu + T_i + e_{ij}.$$

Where,

X_{ij} = individual observation on j^{th} rabbit in the i^{th} treatment.

μ = population mean

T_i = effect of the i^{th} treatment diet on the weight changes, blood chemistry or reproductive parameters.

e_{ij} = random error associated with the X_{ij}.

Data obtained from the experiment were analyzed using the general linear model procedure of SAS while means separation was done using pairwise difference (Pdiff) method (SAS, 2001). Testicular sperm production per animal was estimated using the formula:

Daily sperm production/g parenchyma = Gonadal sperm reserve / Gross testes weight- tunica albuginea×1/3.43 (Amao *et al*., 2012)

Results and Discussion

The significantly ($p < 0.05$) highest right testis weight obtained for the MOLM fed bucks (Table 4) agrees with the work by Cajuday & Pocsidio (2010) that MOLM increased testis weight. The left testis weight was significantly ($p < 0.05$) lower in the MOLM fed bucks compared with the control bucks. Contrary to this, Cajuday & Pocsidio (2010) reported that MOLM increased testis weight. Significantly ($p < 0.05$) lowest left and total testes weight obtained from the MOLM + GR + GG fed bucks compared with the MOLM, MOLM + GR + BP or MOLM + GG + BP fed bucks could not be related to any specific cause, as literature on similar work is scarce. It could likely be that the combination of garlic and ginger has an inhibitory effect on testis weight in rabbits. Significantly ($p < 0.05$) higher right testis volume obtained for the MOLM fed bucks compared with the control bucks might indicate an enhancement in lumen formation. This might be related to the fact that testicular lumen formation was enhanced in MOLM fed mice (Priyadarshani & Varma, 2014).

Rabbit bucks on MOLM + GR + BP had significantly ($p < 0.05$) highest right testis volume compared with the MOLM, MOLM + GR + GG or MOLM + GG + BP fed bucks, which might suggest enhanced potential for sperm production by the combination of garlic and black pepper. Matthew (2009) reported that garlic increased sperm output. Piper species were also reported to increase testes weight and sperm number (Mohammadi *et al*., 2013). Significantly ($p < 0.05$) lowest left testis volume obtained from MOLM + GR + GG fed bucks could be attributable to the phytochemical components in garlic and ginger mixture, which had inhibitory effect on testis volume. Total testis volume was significantly ($p < 0.05$) highest in MOLM + GR + BP fed bucks, which might indicate a likely capacity of the combination of garlic and black pepper, in enhancing sperm production and thereby fertility. Tijani *et al*. (2014) reported that testicular volume correlated with sperm density. This is because testicular tissue consists majorly of seminiferous tubules (Ku *et al*., 2002), hence testis volume is associated with testicular function which is spermatogenesis.

TABLE 4
Gross testicular anatomy of rabbit bucks fed MOLM, MOLM supplemented with mixtures of garlic and ginger, garlic and black pepper or ginger and black pepper

Parameter	Control	MOLM	MOLM+GR+GG	MOLM+GR+BP	MOLM+ GG+BP	SE	P
Testis Wt. (g):							
Right	1.20[b]	1.70[a]	1.10[b]	1.20 [b]	1.20[b]	0.08	0.04
Left	4.02[a]	3.30[b]	2.30 [c]	4.20 [a]	4.20[a]	0.09	0.05
Total	5.40[a]	5.00 [a]	3.40[b]	5.40 [a]	5.40[a]	0.97	0.05
Percent	0.20[a]	0.10[b]	0.10[b]	0.10[b]	0.20[a]	0.02	0.05
Testis vol (ml):							
Right	2.30[d]	3.20[c]	2.00 [d]	4.30[a]	3.70[b]	0.22	0.01
Left	4.20[a]	3.30[b]	2.30[c]	4.20[a]	4.20[a]	0.24	0.02
Total	6.50[c]	6.50[c]	4.30[d]	8.50[a]	7.90[b]	0.46	0.02
Testis L (cm):							
Right	3.00	2.60	2.70	2.30	2.30	1.17	0.28
Left	2.30	3.70	1.90	2.60	2.40	1.16	0.01
TestesD (g/ml)	0.80 [a]	0.77 [a]	0.79[a]	0.64 [b]	0.68 [b]	0.02	0.01

[abcd]means across rows with different superscripts are significantly ($p \leq 0.05$) different. Wt= Weight; L = Length; Vol = Volume; D = Density. SE = Standard Error; P = Probability

Results for gross epididymal anatomy of rabbit bucks fed 6% MOLM or 6% MOLM plus mixtures of GR, GG or BP is shown in Table 5. Significantly ($p < 0.05$) higher right caput epididymide length obtained from MOLM fed bucks compared with the control fed bucks and significantly higher left caput epididymide length obtained from MOLM + GR + GG fed bucks compared with the MOLM fed bucks could indicate the effect of phytochemical components in the spices; however, there is a dearth of literature on similar works. Significantly ($p < 0.05$) lower total, right and left epididymides length obtained for the MOLM + GR + BP fed bucks could indicate an inhibitory effect of these spices on the growth of the epididymides. The right caput, right caudal, left caudal, total left epididymides and epididididymidis weight were significantly ($p < 0.05$) higher for the MOLM fed bucks than those on the mixed spices. Sudha *et al*. (2010) also reported that MOLM increased epididymal weight.

TABLE 5
Epididymal parameters of rabbit bucks fed MOLM, MOLM supplemented with mixtures of garlic and ginger, garlic and black pepper or ginger and black pepper

Parameter	Control	MOLM	MOLM+GR+GG	MOLM+GR+BP	MOLM+G-G+BP	SE	P
Epididymides Length (cm):							
Right caput	0.40^{c}	1.00^{b}	1.40^{a}	0.90 ^{b}	0.50 ^{d}	0.08	0.03
Right corpus	2.80^{a}	2.10^{b}	2.30^{b}	2.00^{c}	1.90 ^{c}	0.14	0.05
Right caudal	3.00 ^{a}	2.60 ^{bc}	2.70 ^{ab}	0.40^{d}	2.30 ^{c}	0.16	0.01
Total (Right Epididymis)	6.20^{a}	5.70^{a}	6.40 ^{a}	3.300 ^{c}	4.70 ^{b}	0.38	0.04
Left caput	2.30^{a}	1.00^{b}	1.90 ^{ab}	1.10^{b}	1.00 ^{b}	0.12	0.04
Left corpus	2.30^{b}	2.00 ^{b}	2.00 ^{b}	3.00^{a}	2.80^{a}	0.16	0.04
Left caudal	2.80^{b}	3.70^{a}	1.90^{b}	0.50 ^{c}	2.30 ^{b}	0.34	0.01
Total (Left Epididymis)	7.40^{a}	6.70^{ab}	5.80 ^{b}	4.60 ^{c}	6.10^{b}	0.47	0.02
Epididymides weight (g):							
Right caput	0.10^{d}	0.40^{a}	0.30 ^{b}	0.10^{d}	0.20^{c}	0.01	0.03
Right corpus	0.02	0.02	0.01	0.03	0.04	0.03	0.12
Right caudal	0.30^{c}	0.50^{a}	0.4^{b}	0.50 ^{a}	0.40^{b}	0.03	0.03
Total (Right Epididymis)	0.42	0.92	0.71	0.63	0.64	0.44	0.18
Left caput	0.10^{c}	0.20 ^{b}	0.30 ^{a}	0.10 ^{c}	0.10^{c}	0.01	0.05
Left corpus	0.02	0.01	0.02	0.02	0.03	0.02	0.12
Left caudal	0.30 ^{b}	0.50 ^{a}	0.3 ^{b}	0.50^{a}	0.30^{b}	0.03	0.02
Total (Left Epididymis)	0.42^{c}	0.71 ^{a}	0.62 ^{b}	0.62 ^{b}	0.43^{c}	0.03	0.05
Epididymidis weight (g)	0.90 ^{c}	1.70^{a}	1.30 ^{b}	1.20 ^{b}	1.00^{c}	0.06	0.05
% Epididymidis weight (g)	0.04	0.08	0.07	0.06	0.06	0.04	0.20

^{abcd}Means across rows with different superscripts are significantly ($p \leq 0.05$) different. SE = Standard Error; P = Probability

Gonadal and extra gonadal sperm reserves and sperm production of rabbit bucks fed MOLM, MOLM supplemented with mixtures of garlic, ginger and black pepper is shown in Table 6. The significantly ($p < 0.05$) lower left caput epididymide sperm reserves obtained for the MOLM fed bucks compared with the bucks fed MOLM + Garlic, Ginger or Black Pepper diets suggests these spices with antioxidative properties have a positive effect on spermatogenesis, as antioxidants are reported to increase epididymal sperm concentration (Sonmez *et al*., 2005). In line with this, Matthew (2009) reported that garlic improved gonadal and extra gonadal sperm reserves.

Ginger was also reported to stimulate spermatogenesis (Morakinyo *et al*., 2010) and improve spermatogenesis (Toader, 2014), which could hence increase corpus epididymal sperm reserve. Black pepper was also reported to enhance spermatogenesis (Kumar *et al*., 2012). Furthermore, increase in epididymal sperm concentration, spermatocyte counts, spermatid counts and weight of epididymis tubules was reported in male mice fed black pepper (Sutyarso *et al*., 2016). The right, left and complete corpus epididymides were significantly ($p < 0.05$) lower for MOLM fed bucks compared with the control bucks, indicating an inhibitory effect of Moringa on development of the corpus epididymides.

This result is contrary to the report of Priyadarshani & Varma (2014) that MOLM increased spermatogenesis through increased lumen formation in the testis. This disparity might be due to the level of MOLM fed or the method of processing the MOLM. Rabbit bucks fed MOLM + GR + BP had significantly ($p < 0.05$) highest left and complete corpus epididymides, while right corpus epididymides was highest ($p < 0.05$) for the MOLM + GG + BP fed bucks compared with bucks fed MOLM diet. It is likely that black pepper has synergistic effect with garlic and ginger on growth and development of the corpus epididymal tissue.

This result could be related to the report of Morakinyo *et al*. (2010) that ginger stimulates spermatogenesis. Significantly ($p < 0.05$) highest right, left and complete caudal epididymides sperm reserves obtained for the MOLM fed bucks in this study indicates a possible enhancement in the development of the lumen of the epididymides by MOLM and a possible inhibition by the spices used. This is in line with the study of Priyadarshani & Varma (2014) that MOLM increased testicular lumen formation which enhances spermatogenesis. Significantly ($p < 0.05$) higher epididymidis sperm reserve obtained for the MOLM fed bucks compared with the control, indicates that MOLM has beneficial effect on spermatogenesis which agrees with the findings of Priyadarshani & Varma (2014) that MOLM stimulates spermatogenesis.

The significantly ($p < 0.05$) lowest epididymidis sperm reserve obtained for the MOLM + GG + BP fed bucks might mean that black pepper (BP) had some negative effects on spermatogenesis at the level fed, especially as the combination of ginger and garlic did not give a similar result. In line with this, D'Cruz & Mathur (2005) reported that piperine from black pepper could damage the epididymal environment. Daily sperm production (DSP) was non-significantly ($p > 0.05$) affected by MOLM or addition of the spices to the diet of the bucks. This shows that MOLM and the phytochemicals did not improve sperm production and hence at the level fed might not be beneficial to DSP. This result is not in line with the report of Cajuday & Pocsidio (2010) that MOLM induced sperm production in mice.

Garlic is reported to cause a dose-dependent increase in the percentage of empty seminiferous tubules, thereby altering spermatogenesis and reducing testosterone secretion (Benerjee *et al*., 2001). It is also associated with the inhibition of Leydig steroidogenic enzyme expression and Sertoli cell markers, which are capable of inducing apoptosis in testicular germ cells that is the spermatocytes and spermatids (Omotoso *et al*., 2009). This contradicts Matthew, (2009) that garlic increased sperm output. This disparity might be as a result of the level of garlic used. However, in relation to this study, garlic was reported to have inhibitory effect on spermatogenesis (Dixit & Joshi, 1983; Hammami *et al*., 2008).

Daily sperm production per gramme parenchyma was also not affected by the MOLM or addition of the spices. Though black pepper is believed to enhance efficiency of sperm production. This did not happen in this study especially as black pepper is reported to maintain and enhance the levels and efficacy of important antioxidant compounds because it contains several powerful antioxidants and is thus one of the most important spices for preventing and curtailing oxidative stress (Vijayan & Thampuran, 2000). Black pepper also has high content of zinc (Bouba *et al*., 2012), which helps in testicular growth, development of seminiferous tubules and as such enhancing spermatogenesis (Kumar *et al*., 2012).

TABLE 6

Gonadal and extra gonadal sperm reserves and sperm production (×10⁶/ml) of rabbit bucks fed MOLM, MOLM supplemented with mixtures of garlic and ginger, garlic and black pepper or ginger and black pepper

Parameter	Control	MOLM	MOLM+GR+GG	MOLM+GR+BP	MOLM+G-G+BP	SE	P
Testis:							
Right	6.00 [b]	3 .00[d]	7.50 [a]	3.00 [d]	4.50 [c]	0.68	0.04
Left	7.00 [b]	9.50 [a]	5.50[d]	5.00[d]	6.50[c]	0.71	0.01
Complete	13.00	12.50	13.00	8 .00	11.00	3.38	0.31
Caput Epididymides:							
Right	2.00 [a]	0.00 [c]	2.00 [a]	0.00 [c]	1.00 [b]	0.46	0.04
Left	1.00 [c]	2.00 [b]	0.20 [d]	4.50 [a]	2.00 [b]	0.18	0.02
Complete	3.00 [b]	2.00 [c]	2.00 [c]	4.50 [a]	3.00 [b]	0.18	0.03
Corpus Epididymides:							
Right	0.00 [c]	0.00 [c]	0.00 [c]	1.00[b]	2.00 [a]	0.14	0.03
Left	0.00 [c]	2.00 [a]	1.00 [b]	2.00 [a]	0.00 [c]	0.16	0.04
Complete	0.00 [d]	2.00[b]	1.00 [c]	3.00 [a]	2.00 [b]	0.13	0.04
Caudal Epididymides:							
Right	27.50 [c]	87.00 [a]	30.50 [c]	38.00 [b]	11.00 [d]	2.35	0.01
Left	34.00 [d]	57.50 [a]	29.00 [c]	53.00 [b]	9.00 [e]	1.76	0.01
Complete	61.50 [d]	144.50 [a]	59.50 [c]	91.00 [b]	20.00 [e]	4.03	0.01
Epididymidis	64.50 [c]	148.50[a]	62.50 [c]	98.50[b]	25.00 [d]	4.12	0.01
Daily Sperm Production	3.80	3.70	3.80	2.30	3.20	1.40	0.31
DSP/gP	1.40	1.10	1.80	0.90	1.30	1.22	0.33

[abcde]means across rows with different superscripts are significantly ($p \leq 0.05$) different. SE = Standard Error; P = Probability; DSP/gP = Daily Sperm Production per gram parenchyma

Conclusion and Recommendation

Based on the results obtained in this study, it is concluded that the MOLM fed bucks had significantly ($p < 0.05$) highest right testis weight and volume, right caput epididymide length, complete caudal epididymides sperm reserves, caput epididymide sperm reserves. The MOLM + GR + GG fed bucks had significantly ($p < 0.05$) lowest left and total testes weight, left testis volume, left caput epididymide length, epididymidis sperm reserve, and complete corpus epididymides. The MOLM + GR + BP bucks had significantly ($p < 0.05$) highest total testis volume complete corpus epididymides and lower ($p < 0.05$) total, right and left epididymides length. Significantly ($p < 0.05$) highest right corpus epididymides was obtained for MOLM + GG + BP fed bucks. Daily sperm production and daily sperm production per gramme parenchyma were not ($p > 0.05$) affected by MOLM or addition of the spices to the diet of the bucks. It is recommended that crossbred rabbit bucks should be fed MOLM or 6% MOLM + 3.5 g GR + 3.5 g BP per kg diet for increased testes volume and caudal epididymides sperm reserves.

Acknowledgment

The authors wish to thank the Management of the National Animal Production Research Institute (NAPRI), Shika, Zaria, Nigeria for providing the experimental space. The assistance of the technical staff of the Swine and Rabbit Research Programme in the conduct of the study and that of the technical staff of the Fertility section, Artificial Insemination Unit, Animal Biotechnology Programme, NAPRI, in the testicular analysis is also acknowledged.

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