

**Original Article****Additive Effect of Ethanolic Extract of Black Pepper (*Piper Nigrum*)
On Gonadosomatic Weight of Testes and Testicular
Histomorphometry: In Relation to Spermatogenesis in Rabbit Bucks****R.E. Ere¹, J.A. Imasuen¹, and A.A. Ere-Richard²**¹Department of Animal Science, Faculty of Agriculture, University of Benin, Nigeria²Department of Animal Physiology, College of Animal Science and livestock production,
Federal University of Agriculture, Abeokuta, Nigeria**ABSTRACT**

This study investigated the additive effect of ethanolic extract of Black Pepper (*Piper nigrum*) on gonadosomatic index of the testes and testicular histology as well as testicular morphometry of Rabbit bucks. A total of twenty (20) composite Rabbit bucks aged 18 – 20 weeks were used for this study in a completely Randomized design. Experimental animals were divided into 5 groups represented as A, B, C, D, and E consisting of 4 bucks each and replicated four (4) times with a buck per replicate. The first group A (Control) was given pelleted feed that did not contain extract of black pepper. The second, third, fourth and fifth groups (B, C, D, E) had in their diet (pelleted feed) inclusion levels of black pepper extract at 100mg/kg feed, 150mg/kg feed, 200mg/kg feed (optimum) and 250mg/kg feed (above optimum). The treatment (test diet) was administered for 90 days. Ethanolic extract of Black pepper inclusion at optimum and above optimum showed statistical evidence of higher values for optimum and lower values for above optimum. Testicular length of Rabbits fed at optimum and above optimum showed (6.900±0.20) and (6.067±0.20), testes weight showed (4.47±0.40) and (1.63±0.40), testes volume showed (2.53±0.23) and (0.90±0.23), epididymis length showed (23.23±0.97) and (18.73±0.97), and gonadosomatic index results showed (0.1257±0.01) and (0.0483±0.01). Black pepper

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ethanol extract enhanced testicular morphometric parameters of Rabbit bucks fed at optimum inclusion level of 200mg/kg feed. It was also observed to be toxic to testis development at higher doses (above optimum) based on the Gonadosomatic index results, and testicular histological slides showed that inclusion levels of ethanolic extract of Black pepper above optimum levels causes destruction of seminiferous tubules and negatively affected spermatogenesis.

Keywords: Histology, morphometry, *Piper nigrum*, Rabbit, spermatogenesis, testes.

INTRODUCTION

Rabbit meat production can be used to bridge the gap in short supply of animal protein especially in developing nations with progressively increasing population. Rabbit meat is a ready source of high value animal protein to man, it is a white meat with low level of cholesterol unlike beef. Rabbit meat is sixth after beef, fish, mutton, goat meat (chevon) and bush meat or game animals in the parametric assessment of meat animal production and consumption in Nigeria (Onifade *et al.*, 1999). Aduku and Olukosi (1990) and Onifade *et al.* (1999) reported that rabbit production has a lot of benefits such as high adaptability, easiness to handle and manage, high growth rate, high efficiency in converting forage to meat, short gestation period and very high prolificacy. Rabbit production can be improved by manipulation of its reproductive potential. The reproductive potential of rabbit buck is determined to a great extent by the quality of semen produced (Ewuola *et al.*, 2011). The prediction of Rabbit sperm production potential requires knowledge of testicular morphometry and testicular tissue histology which is related to the potential capacity of sperm production. The testes are the primary reproductive organs in the male due to the specific role they play in the production of male gametes (spermatozoa) in a process called spermatogenesis (NseAbasi, 2015). Testicular parameters are the most precise tool for measuring the level of semen production from the daily sperm production potential of Rabbit bucks (Hassan *et al.*, 2009).

Some plant extract such as Black pepper (*Piper nigrum*) has been reported to improve Reproductive functions (Sutyarso *et al.*, 2016; Mohammadi *et al.*, 2013; Chauhan *et al.*, 2007), while others balance the levels of hormones in the hypothalamic- pituitary gonadal axis of male and female animals (Asuquo *et al.*, 2013).

Abdallah and Abdalla, 2018, reported that phytochemical screening of Black Pepper showed secondary metabolites of plants such as tannins, flavonoids, alkaloid etc. in the plant extract through a simple chemical test. These secondary metabolites give plants their bioactive properties and thus referred to as active constituents. Piperine (1-Piperoyl piperidine) is a major alkaloid of Black pepper (*Piper nigrum*) which is responsible for its pungency (Abdallah and Abdalla, 2018).

The aim of this study was to examine the additive effect of ethanolic extract of black pepper on testicular histology and morphometric characteristics of Rabbit buck testis as it affects spermatogenesis.

Experimental Materials and Management

The rabbits were managed intensively in a hutch and quarantined for four (4) weeks, during which they were treated with Ivomec® injection for the control of haemoparasite, internal and external parasites. The rabbits were allowed *ad libitum* access to water and feed (commercial growers' diet) supplemented with forages.

Experimental Design

A total of Twenty (20) composite rabbit bucks between the ages of 18 – 20 weeks were used in this study in a completely Randomized design. The experimental animals were divided into 5 groups (A, B, C, D, and E) consisting of 4 bucks each and replicated four times with a buck per replicate. The control group (A) was given pelleted commercial growers feed without black pepper extract while the other groups (B, C, D and E) had in their diet (pelleted commercial growers feed) 100mg/kg feed, 150mg/kg feed, 200mg/kg feed and 250mg/kg feed inclusion levels of black pepper extract respectively.

Procurement and preparation of Black pepper

Black pepper (*Piper nigrum*) seeds were sourced from new Benin market, Benin City, Edo state, Nigeria and prepared using standard procedure of phytochemical extraction as described by (Odey *et al.*, 2012; Ismail *et al.*, 2012; Sutyarso *et al.*, 2016). 2kg of Black pepper seeds were weighed using a weighing scale and ground into fine powder with the aid of an electric blender. Alcoholic extract of Black pepper was obtained by soaking the powder in 95% ethanol (950ML of absolute alcohol plus 50ML of distilled water) at room temperature. 1kg of Black pepper powder was soaked with 5000ML of Hydroalcoholic solvent (95% ethanol) in a screw capped container and stirred every four (4) hours. The mixture (hydroalcoholic extracts) was filtered with a muslin cloth first and secondly with whatman No1 filter paper (24cm). The supernatant collected every 24 hours for three days (72 hours) was evaporated under low pressure using a Rotary evaporator to 10% of its original volume at 37-40°C until a brownish-viscous extract was formed. The Black pepper extract (BPE) formed was preserved in a sterile airtight container and stored in a refrigerator until its use.

Preparation of test diet

The Black pepper extract (BPE) was completely mixed with commercial poultry growers mash at varying inclusion levels within the range of tolerable limit as described by Chanda *et al.*, 2009. The experimental diets are as follows:

Diet 1 (control)= 0mg of BPE/kg of feed

Diet 2 = 100mg of BPE/kg feed

Diet 3 = 150mg of BPE/kg feed

Diet 4 = 200mg of BPE/kg feed

Diet 5 = 250mg of BPE/kg feed

The experimental diet was then pelleted into four (4) MM size of feed ideal for Rabbit consumption. The treatment (test diet) was administered to all groups for a period of 90 days.

Data collection

Morphometric evaluation of Buck testes

At the end of the experiment three (3) bucks from each treatment were randomly selected from the experimental bucks for morphometric evaluation as described by (Ansa *et al.*, 2017).

The bucks were euthanized and the scrotal sacs incised to exteriorize the testes, and the epididymis was separated from the testes and other adhering tissues. The testes and epididymis weight were measured using an electronic scale and the testes volumes determined volumetrically by water displacement in a measuring cylinder according to Archimedes principle. The testes density was derived with the equation:

Testes density = Testes weight (g)/ Testes volume (cm³).

The length and circumference of the testes and epididymis were measured using a thread and meter ruler.

Histopathological evaluation of buck testes

Histopathological examination of the harvested buck testes was carried out as described by (Ansa *et al.*, 2017). The testes recovered from testicular morphometry was fixed in Bouin's fluid for 24hours. The tissues were washed in ascending grades of ethanol (50, 75, and 100%) and cleared with xylene. They were embedded in paraffin wax and then sectioned using a microtome at 4-5micron thickness. De-waxed sections were stained with Haematoxylin and Eosin conducted at the Histology Laboratory, University of Benin Teaching Hospital. The slides were covered with Distyrene, plasticizer and Xylene mountant to increase refractive index of the stained preparation and then covered with slides to prevent scratches. All sections were examined under light microscope using x100 and x400 magnification. Photomicrographs of the testicular tissues was taken under microscopic view and documentation of histopathology.

Statistical Analysis

Data collected from testicular Morphometric and Histopathological evaluation and all other parameters evaluated were subjected to one-way statistical Analysis of Variance

(ANOVA) using Genstat 2009 (12th Edition) statistical package. Significant means between treatment groups was separated by Duncan multiple range test (DMRT).

RESULTS

Effect of BPE on Morphometric measurement of Testes, Epididymis and gonadosomatic index of Mature Rabbit bucks

The Effect of BPE on Morphometric measurement of Testes and Epididymis of Mature Rabbit bucks are shown in table 1. Results on Buck weight showed that Rabbits fed Diets 1 and 4 were not significantly different from each other. Rabbits fed Diets 3 and 5 were also not significantly different from each other. However, weight of Rabbits fed Diets 1 and 4 were significantly ($P<0.05$) higher compared to others, whereas, weight of Rabbits fed Diet 2 was significantly ($P<0.05$) lower compared to the weight of Rabbits fed other Diets.

Testes weight results revealed that testicular weight of Rabbits fed Diet 1 was significantly different from testicular weight of Rabbits fed Diets 2,3,4 and 5. However, testicular weight of Rabbits fed Diets 2 and 3 were also not significantly different from each other while testicular weight of Rabbits fed Diet 4 was significantly ($P<0.05$) higher compared to testes weight of Rabbits fed all other Diets. Whereas, testicular weight of Rabbits fed Diet 5 was significantly ($P<0.05$) lower compared to the testes weight of Rabbits fed other Diets.

It was evident from the results that testicular length of Rabbits fed Diet 1 was significantly ($P<0.05$) lower compared to Rabbits fed Diets 2,3,4 and 5, though the testicular length of Rabbits fed Diets 2,3,4 and 5 were significantly different from each other and those of Diet 1. However, testicular length of Rabbits fed Diets 4 was significantly ($P<0.05$) higher compared to those fed Diets 1,2,3 and 5.

The result revealed that testicular circumference of Rabbits fed Diets 2,3,4 and 5 were not significantly different from the testicular circumference of Rabbits fed Diet 1.

Results on testicular volume showed that testicular volume of Rabbits fed Diets 1 and 2 were not significantly different from each other, while, that of Diets 3,4 and 5 are significantly different from each other. Whereas, testicular volume of Rabbits fed Diet 4 was significantly ($P<0.05$) higher compared to those of Diets 1,2, 3 and 5, while, testicular volume of Rabbits fed Diet 5 was significantly ($P<0.05$) lower compared to all others.

Testes density parameter showed no significant difference between the testicular density of Rabbits fed Diet 1 and those of Diets 2,3,4 and 5.

Epididymis weight results also showed no statistical significant difference between the epididymal weight of Rabbits fed Diets 1 and those of Diets 2,3,4 and 5.

Table 1. Effect of black pepper extract on Morphometric measurement of Testes and Epididymis of Mature rabbit bucks

Parameters	Level of inclusion				
	Control	100mg/kg	150mg/kg	200mg/kg	250mg/kg
Buck weight (g)	1812±48.7 ^a	1572±48.7 ^{bc}	1678±48.7 ^{ab}	1763±48.7 ^a	1687±48.7 ^{ab}
Testes weight (g)	4.50±0.40 ^a	3.83±0.40 ^{abc}	3.90±0.40 ^{abc}	4.47±0.40 ^{ab}	1.63±0.40 ^{bcd}
Testes length (cm)	5.400±0.20 ^{bcd}	6.533±0.20 ^{abc}	6.600±0.20 ^{ab}	6.900±0.20 ^a	6.067±0.20 ^{bc}
Testes circumference (cm)	5.33±0.36 ^a	6.10±0.36 ^a	6.00±0.36 ^a	6.23±0.36 ^a	5.20±0.36 ^a
Testes volume (ml)	2.20±0.23 ^{abc}	2.20±0.23 ^{abc}	2.23±0.23 ^{ab}	2.53±0.23 ^a	0.90±0.23 ^{bcd}
Testes density (g/ml)	1.722±0.20 ^a	1.743±0.20 ^a	1.747±0.20 ^a	1.760±0.20 ^a	1.938±0.20 ^a
Epididymis weight (g)	0.933±0.06 ^a	1.000±0.06 ^a	0.933±0.06 ^a	1.100±0.06 ^a	1.033±0.06 ^a
Epididymis length (cm)	19.73±0.97 ^{bc}	18.77±0.97 ^{bc}	21.80±0.97 ^{ab}	23.23±0.97 ^a	18.73±0.97 ^{bc}
Epididymis reserve (ml)	0.800±0.09 ^{abc}	0.800±0.09 ^{abc}	0.800±0.09 ^{abc}	1.000±0.09 ^a	0.400±0.09 ^{ab}
Gonadosomatic index (%)	0.1240±0.01 ^{ab}	0.1217±0.01 ^{abc}	0.1160±0.01 ^{abc}	0.1257±0.01 ^a	0.0483±0.01 ^{bcd}

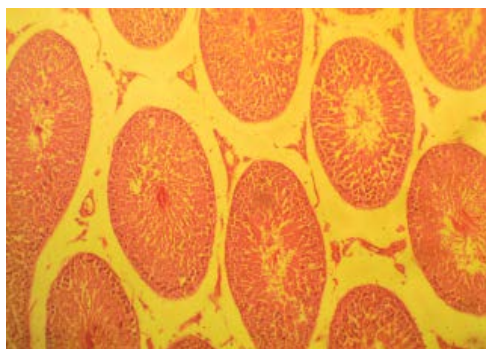
^{abcde} Means in same row with different superscripts differ significantly (P<0.05).

Epididymis length results showed that the epididymal length of Rabbits fed Diets 1,2 and 5 were not significantly different from each other, while those of Diets 3 and 4 were significantly different from each other. However, epididymal weight of Rabbits fed Diets 3 and 4 are also significantly different compared to those of Diets 1, 2 and 5. The epididymal length of Rabbits fed Diet 4 was significantly ($P<0.05$) higher compared to others while, that of Diet 5 was significantly ($P<0.05$) lower compared to all others.

Epididymal reserve parameter revealed that the Epididymis reserve of Rabbits fed Diets 1,2 and 3 were significantly not different from each other, whereas, that of Diet 4 was significantly ($P<0.05$) higher among all, while that of Diet 5 was significantly ($P<0.05$) lower compared to all others.

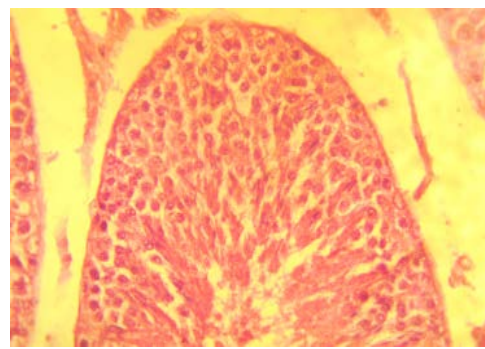
Gonadosomatic index results showed that Gonadosomatic index values for Rabbits fed Diet 1 was significantly different from those of Diets 2,3,4 and 5. However Gonadosomatic index value for Rabbits fed Diet 4 was significantly ($P<0.05$) higher compared to those of Diet 1, while that for Rabbits fed Diet 5 was significantly ($P<0.05$) lower compared all others.

Histological slides showing the effect of BPE on seminiferous tubules, leydig cells and sertoli cells of buck testes



X100

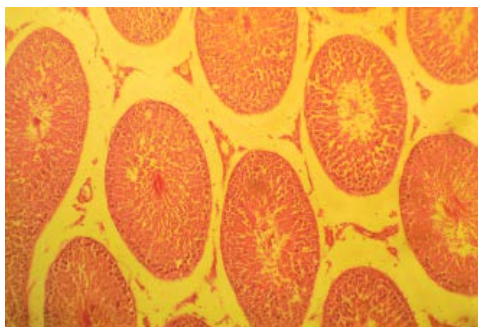
Section shows normal seminiferous tubules



X400

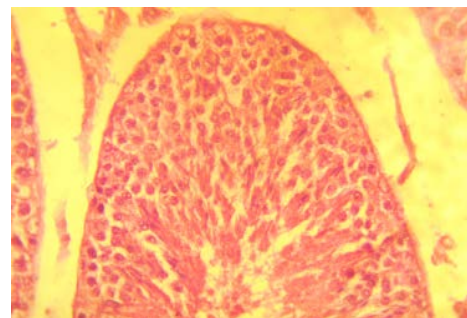
Section shows normal leydig cells, normal sertoli cells and normal spermatogenesis

Figure 1: Testicular histology slide of Rabbits fed Diet 1 (0mg/kg) feed of BPE



X100

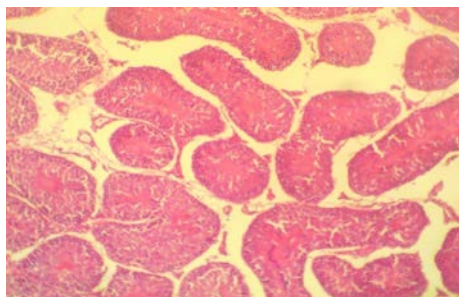
Section shows normal seminiferous tubules



X400

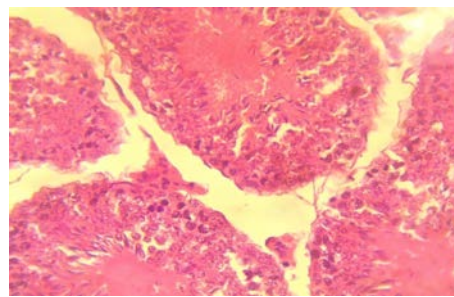
Section shows normal leydig cells, normal sertoli cells and normal spermatogenesis

Figure 2: Testicular histology slide of Rabbits fed Diet 2 (100mg/kg) feed of BPE



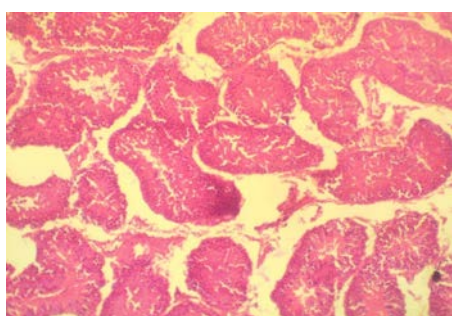
X100

Section shows normal seminiferous tubules



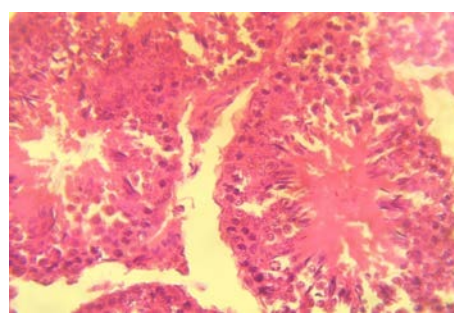
X400

Section shows normal Leydig cells, normal Sertoli cells and normal spermatogenesis

Figure 3: Testicular histology slide of Rabbits fed Diet 3 (150mg/kg) feed of BPE

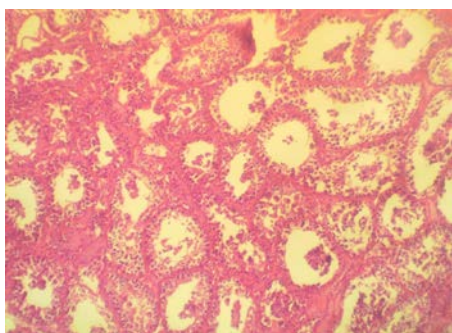
X100

Section shows normal seminiferous tubules



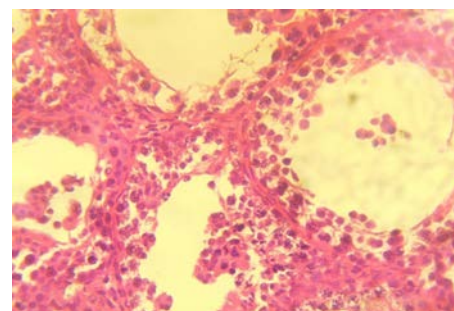
X400

Section shows normal Leydig cells, normal Sertoli cells and normal spermatogenesis

Figure 4: Testicular histology slide of Rabbits fed Diet 4 (200mg/kg) feed of BPE

X100

Section shows normal seminiferous tubules



X400

Section shows normal Leydig cells, normal Sertoli cells and normal spermatogenesis

Figure 5: Testicular histology slide of Rabbits fed Diet 5 (250mg/kg) feed of BPE

DISCUSSION

Buck organ weight is known to have a positive correlation with its body weight, i.e. the bigger the body weight of the animal, the larger the organ size. NseAbasi, 2015, observed a positive correlation between body weight and testicular size. Tia, *et al*, 2018, also reported that decrease in testis weight is associated with body weight loss. The present study showed that only the body weight of Rabbits fed Diet 4 compared favourably with the body weight of Rabbits in the control group (Diet 1). This suggests that inclusion levels of BPE did not significantly increased body weight

gain of animals. This report corresponds with the findings of Chen *et al.*, 2018 who reported that Piperine treatment of rat at 5mg/kg and 10mg/kg body weight did not affect body weight of the animals in the course of treatment. This agreement may be as a result of the high concentration of piperine in BPE fed to the animals.

The weight of the reproductive organs always gives a good measure of the degree of spermatogenesis. Akpa *et al.*, (2012) stated that a positive relationship existed between sperm production and testicular dimension, giving an indication that improvement in one would lead to improvement in the other. Adewumi *et al.*, (2009) reported that increase in the size of the testes might result in improved fertility. Testes size has been regarded as the trait of choice in male animal and may be useful as a selection criterion for improvement of the reproductive ability of bucks (Akpa *et al.*, 2012). Testicular size is the main factor determining the number of sperm and volume of ejaculate (Ashwood, 2009). Results from the present study showed that BPE had minimal effect on testes weight of Rabbits fed Diets 2,3 and 5 compared to the control. It was also observed that the testicular weight of Rabbits fed Diet 4 was significantly higher compared to those on Diet 1. This suggests that BPE enhanced testis growth up to an optimum inclusion level (200mg/kg feed) and beyond this level it exhibited a negative impact on testis weight. This results agreed with the reports of Erdost *et al.*, 2009 who reported that Piper longum (black pepper) treated mice showed a significant increase in the weight of testes. Also Studies carried out by Mohammadi *et al.*, 2013, revealed that mice treated with piper longum exhibited significant increase in the testis weight. The negative effect observed above optimum inclusion levels of 200mg/kg feed could be as a result of the high toxicity level of BPE at high inclusion rate due to the presence of phytochemical bioactive substances such as alkaloids and tannins. Moreover, it is suggested that significant decrease or increase in the absolute or relative weight of an organ after administration of a substance indicates the toxicity of that particular substance. However, another set of researchers Chinta *et al.*, 2017 reported that rats treated with Piperine at dose of 10mg/kg body weight for 60days showed a significant reduction in testicular weight. This discrepancy may suggest differences in the level of dosage administered or mode and duration of administering BPE. It would be agreed with Oyeyemi *et al.*, 2012, that the higher the testicular length (without abnormality), the higher the capacity of cells during spermatogenesis. Also, increase in testicular parameters is followed by a corresponding increase in the sperm production of the related animal. The effect of BPE on the testes length was observed to be significantly higher for Rabbits fed Diet 4 compared to Rabbits fed Diet 1. This shows that BPE treatment at optimum levels positively affected the growth of seminiferous tubules, indicating better sperm production potentials. This results agrees with the reports of Tia *et al.*, 2018 who revealed that BPE at dose 6.66 and 13.32mg/kg Body weight of Male Wister rats caused significant decrease in seminiferous tubule diameter. Similarly, Malini *et*

al.,1999 reported that the administration of Piperine 10mg/kg Body weight of Rats caused a decrease in the diameter of the seminiferous tubules. This is suggestive of the toxic effect caused by high dosage of BPE due to Piperine concentrations. The effect of BPE on testicular circumference was not pronounced. It was observed that the testis circumference of Rabbits fed Diets 2,3,4 and 5 were not significantly different from testis circumference of Rabbits fed Diet 1. This suggest that BPE inclusion at these levels had no negative effect on the testicular circumference of Rabbits. Testis of Rabbits fed Diet 5 showed a significant lower volume compared to the control. The lower testis volume observed may be due to the negative effect of high dose of BPE on testis weight which is an important factor in determining the testis volume. Testis density and epididymis weight of Rabbit bucks fed Diets containing BPE at varying inclusion levels was not significantly different with the control. Hence BPE did not influence these parameters. Treatment of BPE showed a progressive positive effect on the epididymis length of Rabbit bucks fed Diets 2,3 and 4. Epididymis length of Rabbits fed Diet 4 performed better than the Rabbits fed Diet 1. Hence, it can be suggested that the effect of BPE on the epididymis length is also dose dependent as BPE inclusion at higher levels result in a decrease in epididymal length. Epididymis reserve parameter showed a marked increase for Rabbits fed Diet 4 compared to Rabbits fed Diet 1, while a decrease was observed for Rabbits fed Diets 5. Hence, this observation reveals the reason for the very low volume of semen ejaculated by Rabbit bucks fed Diet 5. Gonadosomatic index (GSI) represents percentage of body mass that corresponds to the testes. It is a tool for measuring the sexual maturity of animals in correlation to testes development (NseAbasi, 2015). Results from the present study revealed that Rabbit bucks fed Diet 5 exhibited very poor testis development compared to Rabbit bucks fed Diets 1,2,3 and 4. Hence, it is suggestive that BPE may be toxic to testis development at higher doses. Findings from this research agrees with the reports of Gopichand Chinta *et al.*, 2017 on the toxicity effect of piperine in rats.

CONCLUSION

The present study has revealed that BPE enhanced testicular morphometric parameters of Rabbit bucks at optimum inclusion level of 200mg/kg feed. It was also observed to be toxic to testis development at higher doses above the optimum based on the Gonadosomatic index results, and testicular histological slides showed that inclusion levels of ethanolic extract of Black pepper above optimum levels causes destruction of seminiferous tubules and negatively affect spermatogenesis. It can be concluded from this study that BPE in additive form has some androgenic properties on testis which might be the reason for the increase in testicular and epididymal allometric weight. Thus the indiscriminate use of BPE to enhance male fertility may not be justifiable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abdallah, E.M and Abdalla, W.E. (2018). Black pepper fruit (*Piper nigrum* L.) as antibacterial agent: A mini-review. *Journal of bacteriology and mycology*. 6(2):141-145.
- Adewumi, O. F., Ajalade, R. A., Bayode, D., Osinowo, O.A. and Onagbesan, O. M. (2009). Evaluation of the effect of trail mucus of *Archatina marginata* on the haematology and morphology of wistar rats. *Proc. 34th Annual Conf. Nig. Soc. Anim. Prod*; 206-209.
- Aduku, A.O and Olukosi, J.O. (1990). *Rabbit management in the Tropics: production, processing, utilization, marketing, Economics, Practical training, research and future prospects*. Living Books Services, G.U. Publications. Abuja.
- Akpa, G.N., Yahaya, H.K. and Martin, U.C. (2012). The Effects of Age, Breed, Sire, Body weight and Ejaculate characteristics of Rabbit Bucks. *International Journal of Animal and Veterinary Advances*. 4(3): 191-194.
- Ansa, A.A, Akpere. O and Imasuen, J. A (2017). Semen traits, testicular morphometry and histopathology of cadmium exposed rabbit bucks Administered methanolic extract of *Phoenix dactylifera* fruit. *Acta Scientiarum* 39(2):207-215.
- Ashwood, A. (2009). Bull reproductive soundness: Brahman news. 164. In: NseAbasi NsikakAbasi Etim (2015). Testicular and Epididymal Morphometric Characteristics: Viable Indicators of Reproductive Ability of Farm Animals. *American Journal of Biomedical Science and Engineering* 1(4): 39-44.
- Asuquo, O. R., Oko, O. O., Brownson, E.S., Umoetuk, G.B and Utin, I. S (2013). Effects of ethanolic leaf extract of *Spondias mombin* on the pituitary gonadal axis of female Wistar rats. *Asian Pacific J. Reprod*. 2(3):169-173
- Chanda, D., Shanker, K., Pal, A., Luqman, S., Bawankule, D.U., Mani, D and Darokar, M.P. (2009). Safety evaluation of Trikatu, a generic ayurvedic medicine in charles foster rats. *J. Toxicol. Sci.*, 34:99-108
- Chauhan NS, Rao ChV, Dixit VK (2007) Effect of *Curculigo orchioideis* rhizomes on sexual behavior of male rats. *Fitoterapia* 78:530-534.
- Chen, X., Fei, G., Jianpeng, L., Suhao, B., Yong, C., Dongli, L., Yong, L., Tongliang, H., Xiaofang, C., Qiqi, Z., Qingquan, L and Ren-shan, G. (2018). Diverged effects of piperine on testicular development: stimulating leydig cell development but inhibiting spermatogenesis in rats. *Journal frontiers in pharmacology*. 9:244.
- Chinta, G., Coumar, M.S., and Periyasamy, L. (2017). Reversible testicular toxicity of piperine on male albino rats. *Pharmacogn. Mag.* 13, S525-S532. Doi: 10.4103/pm.pm_405_16.

- Erdost H., Akkoc, CGO., Ozfiliz N., İlhan T., Tutuncu S (2009). The histological investigation on the testes of mice after an application of capsaicin. *Veterinarski arhiv*. 79(5):509-516.
- Ewuola, E. O, Osaiyuwa, O.H, Sokunbi, O.A, Hassan, M.E, Olakanmi, I.G and Halogen, A.S (2011). Semen qualities of normal and frizzle feather strains of domestic Cocks treated with or without tocopherol. *Journal of Trop. Anim. Prod. Invest.* 13(1):30-35.
- GenStat (2009). GenStat getting started version (12th ed.). Lawes Agricultural Trust, Rothamsted Experimental station UK.
- Hassan, H.E., Elamin, K.M., Yausif, L. A., Musa, A.M and Elkhaimy, M.A. (2012). Evaluation of body weight and some morphometry traits at various ages in local rabbits of Sudan. *Journal of Animal Science Advances* 2(4): 407-415.
- Ismail, M.M., Essam, T.M., Mohamed, A.F, and Mourad, F.E (2012). Screening for the antimicrobial activities of alcoholic and aqueous extracts of some common spices in Egypt. *Int. J. Microbiol. Res.*, 3:200-207.
- Malini, T., Manimara, R.R., Arunakaran, J., Aruldas, M.M and Govindarajulu, P (1999). Effect of Piperine on testis of Albino rats. *Journal of Ethnopharmacol.* 64:219-225.
- Mohammadi, F., Nikzad, H., Taherian, A., Mohabadi, J.A., Selehi, M. (2013). Effect of herbal medicine on male infertility. *Anatomical science*. 10(4):3-15.
- NseAbasi NsikakAbasi Etim (2015). Testicular and Epididymal Morphometric Characteristics: Viable Indicators of Reproductive Ability of Farm Animals. *American Journal of Biomedical Science and Engineering* 1(4): 39-44.
- Odey, M.O., Iwara, I.A., Udiba, U.U, Johnson, J.T., Inekwe, U.V., Asenye, M.E and Victor, O (2012). Preparation of plant extract from indigenous medicinal plants. *International journal of science and technology* .1(12): 688-692.
- Onifade, A.A., Abu, O.A., Obiyan, R.I and Abanikannda, O.T.F (1999). Rabbit production in Nigeria: some aspects of current status and promotional strategies. *World Rabbit Science*, 7(2):51-58.
- Oyeyemi, M. O., Fayomi, A. P., Adeniyi, D. A. and Ojo, K. M. (2012). Testicular and epididymal parameters of Sahel buck in the humid zone of Nigeria. *Int. J. Morphol.*, 30 (2): 489 – 492.
- Sutyarso, Muhartono, M. Kanedi (2016). The Effect of Fruit Extracts of Black Pepper on the Fertility Potential of Male Albino Mice. *American Journal of Medical and Biological Research* 4(1):1-4.