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Assessment of Reproductive Function of Rabbit Bucks Administered Black Pepper (*Piper nigrum*) Extract as Feed Additive

Ere, R. E.[1]; Ere-Richard, A. A.[2]; Otto O. B.[3]

[1]Lead Researcher

[1,2]Faculty of Agriculture, Department of Animal Science, University of Port Harcourt, Nigeria.
[3]Faculty of Agriculture, Department of Animal Science, Ignatious Ajuru University of Education, Ndele, River State.

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Abstract: This research examined the effect of ethanolic extract of black pepper (*Piper nigrum*) on the reproductive capabilities of rabbit bucks when used as a feed additive. The study utilized twenty (20) composite-breed of rabbit bucks aged 18-20 weeks in a completely randomized design, divided into five groups (A - E) of four bucks each, with four replicates per group. Group A (control) received standard pelleted feed, while group B – E were supplemented with 100, 150, 200 and 250 mg/kg black pepper extract respectively. Diets were provided for 90 days, within which semen quality (macro- and microscopic traits) and hormonal levels were assessed in the animals.

Results from this study revealed that black pepper extract (BPE) at 100 – 200 mg/kg positively influenced both macroscopic and microscopic semen quality characteristics as well as hormonal profiles of rabbit bucks. However, Inclusion levels at 250mg/kg BPE adversely impacted semen quality characteristics and reduced circulating levels of reproductive hormones (FSH, LH and testosterone) due to its toxicity.

The optimal inclusion rate for best reproductive outcomes was determined to be 200mg/kg and therefore considered the recommended dosage.

***Keywords**: Black Pepper, Semen Parameters, Hormonal Assay, Rabbit Bucks.*

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I. INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are micro-livestock which feed on both Concentrates and forages and are raised mainly for meat and fur production. Rabbit meat offers high-quality protein with lower cholesterol than beef (Hassan *et al.,* 2012). Advantages include strong adaptability, simple management, rapid growth, efficient feed conversion, brief gestation, and high fertility rates (Akpa *et al.,* 2012). Enhancing reproduction involves optimizing semen quality, which largely dictates buck fertility, alongside hormone profiles and testicular measurements (Sokunbi, 2008; Ewuola *et al*., 2011). Certain plant extracts boost libido, mating, and sperm production while regulating hormones like testosterone, LH, and FSH (Chauhan *et al.,* 2007; Asuquo *et al.,* 2013). Phytochemical screening of Black pepper's ethanolic extract revealed that black pepper extract (BPE) contains antioxidants such as phenolics and flavonoids, with piperine providing pungency (Gayatri and Sahu, 2011, Abdallah and Abdalla, 2018). This study addresses the need for substantial data on the effect of BPE supplementation in enhancing the reproductive performance in rabbit bucks.

II. MATERIALS AND METHODS

The trial occurred at the Rabbitary Unit of the Teaching and Research Farm of the University of Benin, Benin City. Nigeria.

- *Experimental Materials and Management*

Bucks were housed intensively, quarantined for four weeks, deparasitized with Ivomec® injection and given *ad libitum* access to water and formulated concentrate feed supplemented with forages.

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➢ *Experimental Design*

Twenty bucks between (18 – 20 weeks old) were assigned to five treatments: Treatment A (control) had no BPE and treatments (B - E) had in their diets 100, 150, 200 and 250 mg inclusion levels of BPE per kilogram of feed respectively. The experiment lasted for 90 days.

➢ *Procurement and preparation of Black pepper*

Black pepper seeds were purchased from new Benin market, Benin City, Nigeria and authenticated by a botanist in the Department of Forestry, University of Benin. The extraction process followed standard procedure of phytochemical extraction as described by (Sutyarso *et al*., 2016). Black pepper seeds were ground, extracted with 95% ethanol, filtered 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. Black pepper extract formed was stored in a sterile airtight container and stored in a refrigerator until its use.

➢ *Preparation of test diet*

The Black pepper extract was thoroughly incorporated into commercial poultry growers mash at varying inclusion levels within tolerable limits as described by Chanda *et al*. (2009).

The experimental diets consisted of:

- Diet 1 (control): 0 mg black pepper extract per 100kg of feed
- Diet 2: 100 mg of black pepper extract per kg feed
- Diet 3: 150 mg of black pepper extract per kg feed
- Diet 4: 200 mg of black pepper extract per kg feed
- Diet 5: 250 mg of black pepper extract per kg feed

The diets were pelleted to 4 mm size suitable for rabbit consumption. All treatment groups received their assigned diets for 90 days, during which semen samples were collected and assessed for macroscopic and microscopic sperm characteristics and blood samples were analyzed for hormonal profile.

III. DATA COLLECTION

➢ *Collection of Semen Samples*

On the 68th day after starting the experimental diets, the 20 bucks in this study began a 3-weeks (21-days) semen collection schedule. Semen was collected twice weekly (Mondays and Thursdays) from each buck between 8.00 am and 1.00 pm., using a mature doe as a teaser to ensure optimal semen quality.

Semen samples from bucks in each treatment group were obtained via a locally improvised artificial Vagina (IAV) as described by Ajuogu and Ajayi (2010). Before collection, the IAV was warmed in warm water to 36° C ± 2.0°C and drained while retaining this average temperature. The doe was introduced into the buck's cage just before mating to stimulate mounting. The buck was watched closely, prior to mounting, the IAV was positioned at the doe's vulva to guide the buck's penis for penetration and ejaculation. Post-ejaculation samples in the collection vessel were immediately transported to the University of Benin Teaching Hospital for evaluation.

➢ *Semen Parameters Evaluated*

Ejaculated semen samples were evaluated as promptly as possible for macroscopic and microscopic semen characteristics.

➢ *Macroscopic Evaluation of Semen*

Macroscopic semen analysis (semen colour, semen odour, ejaculate volume, semen P^H, liquefaction time, semen viscocity) was carried out in the Microbiology Laboratory, University of Benin teaching Hospital as described by (WHO Laboratory manual, 2010).

➢ *Semen Colour*

A visual test was carried out to determine the colour of the semen sample. This was done by unaided eye observation of semen sample. A milky colour indicates a normal sperm colour.

➢ *Semen Odour*

Ejaculates in the collection tube were placed close to the nostrils to observe for raw fresh milk smell indicating normal odour of undiluted semen.

➢ *Semen Volume*

Ejaculate volume was read-off directly in milliliters from the calibrated collection tube attached to the artificial vagina.

➢ *Liquefaction Time*

After semen collection the sample were kept erect at room temperature without agitation in the collection tube. The time of liquefaction (period when semi solid coagulated mass begins to liquefy) was observed using a stop watch.

➢ *Semen Viscosity*

This was determined immediately after liquefaction. It was estimated by gently aspirating the semen sample with a disposable plastic pipette. The semen was allowed to drop by gravity and the length of the thread was observed. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal the drop will form a thread more than 2cm.

➢ *Semen pH*

The pH was measured within one hour after semen liquefaction. Samples were thoroughly mixed, and drops were evenly spread on pH paper, then allowed to sit for about 30minutes until the colour in the impregnated zone stabilized. This colour was compared to the calibration strip. A semen pH of 7.0-10.0 indicates normal acidity.

➢ *Microscopic Evaluation of Semen*

Microscopic evaluation of Semen was carried out at the Medical Microbiology Laboratory of the University of Benin teaching Hospital for; sperm motility, concentration (sperm count), morphology, abnormality, viability (sperm live to dead ratio) and membrane integrity.

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➢ *Sperm Progressive Motility*

Sperm progressive motility was assessed following the method outlined by Igbokwe *et al*. (2018). To evaluate sperm motility, a 5 µL aliquot of raw semen was diluted in 100 µL of physiological saline (0.9% NaCl solution), using a micropipette, a drop of this diluted sample was placed on a warm microscopic slide covered with a 22 x 22 mm cover slip and examined under a Celestron PentaView microscope (LCD-44348, RoHS, China) at 400x magnification. Each sample was prepared on two slides, with five fields per slide observed for progressive motility. Progressive motility is defined as spermatozoa moving forward in a straight line. Three observers counted the progressively motile sperm across these fields, and the average from the five evaluations was recorded as the final score. Percentage motility was computed according to (Salmin 2000) using this formula; sperm motility (%) = [(total number of sperm observed – Total of non-progressively motile sperm)/ (Total number of sperm observed)] × 100 %.

➢ *Viability/ Live: Dead Ratio*

This was done to obtain the percentage of live and dead spermatozoa. Viability evaluation was carried out as described by Bearden *et al*. (2004). A thin smear of mixture of semen and eosin-nigrosin solution was drawn across the slide and dried. The samples were observed under Celestron Penta View LCD microscope (400 x magnification) for live and dead spermatozoa. Spermatozoa that appeared white were regarded as live spermatozoa while those that picked up the stain were regarded as dead spermatozoa.

The calculation of viable sperm was based on the percentage of live spermatozoa through differential staining with eosin-nigrosin stain (Bearden *et al*., 2004) using the formula: Sperm livability (%) = [(Total of live sperm) / (Total number of sperm observed)] x 100%

➢ *Spermatozoa Concentration*

Sperm cell concentration (cell/ml) x 10^6 was determined as described by Llyas (2007). 1ml diluted semen sample was prepared from the semen sample collected. This was done by adding 0.9ml of saline water to 0.1ml of semen sample. With the aid of a micropipette 50 microliters of diluted semen sample was drawn and was loaded unto the haemocytometer chamber and placed under the microscope to view at 400 magnifications. The number of sperm cells with head and tail within the gridline were counted, the sperm number count per chamber was recorded and repeated from aliquot sample to get the average. To get the number of cells counted, the value obtained for the average number of cells counted under the microscope was multiplied by the number of chambers of the haemocytometer, i.e. 5. And to get concentration of sperm cells, the dilution factor of hundred was multiplied by the value of sperm number, the constant value for concentration (0.05) and by 10^6.

➢ *Sperm Morphology*

Sperm morphology was evaluated by counting normal and abnormal sperm types in eosin-nigrosin stained samples. A semen droplet was placed on a slide, smeared with eosin-nigrosin stain, and allowed to air-dry. The prepared slide was then viewed under a bright-field microscope at 1000 x magnification using oil immersion. Around 200 sperm per replicate were examined to determine the percentage of normal versus abnormal forms.

➢ *Sperm Abnormality*

Abnormal sperm calculations were based on the percentage of morphologically abnormal spermatozoa through differential staining with eosin-nigrosin stain (Bearden *et al.*, 2004) using the formula: sperm abnormality (%) = [(morphologically abnormal sperm) / (Total number of sperm observed)] x 100%. A thin smear of mixture of semen and eosin-nigrosin solution was drawn across slides for the respective treatments and dried. The percentage of morphologically abnormal spermatozoa with defects in the head, midpiece and tail were observed under Celestron Penta View LCD microscope (400 x magnification) and recorded.

➢ *Plasma Membrane Integrity of Sperm*

Sperm plasma membrane integrity was evaluated via the hypo-osmotic swelling (HOS) assay, following the protocol of Nie and Wenzel (2001). This involved mixing 10 µl of the spermatozoa sample with 100 µl of hypo-osmotic solution (containing 7.35g sodium citrate [0.0285M] and 13.5g fructose [0.075M]) and incubating at 37^0Cfor 30 minutes. Next, 0.1ml of the mixture was placed on a pre-warmed slide, covered with a coverslip, and examined under a Celestron Penta View LCD-44348 digital microscope at 400 x magnification. A total of 200 spermatozoa were assessed; those showing positive HOS response (with curled tails, indicating intact membranes) were recorded as a percentage, while those without swelling (straight tails) were deemed to have damaged membrane integrity.

➢ *Blood Sampling for Hormonal Assay*

At the conclusion of the feeding trial, 3 mL of blood samples were drawn from three bucks per group via marginal ear venipuncture with a 25-gauge hypodermic needle and placed into sample bottles for hormonal profiling.

The samples were immediately chilled in iced water, transported to the laboratory, and refrigerated at 4^0C for 1hour. Serum was then separated by centrifugation at 500 rpm for 10 minutes and stored at -20^0C. Levels of testosterone, luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) in the serum were quantified at the chemical pathology laboratory, University of Benin Teaching Hospital, using ELISA method, following the protocols in the Elabscience ELISA manual as describe by Micallef *et al*. (1995).

➢ *Statistical Analysis*

Data collected on macroscopic and microscopic semen parameters were subjected to one-way statistical Analysis of Variance (ANOVA) using Genstat 2009 (12^{th} Edition) statistical package. Significant means between treatment groups was separated by Duncan multiple range test (DMRT).

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IV. RESULTS

➢ *Effect of Black Pepper Extract on Macroscopic Semen Characteristics of Rabbit Bucks*

Table 1. shows the effect of black pepper extract on macroscopic semen characteristics of Rabbit Bucks. Highest (P<0.005) liquefaction time was recorded in rabbits fed 250 mg/kg of BPE compared to the control and other inclusion levels of BPE while rabbits in the control gave the lowest (P<0.005) liquefaction time compared to rabbits in other inclusion levels of BPE. Semen pH of rabbits in the control was neutral (P<0.005) while rabbits in other inclusions levels of BPE had slightly acid pH. Results showed that there was no significant difference between the semen volume of rabbits fed the control diet and semen volume of those fed 100 mg, 200 mg and 200 mg/kg of BPE respectively. However, the lowest (P<0.005) semen volume was observed in rabbits fed 250 mg/kg of BPE compared to rabbits fed the control diet and other levels if BPE inclusion. Moderate (P<0.005) semen viscosity was recorded in rabbits fed 200 mg/kg of BPE compared to rabbits in the control and other inclusions levels which recorded low semen viscosity. There was no significant difference in semen colour and odour of rabbits in the control and other groups.

Table 1 Effect of Black Pepper Extract on Macroscopic Semen Characteristics of Rabbit Bucks.

Parameters			**Level of Inclusion**		
	Control	**100 mg/kg**	**150 mg/kg**	**200 mg/kg**	**250 mg/kg**
Liquefaction (min)	17.50 ± 1.11^{bc}	21.00 ± 1.11^{ab}	21.00 ± 1.11^{ab}	21.00 ± 1.11^{ab}	24.17 ± 1.11^{a}
Semen P^{H}	7.300 ± 0.06^{a}	6.433 ± 0.06^{b}	6.367 ± 0.06^{b}	6.183 ± 0.06^{bcd}	6.000 ± 0.06^{bcde}
Semen volume (ml)	0.300 ± 0.05^{a}	0.400 ± 0.05^{a}	0.400 ± 0.05^{a}	0.400 ± 0.05^{a}	0.200 ± 0.05^{b}
Viscosity	Low	Low	Low	Moderate	Low
Colour	Milky white	Milky white	Milky white	Milky white	Milky white
Odour	Fresh milk	Fresh milk	Fresh milk	Fresh milk	Fresh milk

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

➢ *Effect of Black Pepper Extract on Microscopic Semen Characteristics of Rabbit Bucks*

The effect of black pepper extract on microscopic semen characteristics of Rabbit Bucks in presented in Table 2. Percentage sperm motility was significantly higher in rabbits fed 200 mg/kg of BPE compared to the control and rabbits fed other inclusion levels of BPE. Lowest (P<0.005) percentage sperm motility was observed in rabbits fed 250mg/kg of BPE. Rabbits fed 200 mg/kg of BPE had the highest (P<0.005) sperm concentration compared to rabbits in the control and rabbits fed other inclusion levels of BPE. Lowest (P<0.005) sperm concentration was recorded in rabbits fed 250 mg/kg of BPE. Highest percentage sperm membrane integrity was observed in rabbits fed 200 mg/kg of BPE compared to the control and other levels of BPE inclusion levels while rabbits fed 250 mg/kg of BPE had the lowest (P<0.005) percentage sperm membrane integrity. There was however no significant difference in the percentage membrane integrity of rabbits fed the control diet compared to those fed 100 mg/kg and 250 mg/kg of BPE. Rabbits fed 200 mg/kg of BPE had the highest (P<0.05) percentage sperm livability while the lowest (P<0.05) percentage livability was recorded in rabbits that were fed 250 mg/kg of BPE compared to rabbits in the control and rabbits in other inclusion levels of BPE. Highest (P<0.05) percentage sperm abnormality was seen in rabbits fed 250 mg/kg of BPE compared to rabbits in the control and rabbits in other inclusion levels of BPE whose percentage sperm abnormality were not significantly different from each other.

Table 2 Additive Effect of Black Pepper Extract on Microscopic Semen Characteristics of Rabbit Bucks.

Parameters	**Level of inclusion**				
	Control	**100 mg/kg**	**150 mg/kg**	**200 mg/kg**	**250 mg/kg**
Motility (%)	50 ± 1.44^{bc}	50 ± 1.44^{bc}	55 ± 1.44^{b}	60 ± 1.44^{a}	40 ± 1.44^{bcd}
Sperm concentration (cell/ml) x 10^{6}	107 ± 18.95^{bcde}	161 ± 18.95^{bcd}	234 ± 18.95^{b}	320 ± 18.95^{a}	87 ± 18.95^{bcde}
Membrane integrity (%)	52 ± 2.38^{bc}	50 ± 2.38^{bc}	58 ± 2.38^{ab}	60 ± 2.38^{a}	45 ± 2.38^{bc}
Livability (%)	75 ± 1.94^{ab}	70 ± 1.94^{bc}	75 ± 1.94^{abc}	80 ± 1.94^{a}	50 ± 1.94^{bcde}
Sperm abnormality (%)	20 ± 2.29^{b}	20 ± 2.29^{b}	20 ± 2.29^{b}	15 ± 2.29^{b}	34.8 ± 2.29^{a}

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

➢ *Effect of Black Pepper Extract on Three Specific Reproductive Hormones of Rabbit Bucks*

Table 3 shows the Effect of varying inclusion levels of black pepper extract on three specific reproductive hormones (Testosterone, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH)) of Newzeland White Rabbit bucks. Rabbit bucks administered 200 mg/kg of BPE had the highest (P<0.005) concentration of testosterone in their blood compared to rabbits in the control and all other inclusion levels of BPE. However, there was no significant difference in circulating testosterone levels of rabbits fed the control diet and rabbits fed 100 mg/kg, 150 mg/kg and 250 mg/kg inclusion levels of BPE respectively. Highest (P<0.05) circulating FSH was recorded in rabbits fed 200 mg/kg of BPE compared to rabbits in the control and other inclusion levels of BPE while lowest (P<0.05) concentration of circulating FSH was observed in rabbits fed 250 mg/kg of BPE. Circulating levels of LH was significantly higher in rabbits fed 200 mg/kg of BPE compared to rabbits in the control and other inclusion levels of BPE, while the lowest

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(P<0.05) levels of circulating LH was observed in rabbits fed the control diet and rabbits fed 150 mg/kg of BPE.

Table 3 Additive Effect of Black Pepper Extract on Three Specific Reproductive Hormones of Rabbit Bucks

Parameters	Level of inclusion				
	Control	100 mg/kg	150 mg/kg	200 mg/kg	250 mg/kg
Testosterone (ng/ml)	0.021 ± 0.007^{b}	0.020 ± 0.007^{b}	0.025 ± 0.007^{b}	0.078 ± 0.007^{a}	0.028 ± 0.007^{b}
FSH (u/l)	1.300 ± 0.064^{ab}	1.100 ± 0.064^{b}	1.200 ± 0.064^{b}	1.500 ± 0.064^{a}	0.800 ± 0.064^{c}
LH (u/l)	0.580 ± 0.031^{bc}	0.510 ± 0.031^{c}	0.560 ± 0.031^{bc}	0.750 ± 0.031^{a}	0.660 ± 0.031^{ab}

^{abc} Means in same row with different superscripts differ significantly (P<0.05).
FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone

V. DISCUSSION

Liquefaction period connotes the time when semen semi solid coagulated mass begins to liquefy. The longer semen liquefaction time observed in rabbits fed varying inclusion levels of BPE compared to the control may be attributed to the effect of the antioxidants present in BPE. Antioxidants inhibits the effect of free radicals such as hydroxyls and superoxides in semen, thus preventing lipid peroxidation and oxidative stress which affects healthy cells and thus causing them to liquefy quickly (Meghwal and Goswami 2012).

Semen pH shows the level of acidity or alkalinity of a semen sample. The slightly acidic semen pH observed in rabbits fed varying levels of BPE may be because the pH of BPE is slightly acidic. Black pepper extract at high concentration increases the acidity level of the medium giving its antimicrobial property. This corroborates the findings of Himabindu and Arukumar (2017), who reported that black pepper increased the acidity level of cottage cheese in order to prolong its shelf life, by inhibiting bacterial activity. Semen sample of rabbits in all the treatment groups however, fell within the optimal pH (7.0 and 8.5) for sperm viability as reported by Ajuogu *et al.* (2018).

Semen volume for the black pepper extract treated rabbit bucks were not significantly different from those of the control. However, an optimum level of 200 mg/kg feed of black pepper extract inclusion influenced semen volume positively whereas inclusion level above the optimum inclusion level revealed a negative effect on semen volume. Rabbit bucks fed 250 mg/kg feed inclusion of the extract produced a relatively low semen volume. The negative results observed above optimum level of inclusion of the extract, still points to the toxic effect of black pepper beyond certain inclusion level.

BPE had no effect on semen viscosity, colour and odour of rabbits as the aforementioned macroscopic parameters were observed to be normal in all the treatment inclusion levels. The semen appearance was milky, with a characteristic fresh egg odour and the viscosity was relatively moderate.

Sperm motility is perhaps the most striking attribute of spermatozoa as it is essential for transportation to the site of fertilization (Williams and Ford, 2001). High motility observed in bucks administered black pepper extract within the optimal inclusion levels could be attributed to the presence of vitamins C and E, and fatty acids in BPE as reported by Meghwal and Goswami (2012). Phenolic antioxidants such as fat-soluble vitamin E (tocopherol) and water-soluble vitamin C (ascorbic acid) are proven to have antioxidant properties that help to oppose the destructive effects of excess reactive oxygen species (Almbro *et al.*, 2011) by inhibiting free radical formation and/or interrupting propagation of autoxidation (Brewer, 2011). The reports of Erdost *et al.* (2009) revealed a significant elevation of percentage sperm motility in rats treated with black pepper extract which corroborates these present findings. It is however important to note that sperm progressive motility increased with increasing concentration of black pepper extract but was latter observed to decrease at highest inclusion level. This suggest that black pepper extract support sperm motility up to an optimum level (200 mg/kg) and beyond this level it exhibited a negative impact on percentage motility. This may be attributed to high concentration of tannins as a result of increased level of inclusion, which thereby hindered progressive movement of spermatozoa. Benhong *et al.* (2012) reported similar observation that some certain actions may occur between tannins and sperm based on the common tannin-protein reaction. He further stated that tannins in pomegranate exhibited inhibitory effect on sperm motility.

The assessment of the percentage of live and dead sperm cells is based on the assumption that dead sperm cells possess disintegrated plasma membrane, therefore allowing eosin penetration (Rodriguez-Martinez, 2003), thus the percentage of eosin positive cells stained with eosin-nigrosin stain is considered as percentage of dead cells while the cells that do not pick up stain are considered as live spermatozoa. Increased percentage livability observed in the semen collected from bucks treated with black pepper extract, indicates that the extract was able to sustain live spermatozoa. The percentage of live sperm cells in this study were still within the range (34.8 – 80.16) recommended for good reproductive potential and fertility in either normal mating or in artificial insemination as reported by Oyeyemi and Okediran (2007). Results of the present study agrees with the reports of Rajesh *et al.*,2014 which revealed similar trend of sperm livability results for goat epididymal sperm administered varying levels of Piperine.

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In this study, the percentage of abnormal sperm cells at suboptimal doses of black pepper extract remained below the 20% upper limit recommended as the minimum threshold for fertilizable semen quality in rabbit bucks (International Rabbit Reproduction Group, 2005). These results contradict Tia *et al*. (2018), who observed an increase in sperm abnormalities following administration of ethanol extract from black pepper fruit. The discrepancies likely stem from differences in dosages used.

Sperm concentration for semen samples collected from rabbit bucks fed black pepper extract below the optimum inclusion level (200 mg/kg BPE) revealed increasing number of sperm concentration. This result disagrees with findings of Tia *et al.* (2018), who reported decreased spermatozoa concentration by administration of ethanol extract of black pepper fruits. This differences are due to dose dependent nature of the active ingredient of black pepper.

The Hypo Osmotic swelling test (HOS) is the most widely used tool for the analysis of functional integrity of sperm membrane. The membrane integrity of sperm cells collected from rabbit bucks fed black pepper extract below the optimum inclusion level of 200 mg/kg feed were influenced positively while those above the optimum inclusion level of the extract was affected negatively. This results corroborates with the findings of Rajesh *et al*. (2014), who reported significant disruption in the functional integrity of goat sperm membrane by piperine at higher doses.

FSH and LH are vital hormone in male reproduction, FSH stimulates spermatogenesis in the sertoli cells while LH stimulates the production of testosterone in the leydig cells of the testes (Sreedhar *et al*., 2016). Circulating FSH and LH levels of rabbits significantly increased from the smallest level of BPE to the optimum level, after which a decline was observed. This report agrees with that of Chinta *et al.* (2017), who reported that piperine significantly increased the level of FSH and LH in rats after 60 days of treatment but disagrees with Chen *et al*. (2018), who reported that Piperine treatment of rats for thirty-days revealed reduced serum FSH and LH levels. This discrepancy may be attributed to the dose dependent effect of BPE on the said reproductive hormones of rabbits and or the duration of treatment and the method of administering the test ingredient.

Testosterone which is the hormone responsible for male sex drive is mainly regulated by FSH and LH (Vijayakumar and Nalini, 2006). It is therefore safe to say that increasing circulating levels of FSH and LH at optimum levels of BPE may be responsible for the corresponding increase in the circulating levels of testosterone at optimum inclusion levels of BPE. This is in line with Sutyarso *et al.* (2016), who reported that the treatment of male albino mice with fruit extract of black pepper resulted in increasing testosterone levels of the mice. The increased level of testosterone secretion in the blood may as well be due to high levels of Piperine, which is the major phenolic compound present in BPE. Hirata (2007) reported that piperine has an inhibitory effect on the enzyme, testosterone 5 α reductase which causes blood circulating levels of testosterone to remain high.

VI. CONCLUSION

➢ The Following Conclusion can be Drawn from This Study:

- Black pepper ethanol extract influenced semen parameters of rabbit bucks positively, however optimum sperm reproductive characteristics were obtained in rabbits fed 200mg/kg BPE.
- Black pepper extract increased circulating blood testosterone, FSH and LH levels at inclusion levels below and at the optimum.
- Levels of BPE above the optimum exhibited a toxic and deleterious effect on the sperm and hormonal parameters of rabbits.

RECOMMENDATIONS

➢ Based on the Results Obtained from This Study, The Following are Recommended:

- Improved reproductive function of Rabbit bucks can be achieved with the use of black pepper ethanol extract within the range of 100-200mg/kg feed.
- Black pepper fruits or extract should not be incorporated into animal feed indiscriminately because it can be toxic to the reproductive function of animals at higher inclusion levels.

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