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Semen Characteristics of Rabbit Bucks Fed Supplementary Walnut Powder *Tetracarpidium conophorum*

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ABSTRACT

Background

Emerging evidence from in vivo as well as in vitro studies indicates that botanicals play noteworthy roles in the treatment, prevention and management of diseases. Use of natural compounds in botanicals has been proposed as potential alternative to conventional therapeutic options. Therefore this study aimed to evaluate the effect of African walnut powder (*Tetracarpidium conophorum*) on semen characteristics of rabbit bucks.

Methods

The semen characteristic of bucks placed on varying levels (0%, 1.5%, 2.0% and 2.5%) of African walnut powder as supplement was examined. A total number of 16 matured bucks were allotted into four treatments. African walnut was obtained from Osun state, Nigeria and processed into powder. The experimental diets were administered after 2 weeks of acclimatization. Progressive motility, acrosome integrity and morphology, semen volume, live dead ratio, semen pH and reaction time were evaluated after semen collection through the use of artificial vagina. Hormones like testosterone, cortisol, thyroxin and triiodo – thyronine were also assayed. Data obtained were subjected to one-way analysis of variance.

Results

Significant differences were observed in semen volume across the treatment ($p<0.05$), semen volume ranged between 0.16ml (2.0%AWNP) and 0.38ml (2.0%AWNP) across the treatment. Result shows that progressive motility ranged from 73.30% (1.5% and 2.0%AWNP) and 83.30% (2.5%AWNP). Acrosome integrity of the animals placed on 2.0% and 2.5%AWNP had mean value of 90%, while 0.0% and 1.5% had 83.33% and 86.66% respectively. Morphology was observed to be statistically uniform across the treatments ($p>0.05$). Live dead ratio had higher mean value (90%) than control animals, (80.0%) though the difference is negligible statistically ($p>0.05$). Reaction time across the treatments showed no significant difference ($p>0.05$). Triiodo-thyronine showed higher mean value of 17.02pg/ml at 2.0%AWNP while animal on 2.5%AWNP had the least value of 12.98pg/ml. Thyroxine and cortisol had similar trend of increased value with increased value of AWNP. Testosterone showed significant difference across the treatment ($p<0.05$) with 2.0%AWNP having the highest value (42.5pg/ml) while the least value was obtained from 1.5%AWNP.

Conclusions

To improve bucks semen characteristic and fertility, 2.0% level of AWNP is recommended based on the result of progressive motility, morphology, acrosome integrity and testosterone which had an increase in value more than the control which was lower compare to other percentage levels of AWNP.

List of Abbreviations

AWNP: African Walnut Powder
PKC: Palm Kernel Cake
ANOVA: Analysis of Variance

INTRODUCTION

Rabbits are induced breeder but have an annual cycle of reproduction which is shown by change in conception rate, acceptance of male and ovulation rate and in male by significant seasonal change in testicular size (Habbeb *et al*., 1992). The most obvious limitation to rabbit production in hot climate area is their susceptibility to heat stress that evoke a series of drastic changes in their biological function which in turn end with impairment of production and reproduction. Such detrimental effect is punctuated during summer which are reflected in limiting the breeding season of rabbit to be normal from September to May each year, (Marai *et al*., 2002a, and Marai *et al*., 2002b). Abd El-Azim and EL- Kamash (2015) reported the spermatozoa behavior during transit along the female reproductive tract (Castellini, 2008). Variations in the seminal characteristic contribute to the large variability in semen quality trait. (Alvarino, *2000*), it is important to evaluate seminal inclusion to improve semen output. Oxidative stress results from increased production of free radicals and reactive oxygen species and a decrease in antioxidant defenses. (Trevasan, *et al*., 2001; William, *et al*., 2002). *Tetracarpidium conophorum* like many other plants in Africa and other part of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial value over the years. Phytochemical analysis of Africa walnut indicates that it contains bioactive compounds such as oxalates, phytates, tannins, saponins and alkaloids, which partly show the use of the seed, leaves and roots in herbal medicine *(*Ojobor *et al.,* 2015 *and* Ayolola *et al.,* 2011). The presence of tannin, support its anti-inflammatory property. As a rich source of alkaloids, couple with the presence of the essential vitamins and minerals*, T.conophorum* can be seen as a potential source of useful food and drugs. High content of ascorbic acid also indicates that the plant can also be used to prevent or at least minimize the formation of carcinogenic substance from dietary materials (Ojobor *et al.,* 2015). Studies have shown that the nuts are rich in protein, carbohydrate, fat and oil, vitamin and minerals *(*Ojobo *et al., 2015,* Onawumi *et al., 2013* and Ihemeje *et al., 2010)*.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the rabbit unit of Department of Animal Production, The Oke-Ogun Polytechnic Saki (TOPS). The pen was adequately and thoroughly cleaned by removing the cobweb, rabbit waste and the cage was thoroughly disinfected with the use of formalin 2 days before the arrival of the experimental animals.

Experimental Animal and Management

16 matured male rabbit (bucks) were obtained from a reputable farm in Saki, Oyo state, Nigeria and they were weighed individually and distributed randomly (Completely randomized design was used) into four experimental treatments with 4 bucks to each of the treatment. They were reared in cage system under similar environmental condition. The animals were acclimatized for two weeks; they were given prophylactic treatment which includes deworming and antibiotics administration. The bucks were weighed at the beginning of the study to obtain their initial body weight and they were individually weighed into the nearest gram at weekly intervals

Experimental diet

The experiment comprised of four treatments viz, 0% AWNP, 1.5% AWNP, 2.0% AWNP and 2.5% AWNP as supplement in the bucks' diet.

Table 1: Gross formulation for the experiment

Ingredient	0% AWNP	1.5% AWNP	2.0% AWNP	2.5% AWNP
Maize	20.00	20.00	20.00	20.00
Cornbran	50.50	50.50	50.50	50.50
Soya meal	20.00	20.00	20.00	20.00
PKC	05.00	05.00	05.00	05.00
Premix	00.25	00.25	00.25	00.25
Bone meal	03.00	03.00	03.00	03.00
Salt	00.25	00.25	00.25	00.25
Fish meal	01.00	01.00	01.00	01.00
Walnut powder	0.00	1.00	1.30	1.65
Total(Kg)	100	100	100	100
Calculated nutrient				
Crude protein	17.00	17.00	17.00	17.00
Metabolizable energy(ME)(kcal/kg)	2502.42	2505.42	2505.42	2505.42
Crude fiber (%)	11.36	11.36	11.36	11.36

Table: 2 Proximate composition of African walnut powder

Parameter	%
Moisture	31.40
Ash	6.01
Crude fiber	8.66
Crude protein	28.85
Carbohydrate	21.30
Energy	234.57kcal

(Nnerom *et al*., 2013)

Sources and Processing of African Walnut Powder

T. conophorum (African walnut) was obtained from Osun state Nigeria. The walnuts were sliced into smaller size and it was dried. The dried nut was then milled using pestle and mortal to obtain AWNP powder. The AWNP was kept in airtight container till the period of usage.

Materials Used in Semen Collection and Semen Processing

An artificial vagina was made for the collection of the semen from the buck without causing any injury to the animal. Ejaculate were submerged into 37^0C water bath before evaluation. The semen samples were examined for consistency, color, spermatozoa motility, volume, mass activity, morphology and concentration. The ejaculate volume was determined by collecting semen into a graduated tube. Color was evaluated by visual observation. The consistency was scored as 1= watery cloudy, 2=milky, 3=thin creamy, 4=creamy and 5=creamy-grany (Shamsudeen *et al*., 1994). To evaluate mass activity (wave motion) a drop of (2ul) of undiluted semen was placed on a pre-warmed slide at 37^0C, without a cover slip and examined under phase contrast microscope (100X magnification) (Nikon, Eclipes, E200, Japan). The mass activity was scored 0 = no motility, 1 = weak spermatozoa with weak movement (<20%), 2 = some motile spermatozoa (20 - 40%) without wave movement, 3 = slow wave movement (40 - 60%) with motile spermatozoa, 4 = rapid wave movement without whirl pool (60 - 80%) with motile spermatozoa and 5 = very rapid wave movement with clear whirlpools (>80%) with motile spermatozoa (Avudi *et al*., 2004). The spermatozoa motility was estimated subjectively by preparing a wet mount of diluted semen (normal saline) by placing a 5ul drop of fresh semen under coverslip with magnification of 200X using a phase contrast microscope (Nikon, Eclipse, E200, Japan). The spermatozoa quality assay (motility, viability, acrosome integrity, morphology, and pH) were performed. A drop of semen sample was placed on a pre-warmed glass slide and a cover slip. Progressive motility was assessed with phase contrast microscope at X 400 at 37^0C. The motility was examined at least with 200 spermatozoa selected randomly from a minimum of four microscopic fields. Eosin-nigrosin stain was used to examine the morphologically normal spermatozoa (Evans and Maxwell, 1997). Spermatozoa viability was evaluated using eosin-nigrosin stain (Evan and Maxwell, 1987). The acrosome integrity (normal apical ridges) was determine from semen smear with nigrosin-eosin examined under phase contrast microscope 1000x magnification under oil immersion (Yildiz *et al*., 2000). A total of 200 spermatozoa were counted in a least four microscopic field.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) of completely randomized design using SAS (1999) package and the means were separated using Duncan multiple range test of the same software at 5% level of significance.

RESULTS AND DISCUSSION

Semen volume of the experimental bucks ranged from 0.16 - 0.38ml and this is lower than the semen value reported by Iwuji and Herbert (2012) who reported the range of 0.60 - 0.78ml, the disparity might result from breed differences. This result shows that African walnut powder did not significantly ($p>0.05$) influence mass activity of the spermatozoa of rabbit buck and is in agreement with the report of Ajayi *et al*., (2009) who fed rabbits with blood sun-flower meal. However, the mass activity value that ranged from 76.66 – 85.0% is higher than the value (66 – 77%) obtained by Ajayi *et al*,. (2009). Similarly, the result of the study is much higher than those reported by Abu and Uchende (2010) who study anti spermatogenesis effect of aqueous and ethanolic extract of Hymenocardia acida stem bark on sperm activities of laboratory rodent and obtain value of 23 – 28%. The present investigation shows significant difference ($p<0.05$) in the progressive motility of bucks treated with different levels of AWNP which ranges from 73.33 – 83.33%. In farm animals, diverse studies reveal a good correlation between several sperm quality parameters (progressive motility and velocity) according to the straight path, damage acrosome and apoptotic cells and scores of penetration test (Richardson *et al*; 2011; Martins-Rodriguez *et al* 2012). Animals treated with 0.0% supplementary levels of walnut powder recorded the lowest mean value of percentage live spermatozoa (80%). Result of this experiment also demonstrated that 1.5%, 2.0%, 2.5% supplementary level of walnut powder to rabbit buck achieve the maximum percentage of live spermatozoa (90%). Table 3 showed significant difference ($p<0.05$) in the acrosome integrity of the bucks across the treatments with values ranging from 83.33 (0%AWNP) to 90% (2.0 and 2.5%AWNP). This is similar to the work of Roca *et al* (2005) who registered similar value for acrosome integrity (90.0%)

in hybrid male rabbit. However, Lavara *et al* (2005) observed lower acrosome integrity (80.2%). This means that AWNP has significant effect on the acrosome integrity of rabbit bucks on 2.0% and 2.5% which has the highest values. Reaction time (seconds) as an index for libido (sexual drive) showed no significant difference ($p>0.05$) among the experimental animals. Reaction time value ranged from 50.33(0.0%AWNP) to 172 (2.5%AWNP). This is not in agreement with the studies by Iwuji and Herbert (2012) who reported the range of 11.00 - 27.75s in animals treated with Garcina kola. No significant difference ($p>0.05$) was observed in the morphology of bucks fed supplementary walnut powder. Morphometric parameters of rabbit spermatozoa could be important in predicting buck fertility since they remain fairly constant with time. The pH obtained in table 3 tends to maintain an upward trend with the level of African walnut powder across the treatment. According to Meachem (2002) semen has a high buffering capacity much higher ability than that of most other fluid in the body but the buffering ability tends to decrease shortly after ejaculation as a result of loss of carbon dioxide by sperm cell. The comparable semen pH value in all treatment groups was an indication that African walnut powder supplementation in buck diet up to 2.5% did not affect the semen acid/ alkaline equilibrium. The recommended pH of rabbit semen is 5.40 – 9.60 (Peters and Sally 1976). pH result from this study (7.30 – 8.00) falls within the recommended range, this reveals that administration of AWNP does not alter the semen pH negatively. Triiodothyronine and thyroxine ranges from 12.98 – 17.02 and 23.19 – 25.47 respectively. Table 4 shows that there is significant difference ($p<0.05$) among rabbit buck fed supplementary walnut powder. Triiodothyronine is a thyroid hormone that plays vital roles in reproduction, body's metabolic rate, heart and digestive functions, muscle control, brain development and function, and the maintenance of bones (Maria *et al*., 2021).

Thyroxine is the main hormone secreted into the blood stream by thyroid gland. It is the inactive form and most of it is converted to an active form called triiodothyronine by organs such as the liver and kidney. The thyroid hormone plays vital roles in regulating and digestive function, muscle control, brain development and maintenance of bone. Thyroxine values obtained from this study were lower than the result of who reported Chieriato *et al*., (1997) 32.4- 38.0 ng/ml.

Table 4 shows significant different ($p<0.05$) in testosterone of rabbit buck fed different levels of supplementary walnut powder where the highest recorded values was observed in buck group placed on 2.0% ANWP (42.59). Sajjad *et al* (2007) reported that the level of blood serum testosterone is correlated with scrotal circumference and semen volume correlated with scrotal circumferences. Testosterone is thought to regulate sex drive (libido), bone mass, fat distribution, and muscle mass and strength, also the production of red blood cell and sperm cells. A small amount of circulatory testosterone is converted to estradiol, a form of estrogen. As anima aged, they often make less testosterone, and though they produce less estradiol as well. Thus changes often attributed to testosterone deficiency might be partly or entirely due to accompanying decline in estradiol. Testosterone is produced by the interstitial cell of the testis and necessary for the completion of spermatogenesis. The cortisol ranges from 65.33 (0.0% AWNP) – 67.50 (2.5% AWNP). These shows that AWNP has effect on cortisol hormone on rabbit buck. Cortisol is also called stress hormone because of its connection to the stress response; however, cortisol is much more than just a hormone release during stress. Cortisol is one of the steroid hormones and is made in the adrenal gland. Most cells within the body have cortisol receptors. Secretion is controlled by the hypothalamus, the pituitary gland and the adrenal gland a combination of glands often refers to as HPA axis.

Table 3: Semen characteristic of rabbit bucks fed AWNP

Parameters	0.0% AWNP	1.5% AWNP	2.0% AWNP	2.5% AWNP	SEM
Volume (ml)	0.33^{ab}	0.38^{a}	0.16^{b}	0.30^{ab}	0.03
Mass activity (%)	80.00	80.00	76.66	85.00	2.40
Progressive motility (%)	80.00^{ab}	73.33^{b}	73.33^{b}	83.33^{a}	1.44
Live:dead (%)	80.00^{ab}	90.00^{a}	90.00^{a}	90.00^{a}	0.00
Acrosome integrity (%)	83.33^{b}	86.66^{ab}	90.00^{a}	90.00^{a}	1.07
Reaction time (seconds)	50.33	95.00	101.33	172.00	40.14
Morphology (%)	81.66	80.00	83.33	80.00	3.29
pH	8.00^{a}	7.30^{c}	7.50^{b}	7.50^{b}	0.00
Semen color					

a,b,c,d mean having different superscript letters in the same row differ significantly ($p<0.05$)

Table 4: Hormonal profile of rabbit bucks fed AWNP

Parameters	0.0% AWNP	1.5% AWNP	2.0% AWNP	2.5% AWNP	SEM
Triiodothyronine (pg/ml)	15.77^{ab}	16.03^{ab}	17.02^{a}	12.98^{b}	0.62
Thyroxine (pg/ml)	23.60^{b}	23.55^{b}	23.19^{b}	25.47^{a}	0.31
Testosterone (pg/ml)	39.95^{c}	39.54^{c}	42.59^{a}	41.03^{b}	0.12
Cortisol (pg/ml)	65.33^{b}	66.42^{ab}	66.49^{ab}	67.50^{a}	0.29

a,b,c,d mean having different superscript letters in the same row differ significantly ($p<0.05$)

CONCLUSION

To improve bucks semen characteristic and fertility, 2.0% level of AWNP is recommended based on the result of progressive motility, morphology, acrosome integrity and testosterone which had an increase in value more than the control which was lower compare to other percentage levels of AWNP.

Hence AWNP could be supplemented into the diet of buck at 2% level to improve fertility and productivity of bucks.

Disclosure of Conflict of Interest:

The authors declare no conflicts of Interest regarding the publication of this paper.

Competing interests:

The authors declare no competing of Interest regarding the publication of this paper.

Author's Contribution:

Amao, Emmanuel Ayodele: Conceived and designed the experiment and manuscript preparation.
Amao, Oyetoun Dunmola: Made available animals and feed for the animals throughout the period of experiment.
Tijani, Haulat Temitope: Management of the animals and data collection.
Agbaye, Folorunso Peter: Statistical analysis and interpretation of the results.
Afolabi, Ojo Samuel: Laboratory analysis of blood samples.

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