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Semen and Extra Gonadal Characteristics of Cocks Fed Varying Levels of Nutmeg Seed Meal, Clove Leaf Meal and their Composite

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to assess the effect of nutmeg, clove and their composite as additives on the semen characteristics, gonadal and extra-gonadal sperm reserves of the cocks. 168 matured cocks (Isa Brown breed) were randomly assigned to 7 dietary treatments where the control diet without the test and 6 other treatment ingredients were tagged Diet I to VII. They were 250 mg nutmeg, 500mg nutmeg, 250mg clove, 500mg clove and their composites at 250 mg nutmeg-clove, 500mg nutmeg-clove, respectively. Each dietary treatment has 4 replicates of 6 birds per replicate. The feeding trial lasted for 10 weeks. At the end of the feeding trial, 3 cocks per replicate were humanely sacrificed and their reproductive tracts were dissected. All data obtained were subjected to statistical analysis of variance (ANOVA) procedure of SAS (2008). The significant treatment means were compared using New Duncan Multiple range test option of the same software. The results showed that all the semen characteristics were significantly and positively affected by inclusion of nutmeg and clove up to 0.50 g.kg^{-1} diet. The paired epididymis (5.30±0.21, 5.20±0.29, 5.37±0.11, 5.40±0.08, 5.60±0.18, 5.52±0.27, 5.79±0.27) and *vas deferens* weights (1.38±0.24, 1.12±0.08, 1.13±0.14, 1.07±0.09, 0.91±0.10, 1.17±0.03, 1.19±0.03) were significantly ($P < 0.05$) reduced by the inclusion levels of composite of nutmeg and clove up to 0.50 g.kg-1 diet, while vas deferens lengths (20.47±0.48, 18.98±1.15, 17.68±0.57, 18.52±0.49, 15.09±0.79, 13.64±0.21, 13.64±0.21) were significantly ($P < 0.05$) reduced with 0.25 and 0.50 g nutmeg and clove kg^{-1} diet.

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Nevertheless, the testicular parameters were significantly (P < 0.05) increased by the varying inclusion levels of nutmeg and clove when compared with the control. The paired testicular sperm reserves (TSR/testis (2.10±0.15, 2.01±0.12, 2.30±0.03, 2.24±0.16, 2.03±0.02, 1.80±0.03, 1.80±0.03) and TSR/g testis (1.72±0.18, 1.64±0.11, 1.64±0.05, 1.47±0.14, 1.50±0.11, 1.28±0.15, 1.28±0.15) were significantly (P < 0.05) influenced by the nutmeg and clove inclusion when compared with the control diet. However, the paired epididymis sperm reserves were significantly higher at the inclusion levels of 0.25 and 0.50 g nutmeg supplementation of cocks' diets with Nutmeg and clove up to 0.5g.kg^{-1} did not compromise the semen characteristics, gonadal and extra-gonadal sperm reserves in the treated birds. This study reveals that composite of nutmeg and clove up to 0.50g/kg in poultry cocks diets is a potential toxicant that has pathophysiological effects on the reproductive potential of cocks as it significantly reduced the daily sperm production and gonadal reserves of cocks. Composite of nutmeg and clove up to 0.50g/kg diet should not be added with cocks feed used for breeding purposes.

Keywords: Clove leaf meal; extra gonadal reserves; semen; testicular sperm reserve; nutmeg seed meal.

1. INTRODUCTION

In many parts of the world, the poultry industry occupies a leading role among agricultural industries, as it is the main supplier of animal protein for human population. However, this sector is still confronted with many problems like diseases outbreaks, harsh climatic conditions, and high cost of feeding, malnutrition, which are hindering its progress [1]. The cost of the feed covers about 60% of the total cost of production in most poultry production enterprises especially broiler [2]. These days, poultry production has developed and occupies a place of pride among the livestock enterprises due to its rapid monetary turnover [1]. Recently, the use of antibiotic growth promoters (AGPs) in poultry diets has raised concern about the development of antibiotic-resistant microbes and their effect on human health [3]. Consequently, there is a trend in many investigations to seek for possible replacements for AGPs in broiler chicken diets, such as probiotics, prebiotics, organic acids and enzymes [4], plant extracts, herbs and medicinal plants [5,6,7] and spices such as cloves and nutmeg [8].

During the last years, various dietary supplements were used to improve the quality of livestock feeds. Their main purpose is to enhance improved feed intake, thus higher growth performance of different species of poultry birds. Plant extracts are promising not only because they are inexpensive, but they are also natural products that are safe in the environment [9,10]. The practice of feeding livestock with sub-therapeutic levels of antibiotics has been in use for over fifty years [5]. Antibiotics affect microflora by influencing the metabolism of the microorganisms, and suppressing pathogen microbial growth in the gut [11]. Usage of antibiotics mostly has negative effects on animals and production such as residues in tissues, withdrawal period, and development of resistance in microorganisms. Therefore, the use of antibiotic growth promoters has been banned in many countries, especially in the European Union [11]

The sole purpose of going into poultry production is profitability. Considering the fact that more than 70% of the cost of production accounts for feeding cost. Any feed technology that may enhance optimum productivity on the part of the birds and consequent profitability on the part of the farmer without endangering the health safety of the consumers, but rather offer value addition in terms of value for money on the part of the consumer will be a novelty.

Therefore, fortifying poultry feeds with flavour enhancer such as these spices in order to increase palatability of such feeds for better acceptability and subsequent improved productivity, such as: increased body weight, improved hen day production, enhanced reproductive potentials, among others will be highly welcomed. Thus, this study is to evaluate the semen quality, testicular and Epididymal Morphometry of cocks fed diet supplemented with Nutmeg (*Myristica fragrans*), Cloves (*Syzygium aromaticum*) and their composite.

2. MATERIALS AND METHODS

2.1 Experimental Sites and Operations

The research work was carried out at the Poultry Unit of Teaching and Research Farm (Livestock section) of the Federal University of Technology,

Akure, and Ondo State, Nigeria. The farm is located in the rainforest vegetation belt of Nigeria. It is characterised by two rainfall peaks and high humidity during the raining season. It has an average rainfall of 1524mm annually and the rainy season last for 7 months (April – October). It is located on latitude $07^{o}25^{'}$N and longitude $05^{o}14^{'}$E with average temperature 27.5^{o}C and a mean annual relative humidity of over 75% (Adeleke and Olabode, 2017 [12]).

2.2 Number of Birds and Experimental Model

One hundred and sixty eight (168) matured cocks (Isa Brown breed) were randomly assigned to seven (7) dietary treatments where the control diet without the test ingredients is tagged Diet I and other six (6) other treatments were formulated with various proportions of Nutmeg (*Myristica fragrans*) and Cloves (*Syzygium aromaticum*) at 250 mg nutmeg, 500 mg nutmeg, 250 mg clove, 500 mg clove and their composites at 250 mg nutmeg-clove, 500 mg nutmeg-clove, respectively and tagged as Diets II, III, IV, V, VI and VII. Each dietary treatment has four (4) replicates of six (6) birds per replicate. The feeding trial lasted for ten (10) weeks. Water was provided throughout the experimental period. At the end of the feeding trial, three (3) cocks per replicate were humanely sacrificed and their reproductive tracts were dissected. The testes and epididymis were carefully sampled, weighed and processed. The gross composition of the basal diet is presented in Table 1.

2.3 Laboratory Analysis

The laboratory analysis was carried out at the Animal and Reproductive Physiology Laboratory of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria.

2.4 Semen Collection

Cocks were trained for semen collection using five minutes abdominal sexual massage (5ASM) technique [13]. Semen were collected from mature cocks once a week at 9:30am throughout the duration of the experiment, using 5ASM method.

Table 1. Basal composition of experimental diets

Ingredients (kg)	Diet I
Maize	330.00
Soybean meal	28.00
Groundnut cake	35.00
Corn Bran	110.00
Palm kernel cake	160.00
Wheat offal	300.00
Limestone	16.00
Bone meal	14.00
Lysine %	1.00
Methionine	1.00
Salt	2.50
Premix	2.50
Total	**1000.00**
Calculated Nutrients	
Crude Protein %	15.13
Metabolizable Energy (kcal/kg)	2512.06
Calcium %	1.43
Total Phosphorous	0.41
Crude Fibre %	5.23
Crude Fat %	6.88
Methionine %	0.40
Lysine %	1.07

2.5 Estimation of Semen Characteristics

Semen evaluation involved the estimation of both the microscopic and macroscopic indices. Progressive sperm motility percentage score were subjectively assessed in a drop of fresh semen on a warm glass slide covered with a warm cover slip and examined under a low magnification (x400) using a warm stage adjusted at 37^{o}C, according to the procedure arranged by [13]. Ejaculate volume were read off directly in millimetres in calibrated collection cup.

2.6 Percentage Sperm Abnormalities

Percentage sperm abnormalities were determined in the laboratory in the same smears prepared for live/dead ratio using a low magnification (x400). Percentage acrosomal abnormalities were estimated by a drop of fresh extended semen on a pre-warmed slide and dried in a current of warm air. The smears were fixed by immersion in buffered normal saline in a water bath at 37^{o}C for 15 minutes. The slides were washed in a running tap water for 15 - 20 minutes. The smears were dried and immersed in buffered Giemsa stain for 90 minutes after which it was rinsed in distilled water and dried. One hundred stained spermatozoa were examined for each sample under a low magnification (x400) to estimate the percentage of spermatozoa with acrosomal abnormalities.

2.7 Sperm Cell Concentration

Sperm cell concentration (x 10^{7}/mm^{3}) were determined in the laboratory using haemacytometer after a dilution of 1 in 200 in a solution of 45ml normal saline and 5ml formalin. Total sperm (x 10^{9} per ejaculate) were determined by multiplying the semen ejaculate volume by the sperm cell concentration.

2.8 Evaluation of Mass Activity Grade of Sperm Cells

Assessment of mass activity grade was carried out by the placing a drop of semen on a warmed slide under x100 magnification electronic microscope. The swirling movement of the sperm cells were observed and graded according to [13,14] as:

Rapid Swirling	Very Good	+++
Slower Swirling	Good	++
Generalised Oscillation	Fair	+
Sporadic Oscillation	Poor	-

2.9 Organ, Testicular and Epididymal Morphometric

Three (3) birds per treatment were randomly selected and fasted overnight before slaughter. After slaughtering they were eviscerated for gross examination *in situ* and reproductive systems were carefully dissected. Organs such as the testes and epididymis were carefully collected; adhering tissues were trimmed off and then weighed using a sensitive electronic weighing balance.

Testicular and epididymal morphometric parameters: testis length, testis width, testis volume and epididymal length were measured. Testis length, testis width and epididymal length were measured with the aid of a vernier calliper, while testis volumes were determined by water displacement method according to Archimedes principle [15]. Paired and mean testicular and epididymal parameters will be computed from data from left and right testes and epididymis.

2.10 Statistical Analysis

The experiment was a Completely Randomized Design (CRD). All data obtained were subjected to statistical analysis of variance (ANOVA) procedure of SAS [16]. The significant treatment means were compared using New Duncan Multiple range test option of the same software.

3. RESULTS

The semen characteristics of the cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite are as shown in Table 2. From the result of the experiment, it was observed that there were significant ($P<0.05$) differences in all the parameters considered. It was observed that the cocks on Diet VII (0.50 g nutmeg and clove diet) recorded the highest ejaculate volume (0.66ml) compared to the control with 0.52ml. The values for the sperm motility showed a significant ($P<0.05$) difference among themselves as the cocks fed diet V (0.50 clove) recorded the highest mean value of 97.70, while 94.50, 91.00, 89.30, 93.30, 93.30 and 77.70 are for diets I,II,III,IV,V,VI and VII respectively. The values for the semen concentration ranged from 6.32 (control) to 9.96 x 10^{8} (diet IV and VI). The cocks fed diet II (0.25 nutmeg) recorded the highest mean of 96.80,

Table 2. Semen characteristics of cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite

	Control	Nutmeg		Clove		Composite (Nutmeg/Clove)		
Parameters	**I (0 mg)**	**II (250 mg)**	**III (500 mg)**	**IV (250 mg)**	**V(500 mg)**	**VI (250 mg)**	**VII (500 mg)**	**P-Value**
Ejaculate Volume (ml)	0.52 ± 0.01^{c}	0.58 ± 0.18^{b}	0.53 ± 0.12^{c}	0.62 ± 0.09^{ab}	0.62 ± 0.031^{ab}	0.64 ± 0.09^{a}	0.66 ± 0.031^{a}	0.04
Sperm Motility (%)	94.30 ± 1.17^{b}	91.00 ± 4.09^{c}	89.30 ± 1.17^{c}	93.30 ± 4.41^{b}	97.70 ± 4.80^{a}	93.30 ± 4.41^{b}	87.70 ± 4.80^{d}	0.01
Sperm Concentration ($x10^{8}ml^{-1}$)	6.32 ± 0.72^{b}	6.53 ± 0.29^{b}	6.47 ± 0.27^{b}	9.96 ± 1.53^{a}	7.53 ± 0.56^{b}	9.96 ± 1.53^{a}	8.53 ± 0.56^{ab}	0.04
Sperm viability (%)	93.30 ± 0.71^{ab}	96.80 ± 0.86^{a}	91.10 ± 1.62^{ab}	83.10 ± 2.11^{b}	95.30 ± 7.15^{a}	93.10 ± 2.11^{ab}	59.30 ± 7.15^{c}	0.04
Total Sperm Cell/ejaculate (ml) ($x10^{8}$)	4.32±0.72	4.53±0.29	4.47±0.27	4.56±1.53	4.53±0.56	4.56±1.53	4.53±0.56	0.07
Total Live Cells/ml ($x10^{8}$)	7.24 ± 1.67^{b}	8.34 ± 1.02^{a}	8.00 ± 1.80^{a}	7.73 ± 1.84^{ab}	8.28 ± 0.09^{a}	7.83 ± 1.84^{ab}	6.28 ± 0.09^{c}	0.01
Total Mobile Cells/ml ($x10^{8}$)	6.89 ± 1.54^{b}	6.43 ± 1.02^{b}	6.88 ± 1.82^{b}	7.76 ± 1.05^{ab}	8.29 ± 0.32^{a}	8.76 ± 1.05^{a}	7.29 ± 0.43^{ab}	0.03
Mass Activity Grade	+++	+++	+++	+++	+++	+++	+++	

Values are means ± SEM; Means in a row with different superscripts are significantly (P<0.05) different.

Table 3. Extra Gonadal Lengths and weights of cocks fedvarying levels of nutmeg seed meal, clove leaf meal and their composite

	Control	Nutmeg		Clove		Composite (Nutmeg/Clove)		
Parameters	**I (0 mg)**	**II (250 mg)**	**III (500 mg)**	**IV (250 mg)**	**V(500 mg)**	**VI (250 mg)**	**VII (500 mg)**	**P-Value**
Extra Gonadal Length (cm)								
Epididymis								
Left	2.83±0.12	2.80±0.14	2.70±0.14	2.87±0.06	2.97±0.02	2.87±0.02	2.92±0.02	0.061
Right	2.47 ± 0.12^{c}	2.40 ± 0.18^{c}	2.67 ± 0.07^{b}	2.63 ± 0.27^{b}	2.63 ± 0.17^{b}	2.65 ± 0.06^{b}	2.87 ± 0.06^{a}	0.001^{*}
Paired	5.30±0.21	5.20±0.29	5.37±0.11	5.50±0.08	5.60±0.18	5.52±0.27	5.79±0.27	0.061
Vas deferens								
Left	10.96 ± 0.19^{a}	9.58 ± 0.19^{ab}	9.35 ± 0.35^{ab}	9.76 ± 0.71^{a}	8.74 ± 0.19^{b}	9.57 ± 0.03^{ab}	9.59 ± 0.03^{ab}	0.009
Right	9.94 ± 1.26^{a}	9.40 ± 0.46^{a}	8.33 ± 0.18^{c}	8.74 ± 0.36^{b}	8.47 ± 0.82^{c}	8.97 ± 0.21^{bc}	8.97 ± 0.21^{bc}	0.002
Paired	20.47 ± 0.48^{a}	18.98 ± 1.15^{ab}	17.68 ± 0.57^{b}	18.52 ± 0.49^{b}	17.21 ± 0.79^{c}	18.54 ± 0.21^{ab}	18.56 ± 0.21^{ab}	0.002
Extra Gonadal Weight (g)								
Epididymis								
Right	0.63 ± 0.10^{b}	0.60 ± 0.06^{b}	0.63 ± 0.06^{b}	0.67 ± 0.02^{ab}	0.70 ± 0.03^{a}	0.67 ± 0.02^{ab}	0.67 ± 0.02^{ab}	0.052
Left	0.78 ± 0.11^{a}	0.63 ± 0.12^{ab}	0.69 ± 0.06^{ab}	0.69 ± 0.09^{ab}	0.70 ± 0.06^{b}	0.60 ± 0.00^{c}	0.60 ± 0.00^{c}	0.001
Paired	1.41 ± 0.20^{a}	1.23 ± 0.07^{b}	1.32 ± 0.05^{ab}	1.36 ± 0.08^{ab}	1.40 ± 0.03^{a}	1.27 ± 0.02^{b}	1.27 ± 0.02^{b}	0.051

	Control	Nutmeg		Clove		Composite (Nutmeg/Clove)		
Parameters	**I (0 mg)**	**II (250 mg)**	**III (500 mg)**	**IV (250 mg)**	**V(500 mg)**	**VI (250 mg)**	**VII (500 mg)**	**P-Value**
Vas deferens								
Left	0.71±0.02^{a}	0.58±0.07^{ab}	0.57±0.08^{ab}	0.54±0.15^{b}	0.57±0.07^{ab}	0.60±0.02^{ab}	0.60±0.02^{ab}	0.054
Right	0.67±0.14^{a}	0.54±0.03^{b}	0.55±0.09^{b}	0.53±0.07^{b}	0.54±0.15^{b}	0.57±0.08^{ab}	0.59±0.08^{ab}	0.001
Paired	1.38±0.24^{a}	1.12±0.08^{ab}	1.12±0.14^{ab}	1.07±0.09^{b}	1.11±0.10^{b}	1.17±0.03^{ab}	1.19±0.03^{ab}	0.001

Values are means ± SEM; Means in a row with different superscripts are significantly (P<0.05) different.

Table 4. Gonadal weights, volume and density of cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite

	Control	Nutmeg		Clove		NutmegClove		
Parameters	**I (0 mg)**	**II (250 mg)**	**III (500 mg)**	**IV (250 mg)**	**V(500 mg)**	**VI (250 mg)**	**VII (500 mg)**	**P-Value**
Right Testicle (g)								
Whole Weight	15.16±2.40	18.28±1.742	17.96±0.58	17.59±0.86	18.02±1.60	17.12±0.39	18.12±0.39	0.14
Parenchymal Weight	14.10±2.23	17.00±1.62	16.70±0.54	17.50±0.78	17.90±1.49	17.20±0.37	17.20±0.37	0.14
Albuginea Weight	1.06±0.18	1.28±0.12	1.26±0.04	1.09±0.06	1.12±0.11	0.92±0.03	0.92±0.03	0.14
Volume (ml)	16.00±2.57	18.00±1.89	18.00±0.17	17.70±1.20	17.30±1.67	16.90±0.44	18.70±0.44	0.23
Density (g/ml)	0.97±0.03	1.03±0.04	0.96±0.03	0.96±0.06	0.97±0.04	0.99±0.01	0.99±0.01	0.06
Left Testicle (g)								
Whole Weight	15.89±2.03	15.40±0.29	15.64±0.63	15.42±0.69	15.15±1.36	15.20±2.17	15.09±2.17	0.68
Parenchymal Weight	15.00±1.91	14.60±0.27	14.70±0.59	14.50±0.65	14.30±1.28	14.37±2.04	14.20±2.04	0.61
Albuginea Weight	0.89±0.12	0.80±0.02	0.94±0.04	0.92±0.04	0.85±0.08	0.83±0.13	0.89±0.13	0.88
Volume (ml)	15.30±2.20	17.30±0.17	17.70±0.17	16.30±0.44	16.00±1.15	16.00±2.31	16.00±2.31	0.06
Density (g/ml)	0.99±0.03	0.97±0.01	0.98±0.03	0.96±0.07	0.99±0.01	0.96±0.21	0.96±0.21	0.07
Paired Testicle (g)								
Whole Weight	31.05±4.42	33.68±0.48	33.60±1.06	33.01±1.09	33.17±2.84	32.32±3.794	33.21±3.794	0.49
Parenchymal Weight	29.10±4.13	31.60±0.44	31.40±1.03	32.00±1.02	32.20±2.65	31.57±3.55	31.40±3.55	0.50
Albuginea Weight	1.96±0.29	2.08±0.03	2.20±0.03	2.01±0.07	1.97±0.19	1.75±0.24	1.81±0.24	0.46
Volume (ml)	31.30±4.76	35.30±0.50	35.70±0.33	34.00±1.50	33.30±2.73	32.90±4.19	34.70±4.19	0.16
Density (g/ml)	1.96±0.06	2.00±0.01	1.94±0.05	1.92±0.12	1.96±0.04	1.95±0.24	1.95±0.24	0.13

Table 5. Extra Gonadal sperm reserves of cocks fed varying levels of nutmeg seed meal, clove leaf meal and their Composite (x10^7)

	Control	Nutmeg		Clove		NutmegClove		
Parameters	**I (0mg)**	**II (250mg)**	**III (500mg)**	**IV (250mg)**	**V(500mg)**	**VI (250mg)**	**VII (500mg)**	**P-Value**
Right	1.72±0.09	1.73±0.26	1.85±0.43	1.76±0.19	1.85±0.23	1.89±0.23	1.87±0.23	0.485
Left	3.05±0.10^{ab}	3.06±0.46^{c}	3.32±0.16^{b}	3.46±0.27^{b}	3.14±0.25^{c}	3.64±0.25^{a}	3.64±0.25^{a}	0.001
Paired	4.77±0.42^{b}	4.79±0.46^{b}	5.17±0.17^{ab}	5.22±0.17^{a}	4.99±0.30^{ab}	5.53±0.34^{a}	5.51±0.34^{a}	0.003

Values are means ± SEM; Means in a row with different superscripts are significantly (P>0.05) different.

Table 6. Gonadal sperm reserves, daily sperm production of cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite

	Control	Nutmeg		Clove		NutmegClove		
Parameters	**I (0 mg)**	**II (250 mg)**	**III (500 mg)**	**IV (250 mg)**	**V(500 mg)**	**VI (250 mg)**	**VII (500 mg)**	**P-Value**
TSR/g testes (x10^8)								
Left	0.65±0.08^{b}	0.81±0.07^{ab}	1.02±0.08^{a}	0.91±0.10^{ab}	0.59±0.02^{b}	0.71±0.14^{ab}	0.71±0.14^{ab}	0.012
Right	1.07±0.10^{a}	0.83±0.07^{ab}	0.62±0.07^{b}	0.56±0.06^{c}	0.91±0.10^{ab}	0.57±0.08^{c}	0.57±0.08^{c}	0.001
Paired	1.72±0.18	1.64±0.11	1.64±0.05	1.47±0.14	1.50±0.11	1.28±0.15	1.28±0.15	0.207
TSR/testes (x10^9)								
Left	0.78±0.03^{c}	1.01±0.08^{b}	1.45±0.10^{a}	1.32±0.15^{ab}	0.78±0.06^{c}	0.82±0.15^{c}	0.82±0.15^{c}	0.001
Right	1.32±0.12^{a}	1.00±0.07^{ab}	0.85±0.07^{b}	0.92±0.09^{ab}	1.25±0.05^{ab}	0.98±0.14^{ab}	0.98±0.14^{ab}	0.005
Paired	2.10±0.15^{ab}	2.01±0.12^{ab}	2.30±0.03^{a}	2.24±0.16^{a}	2.03±0.02^{ab}	1.80±0.03^{b}	1.80±0.03^{b}	0.014
Sperm Production								
DSP (x10^9) *	1.09±0.08^{ab}	1.16±0.08^{a}	1.19±0.02^{a}	1.05±0.10^{b}	1.04±0.06^{b}	0.93±0.02^{c}	0.93±0.02^{c}	0.0130*

*Values are means ± SEM; Means in a row with different superscripts are significantly (P<0.05) different. Level of significance = ns (not significant) = P≥0.05; * = P< 0.05; TSR/testis (Testicular Sperm Reserve per testis); TSR/g testis (Testicular Sperm Reserve per gramtestis)*

while the others are 93.30, 91.10, 83.10, 95.30, 93.10 and 59.30x10^8 for diets I,II,III,IV,V,VI and VII respectively for the sperm viability and for the total live cells/ml the cocks fed diet B (0.25 clove) recorded the highest mean of 8.34, while others ranges between 7.24, 8.00, 7.73, 8.28, 7.83 and 6.28 respectively for diets I,II,III,IV,V,VI and VII. The cocks fed diet VI (0.25 nutmeg and clove) had the highest mean value of 8.78 for the total mobile cells/ml, while other treatments ranges between 6.89, 6.43, 6.88, 7.76, 8.29 and 7.29 x 10^8 respectively for diets I,II,III,IV,V,VI and VII. The mass activity grade of the cocks fed varying levels of nutmeg and clove were all in turbulent motion for all the cocks.

The results of the extra gonadal lengths and weights for left, right and paired epididymis as well as vas deferens of the cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite are shown in Table 3. It was discovered that all the epididymal and vas deferens lengths of the cocks across all the dietary treatments were significantly (P<0.05) influenced by the varied inclusion levels of nutmeg and clove. The cocks fed varied levels of nutmeg and clove recorded the highest significant (P<0.05) means for the left, right and paired epididymis lengths. The cocks on the control diets recorded the highest significant (P<0.05) means for the left, right and paired vas deferens lengths. Significant (P<0.05) reduction in the vas deferens lengths were observed while the inclusion of nutmeg and clove were elevated above 0.50 g/kg diet with progressive decreases with increasing inclusion of nutmeg and clove in the diets. Similarly, the cocks on the control diets recorded the highest significant (P<0.05) left, right and paired epididymal and vas deferens weights when compared with cocks on the other diets. The cocks on the diets 0.25 to 0.50 g nutmeg and clove /kg diet differ significantly (P<0.05) when the weights recorded by them for the right and left epididymis as well as left and right vas deferens were compared with those on the control diet. The paired epididymis and vas deferens weights of the cocks were significantly (P<0.05) reduced with the inclusion of nutmeg and clove in the diets of the cocks.

The testicular weights, volumes and densities of the cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite are shown in Table 4. It was observed that the cocks on diet II (0.25 nutmeg) had the highest non-significant (P>0.05) values for the right testicular whole weights (18.28g±1.74), albuginea weight (1.28g±0.12), and density (1.03g±0.04). The left testicular whole weights, parenchymal weight, albuginea weight and volume on diet C (0.50 g nutmeg) were the highest but statistically non-significantly (P>0.05) different when compared to the other diets. The values for testicular paired parameters were non-significant (P>0.05) from each other. It was observed that the cocks fed 0.25 and 0.50 composite nutmeg and clove had the highest non-significant (P>0.05) values for paired testicular whole weights, parenchymal weights and albuginea weight while the highest mean weight (1.96g/ml±0.04) for paired testicular density was observed in cocks fed diet V (0.50 g clove).

The epididymal sperm reserves of cock on each of the experimental diets are shown in Table 5. A common trend was noticed for each of the studied parameters as there were as there were elevations in sperm reserves as the inclusion rates of nutmeg and clove in the diets were increased from 0.00 to 0.50 g/kg diet with the cocks on diet VI and VII (0.25 g/kg and 0.50 g/kg composite nutmeg clove) recording the highest non-significant (P>0.05) means for left epididymis sperm reserve (3.64) and diet VI (0.25 g/kg composite nutmeg clove recorded the highest significant (P<0.05) means for right epididymis sperm reserves (1.89). Also, significant decreases were observed in the epididymal sperm reserve of diet II (0.25 g/kg nutmeg) which was 3.06. The control cocks recorded the least significant (P<0.05) values (3.05) for the studied parameters.

The gonadal sperm reserves (GSR) and daily sperm production (DSP) of cocks on diets I to VII are shown in Table 6. The value for the testicular sperm reserve per testis (TSR/testis) for the left testis were found to be significantly (P<0.05) highest on the diet containing 0.50 g/kg nutmeg diet. Testicular sperm reserve/g testis for the right and paired testis were also found to be highest on the control diet while diet III had the highest significant (P<0.05) value for that of the left testis. Daily sperm production was observed to progressively increase with increasing levels of nutmeg with the highest significant (P<0.05) value was recorded on diet III containing 0.50 g/kg of nutmeg and it was observed that there was significant reduction in the daily sperm production of the cocks with the inclusion of clove in the diets of the cocks with the lowest recorded in diet VI and VII containing composite of clove and nutmeg at varied levels.

4. DISCUSSION

Based on the results of this research, it could be noted that the semen characteristics of cocks fed varying levels of nutmeg seed meal, clove leaf meal and their composite did significantly influenced the semen characteristics of the cocks. Consumption of ground nutmeg seed in high doses, from above 10 g per kg body weight, was reported to result in testes structural alterations; hence, reduction in the number of spermatogenic cells, myoid cells and fibroblast in the male animals fed with nutmeg [17]. In this present study, inclusion of nutmeg and clove in the diets of cocks at 0.25 to 0.50 g/kg diets positively affected the ejaculate volume significantly and this is in agreement with the [18] who reported a positive result in the ejaculate volume of cocks which were fed with a spice ingredient at 0.25 to 0.50 g/kg. Furthermore, while clove and nutmeg levels of 0.25 and 0.50 g/kg did significantly affect semen characteristics such as sperm motility, total live cells, sperm concentration and viability, total mobile cells and mass activity grade, no adverse effects were documented for total live cells/ml and total mobile cells/ml in the present study an increase was observed in all the parameters as the inclusion of clove and nutmeg increases in the diets of the cocks from 0.25 to 0.50 g/kg. This contradicts the report of [19] who reported a decrease in ejaculate volume and spermatozoa motility in wistar rats treated with graded doses of *A. sativum* which might be as result of variety of garlic used, dose administered and also species differences. Observation on the effect of nutmeg and clove on sperm concentration has been varied in the present study. There were gradual increases in mean sperm concentration in the treatment groups (B to G), with a significant increase with the inclusion of the treatments in the diets. This corroborates the findings of [20], who evaluated the effect of 100 mg/kg/day of aqueous extract of *A. sativum* on epididymal spermatozoa in mice treated for 3 months and found out that the sperm count was significantly increased in the treatment groups compared to the control group. However, this contradicts the findings of [21], who found out that after the administration of crude garlic at inclusion rate of 5.0, 10, 15 and 30 to Wister rats for 30 days, observed a reduction in sperm concentration. The presence of significant differences in total live cells and total mobile cells among the groups did not agree with [19] who observed a decreased percentage live spermatozoa in Wister rats treated with doses of garlic. The mass activity grade of the cocks fed varying levels of nutmeg and clove were all in turbulent motion for all the cocks which contradicts the findings of [18] who reported a mass activity which ranged from very turbulent motion of the cocks on the control diet to slow motion for those on diet containing 1.00 g nutmeg/kg diet.

The quality and quantity of testicular sperm production as well as storage capacity played a key role in selection for breeding purposes [22]. Testicular and epididymal parameters such as weight and length are usually used in assessing their normality, thus, enhancing the detection of any deviation from normal that might result during experimental process [23]. These parameters are usually positively correlated with the spermatogenic activity of the testis [24]. The significant gradual decrease observed in the paired epididymal and vas deferens weights in the present study suggests that clove and nutmeg might have structural toxic effect on the epididymis and vas deferens as inclusion level of nutmeg and clove increases. This agreed with the findings of [25] which reported significant reductions in absolute and relative weight of epididymis and testis in clove-treated Wister rats at the rate of 4mg/kg body weight but disagreed in the case of testis weight as there were significant difference were observed in this experiment. They opined that the reduction in the epididymal weight and length resulted in reduced sperm count in the epididymis and they may be responsible for the acceleration of the sperm transit time through the epididymis. Acceleration in sperm transit time has been reported to have impairment tendency on sperm maturation and reduction in the number of sperm cells available for ejaculation and fertility [26].

The results of the finding revealed that inclusion of nutmeg and clove in the diets of cocks in comparison of the gross testicular weights, volume, density, parenchymal and albugenea weights of the right, left and paired testes up to 0.50 g/kg diet did not have significant effects on the studied parameters. The result of this finding corroborated with the report of [27] who had similar results for the testis parameters evaluated in clove treated and control rats.

Extra gonadal sperm reserves (ESR) which is the amount of sperm storage in the epididymis is been said to be correlated to sperm production by the testes [28]. In this study, the paired total epididymal sperm reserves of the cocks fed

varied levels of clove and nutmeg significantly reduced with increasing inclusion levels in the diets. This trend is suggestive of dietary influence of clove and nutmeg since all the cocks were fed isocaloric and isonotrogenous diets with only the inclusion levels of clove and nutmeg being the varying factor. The result of the study corroborates the findings of [29] that reported significant decrease in the mean caudal epididymal sperm reserves of the rats that were given medium to high clove and nutmeg relative to the control group and the low dose group.

Important indicator of accessing male fertility potential is the number of spermatids present in the testis, sperm production (SP) and the daily sperm production (DSP) [30]. [25] in their study, reported that the reduction in daily sperm production in nutmeg and clove-treated rats was caused by reduced testicular weight, seminiferous tubular diameter and testicular seminiferous and epithelium height. This present study showed that inclusion of nutmeg and clove differently up to 0.50 g/kg diet was tolerable for the daily sperm production in cocks. The least daily sperm productions were observed among the cocks on the diets containing composite of nutmeg and clove at both 0.25 and 0.50 g/kg diet.

5. CONCLUSION

Supplementation of cocks' diets with nutmeg and clove up to $0.5g.kg^{-1}$did not compromise the semen characteristics, gonadal and extra-gonadal sperm reserves in the treated birds. Enhancing feed palatability for optimum feed utilization could be achieved with nutmeg and clove in cocks' diet if the tolerable limit is not exceeded. This study revealed that the possible taste-enhancing effect of nutmeg and clove in cocks' diet as a feed flavour additive. This study has demonstrated that composite of nutmeg and clove up to 0.50 g/kg, in poultry cocks diets is a potential toxicant that has pathophysiological effects on the reproductive potential of cocks as it significantly reduced the daily sperm production and extra gonadal reserves of cocks. Therefore, it could be concluded that the diets for cocks to be used for breeding purpose could be fortified with nutmeg up to 0.50 g/kg without any deleterious effect on the reproductive performance of the cocks, while supplementation of the cocks' diet with clove has a positive influence on the semen characteristics, gonadal and extra-gonadal sperm reserves of the cocks.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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