SEMEN CHARACTERISTICS, HAEMATOLOGICAL AND SERUM- BIOCHEMICAL INDICES OF COCKS DRENCHED VARYING LEVELS OF CLOVE POWDER (*SYZYGIUM AROMATICUM*)

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ABSTRACT
The use of botanicals has been proposed as a potential alternative to conventional therapeutic options. This study aimed to evaluate the effect of clove powder (*Syzygium aromaticum*) on semen characteristics, haematological and serum-biochemical indices of cocks. Clove powder was drenched in 60 sexually matured (52 weeks old) and healthy, light ecotype Nigerian local cocks (weighing between 1.5 and 1.8 kg) cocks at 0.0, 0.05, 1.00 and 1.5 g. Semen volume, spermatozoa progressive motility, liveability, acrosome integrity, spermatozoa concentration and normal spermatozoa were evaluated for semen characteristics. Haematological parameters measured were: packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). In contrast, serum biochemical parameters evaluated were total protein, albumin, globulin, uric acid, creatinine, cholesterol and glucose. Data obtained were subjected to a one-way analysis of variance. Semen volume (0.44 – 0.47ml), sperm cell progressive motility (80.00 – 93.33%) and acrosome integrity (83.33 – 90.00%) showed significant differences ($p<0.05$), in favour of birds drenched 1.50g CP. The RBC values showed a significant ($p<0.05$) difference (3.64 – 3.76 x 10^6/mL), as the level of CP increases, the value of RBC decreases. There exists a significant difference ($p<0.05$) in Hb (3.64 – 12.86 g/dL) with the highest value in 0.00g CP, MCH (32.79 – 36.86 fl) and MCHC (31.62 – 32.85%) showed significant difference ($p<0.05$) in favour of 1.50g CP. Total protein showed significant difference ($p<0.05$) with values ranging between 4.46 and 5.40 g/dL. There exists a significant difference ($p<0.05$) in the mean value of albumin, with the highest value in 1.50g CP (5.40) in favour of 1.50 g CP. Drenching cocks with clove powder up to 1.5g was discovered posing no harmful effect on cocks.

Keywords: biochemical indices, clove, haematological indices, semen characteristics

1.0 INTRODUCTION

The increasing cost of antibiotics and their residual effects has necessitated the need to research natural plant-based products that could serve as a cheap and effective alternative to commercial (synthetic) antibiotics. Using plants with phytogenic properties as additives in livestock nutrition is becoming popular due to its resultant effect on animals such as improved productivity, reproduction, and quality of animal products through improved health (Olayemi *et al*., 2016).

Cloves are the sweet-smelling dried buds of a tree named botanically as *Eugenia caryophyllata* similarly called Syzgium. This plant is among the richest sources of phenolic compounds such as eugenol, acetate, and gallic acid. It has great potential for pharmaceuticals, cosmetics, food, agriculture, and many other applications. *Syzygium aromaticum* (clove) is a well-known medicinal herb having various proven therapeutic properties according to Shifali *et al*., (2021). Eugenol is the clove's principal constituent responsible for the clove's therapeutic property. It is traditional Ayurvedic's most commonly used spice and food preservative (Shifali *et al*., 2021). Clove has been reported to enhance semen qualities in cocks (Olarotimi and Adu, 2020)

Poultry production has a very crucial role in the economic development of many countries (Kafi *et al*., 2017). Agriculture accounts for about 35.2% of Nigeria's GDP; therefore, it plays a relevant role in reducing poverty and enhancing food security (Heise *et al*., 2015). With the gradual rise in human population, increasing demand for poultry meat as a source of protein is expected soon; as such, poultry health is a significant issue. Due to extremely crowded poultry pens, poor hygiene, and other management problems, antibiotics and other therapeutic chemical agents are used extensively to maintain health and improve poultry growth (Guil-Guerrero, 2017). These chemical agents help to overcome the issues of morbidity and mortality with poultry production; however, they can cause adverse public health issues by developing drug-resistant microflora (Mahesh & Prabhakar, 2018). In addition, the reduction of natural gut microorganisms predisposes the birds to opportunistic infections.

In 2006, the European Union banned antibiotics as feed additives due to their residual effects in animal tissues, subsequently leading to antimicrobial resistance in humans (Gobiraju *et al*., 2017). To avoid the extreme use of antibiotics and other medication, it is essential to find alternative feed supplements, to improve and decrease the cost of production through efficient feed utilisation. Alternatives include phytogenic feed additives, prebiotics, probiotics, enzymes, organic acids, and essential oils. Phytogenic feed additives are obtained from plants with antimicrobial properties (Gobiraju *et al*., 2017). This study is to evaluate the semen quality, blood parameters and serum biochemistry of cocks drenched with varying levels of clove powder.

2.0 MATERIALS AND METHODS

2.1 Procurement and Preparation of Test Ingredient

Clove (*Syzygium aromaticum*) was obtained from Sango market in Saki in Oyo State, Nigeria, in March 2023. Dried clove buds were cleaned and air-dried at room temperature for 24 hours before milling. Thereafter, they were milled using a blender to fine particle sizes and stored in air-tight bags until the period of usage.

2.2 Management of Experimental Animals

A total of 48 sexually matured and healthy light ecotype genotypes of Nigerian local cocks (normal feathered), a sample population of similar age, size, and body weight ranges (between 1.5 and 1.8 kg) used for the study were obtained from the Department of Animal Production Technology Teaching and Research Farm, The Oke - Ogun Polytechnic, Saki. Saki is located in Oyo State, Nigeria, on latitude 8^0 40’ 3.43’’N and longitude 3^0 23’ 38.15’’ E. The birds (52 weeks old) were randomly allotted into 4 treatment (T) groups: birds in T1 were drenched 0.00g CP and this served as a control group, birds in T2, T3, and T4 were drenched 0.50, 1.00 and 1.50g CP respectively. Each treatment had 12 cocks and was replicated 3 times with 4 birds per replicate. The birds were reared and managed intensively in a cage housing system and were observed and acclimatized for 2 weeks before the commencement of the study. Drenching of experimental material which lasted 8 weeks, commenced at the end of the acclimation period. Feed and water were given to the birds *ad libitum.*

2.3 Data Collection

Semen Collection and Evaluation

Semen collection and evaluation were carried out for 8 weeks. Semen was collected by abdominal massage techniques (Hafez, 1978). Semen collection was done twice a week on Mondays and Thursdays between 07:03 and 10:00 am. The birds responded to massage by partially averting their cloaca, and semen was collected from the ventral lip of the vents in calibrated tubes maintained at 35◦C using insulated jackets. Individual ejaculates were collected into a 4 mL graduated collection tube, and ejaculate volumes were read to the nearest 0.1 mL. Following semen collection, the semen was maintained in a 35°C water bath for sperm motility assessment. The physical semen characteristics were analysed as described previously by Peters et al., (2008). For sperm motility, a drop of semen was placed on a microscope slide using a rubber micropipette and then covered with a glass coverslip to spread the semen uniformly on the slide. The slides were placed under a microscope for observation (× 400 magnification). Several microscopic fields were examined for each sample. Motility was expressed as a percentage of the cells that are motile within the observed area. The sperm concentration was measured with an improved Neubauer haemocytometer using a direct cell count method. The haemocytometer consists of specially designed slides that contain 2 counting chambers. The counting chambers are 0.1 mm in depth and have an area of 1.0 mm^2. The squares are further subdivided into 25 smaller squares. One millilitre of the semen was diluted with 0.9 normal saline at the rate of 1:250. The cover slip was moistened with water and affixed to the haemocytometer to enable adhesion. A drop of the diluted semen was placed at both ends of the haemocytometer and allowed to settle. The loaded haemocytometer was then placed under the microscope for observation (×400 magnification). Cells which have their heads within the subdivided smaller squares at the 4 edges and the centre of the haemocytometer were counted and the average was taken for a bird. The sperm concentration was calculated using the formula: C = 50, 000 × N × D where C = concentration of semen per volume (ml), N = Number of spermatozoa counted, and D = dilution rate (Uzochukwu *et al*., 2019). Total spermatozoa were calculated as the concentration of sperm cells in the total volume of ejaculate collected from a cock. The sperm vitality was determined by placing a drop of semen on a microscope slide with a micropipette, and a drop of eosin-nigrosine stain was added, smeared, immediately air-dried, and viewed under a microscope (×400 magnification). The proportions of live (eosin-impermeable) and dead (eosin-permeable) spermatozoa in a sample were assessed based on 200 counted cells. The percentage of normal cells was determined as the percentage of cells with intact and normal morphological features.

Blood Collection

At 42 days of drenching the birds with the test ingredient, blood samples (2.0 ml) were collected with needle and syringe through the brachial wing vein of three cocks per replicate into EDTA bottles and plain bottles (2mls per cock) directly for the determination of haematological and serum biochemical indices using standard procedures, as described by Weiss and Wardrop, (2010). Haematological indices such as red blood cell count (RBC), packed cell volume (PCV), white blood cell count (WBC) and Haemoglobin concentration were evaluated. Haemoglobin concentration was determined photo-metrically using the cyano-haemoglobin method (Elarabany, 2018), and PCV using the Hacksley hematocrit centrifuge (UK) according to the procedure by (Morris *et al*., 2001), PCV results were determined using a micro hematocrit reader. The WBC count was determined using the Neubaer count chamber following the procedure described in previous studies (Fudge, 2000; Cray and Zaias, 2004). Serum total protein was determined using the Biuret method. Albumin was determined using the Bromoscresol Green (BCG) method as described by Peter *et al* (1982). The globulin concentration was obtained by subtracting albumin from total protein.

2.4 Statistical Analysis

At the end of the field trial, data were analysed following a one-way analysis of variance (ANOVA) in completely randomized design (CRD), using the SAS computer analytical package and means were separated with Duncan multiple range test of the same software (SAS, 2003).

3.0 RESULTS AND DISCUSSION

Table 1 shows results of semen characteristics of cock drenched with varying levels of clove powder (CP). There exists a significant difference, (P<0.05) across the treatment with semen volume ranging from 0.44 ml in the control to 0.47 ml on 1.5 g CP. The result shows a progressive increase in the semen volume with the increased level of clove powder. The result deviates from the report of Uzochukwu *et al*., (2019) who report a decrease in semen volume of cocks with increased levels of Ethiopian pepper fruit meal, though the value obtained (0.44 - 0.47ml) in this work is greater than the value (0.18 - 0.34ml) reported by Uzochukwu *et al*., (2019). Sperm cell progressive motility shows a significant difference (P<0.05) with 0.0g CP having the lowest value of 80.00%, followed by 1.0g CP (80%) while 0.5g CP and 1.5g CP had similar sperm progressive motility value of 90.00% and 93.33% respectively. This result corroborates with previous studies that showed an increase in progressive motility of cocks with increased levels of onions and garlic mixture (Victor *et al*., 2016). Sperm cell liveability showed no significant difference across the treatment (P>0.05) with values ranging from 76.66 – 91.66%. Sperm cell liveability showed similar values across the treatments. Acrosome integrity showed a significant difference (P<0.05) across the treatment, 0.0g CP had the least value (83.33%) while 0.5g, 1.0g and 1.5g CP had the same value (90.00%). The result showed that the acrosome in 0.5, 1.0 and 1.5 g CP were well protected compared with the control. There exists no significant difference in sperm cell concentration (P>0.05) with values ranging from 30.00 - 40.00 (x 10^9/ml) since the result is statistically similar clove powder had no detrimental effect on sperm cell concentration. This result agrees not, with the report of Victor *et al*., (2016) who affirmed a significant difference in sperm cell concentration of cocks fed a mixture of onion and garlic. Normal sperm cells showed the highest value in 1.0 g CP (90.00%) followed by 1.5 g CP (86.66%) and 83.33% in 0.0 and 0.5 g CP.

Table 1: Semen characteristics of Cocks drenched with varying levels of Clove Powder

Parameters	0.00g CP	0.50g CP	1.00g CP	1.50g CP	SEM
Semen volume (ml)	0.44^{b}	0.46^{a}	0.47^{a}	0.47^{a}	0.00
Sperm progressive motility (%)	80.00^{c}	90.00^{ab}	83.33^{bc}	93.33^{a}	2.35
Sperm livability (%)	90.00	76.66	90.00	91.66	6.71
Acrosome integrity (%)	83.33^{b}	90.00^{a}	90.00^{a}	90.00^{a}	1.66
Sperm concentration (x 10^8 ml^{-1})	40.00	30.33	33.33	36. 66	3.99
Normal sperm cell (%)	83.33	83.33	90.00	86.66	2.03

$^{a, b, c}$ means with different superscripts within a row are significantly different

Table 2 shows the haematological result of cocks drenched with varying levels of clove powder (CP). Packed cell volume (PCV) values showed no significant difference (P>0.05) across the treatment. Its value ranged from 39.00 - 41.00%, which falls within the normal value of 26.00 - 41.20% (Mitruka and Rawnsley, 1977). This could be interpreted that cloves could be drenched in cocks up to 1.5g without any harmful effect on their packed cell volume. This finding is in agreement with the report

of Ayodele et al., (2021), who reported the harmless effect of phytogenic substances (turmeric and clove) on broiler bird feed at the rate of 0.00, 1.00 and 2.00% for eight (8) week, they also stated that addition of clove did not affect PCV level of broiler birds measured at day-21 and day-42. Clove powder had a significant effect across the treatment on RBC (P>0.05) with a value ranging from 3.64 x 10^6/mL (1.00g CP) to 3.74 x 10^6/mL (0.00 CP), this value falls within the recommended range of (2.90 - 4.10 x 10^6/mL) according to Mitruka and Rawnsley, (1977). Haemoglobin values ranged between 3.64 and 12.86 g/dL. There was no significant difference (p> 0.05) in 0.0 CP and 0.5 g CP (12.86 and 12.33, respectively). The haemoglobin values for 1.00 and 1.50 g CP were lowered (3.64 and 3.65, respectively) than the established normal range of 7.50 – 13.10 g/dL (Mitruka and Rawnsley, 1977). This study reveals that the administration of higher levels of clove in cocks can affect the oxygen transport of the birds. WBC showed no significant (P>0.05) with a value ranging from 18.66 - 19.10 x 10^9/L which is within the range of 9.76 – 31.00 x 10^9/L (Mitruka & Rawnsley, 1977). This finding negates the report of Ayodele *et al*., (2021), who reported a WBC value of 6.10 to 6.65 x 10^9/L for broilers administered 0.00%, 1.00% and 2.00% CP. MCV showed no significant difference (P>0.05) with values ranging from 103.74 (0.00g CP) -112.23 (1.50g CP) which is within the normal range of (100.00 – 128.00 fl) reported by (Mitruka & Rawnsley, 1977). MCH showed a significant difference (P<0.05) with values ranging from 32.80 pg (0.50g CP) - 36.86 pg (1.50g CP) which is higher than the value reported (27.2 - 28.9 pg) by Mitruka and Rawnsley, (1977). There exists a significant difference (P<0.05) in the value of MCHC ranging from 32.16% (0.50 g CP) - 32.85 % (1.5 g CP).

Table 2: Haematological indices of Cocks drenched with varying levels of Clove Powder

Parameters	0.00g CP	0.50g CP	1.00g CP	1.50g CP	SEM
PCV (%)	40.00	39.00	39.00	41.00	1.22
RBC (X10^6/ml)	3.74^a	3.76^a	3.64^b	3.65^b	0.07
Hb (g/dl)	12.86^a	12.33^a	3.64^b	3.65^b	0.53
WBC (X10^9/ml)	19.10	19.00	18.66	19.01	2.85
MCV (fl)	106.95	103.74	107.13	112.23	2.88
MCH (pg)	34.40^{ab}	32.80^b	34.79^{ab}	36.86^a	0.92
MCHC (%)	32.16	32.00	32.24	32.37	0.05

$^{a, b, c}$ means with different superscript within a row are significantly different
Key: PVC – Packed Cell Volume, RBC – Red Blood Cell, WBC – White Blood Cell, Hb – Haemoglobin, MCV –Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration

The total protein on Table 3, showed a significant difference (p<0.05) ranging from 4.46 (1.00g CP) – 5.40 g/dL (1.5g CP). The value for total protein was within the normal range reported by (Mitruka & Rawnsley, 1977). Value for total protein was elevated in 1.50 g CP while value for other treatments has similar results. This could be accredited to high protein digestibility in birds placed on 1.5 g CP since high protein in serum suggested protein sufficiency (Ahamefule *et al*., 2006). There exists a significant difference (p<0.05) in the mean value of albumin, the value increased with the increase in clove powder level with 0.50g CP having the lowest value (0.60 g/dL) and 1.50 g CP having the highest value (5.40 g/dL). A high albumin concentration usually indicates dehydration, while a low concentration may be due to poor liver function due to malnourishment and infection (Esubonteng, 2011). The result of globulin showed a significant (p< 0.05) difference, its value ranged between 3.80 and 4.20g/dL. Globulin values in this study were above the reference range of 2.13 - 3.02 g/dL (Chernecky & Berger, 2008). These raised values could be credited to enhanced host's immune system and improved hepatic function since the liver is the location of protein synthesis. The uric acid observed in this study showed a significant difference (p< 0.05) with values ranging from 0.02 – 0.03 mg/dL. Birds on 0.00 CP, 0.50 g CP and 1.00 g CP had similar results (0.02 mg/dL) while the value for 1.5 g CP was elevated (0.03 mg/dL). Uric acid in the blood is manufactured as a consequence of protein metabolism. Amplified protein metabolism, stress and dehydration affect the concentration of uric acid in the blood (Chernecky and Berger, 2008). High serum uric acid concentrations might be due to ineffective protein deployment (Oduguwa & Ogunmodede, 1995). However, the values were below the reference range (2.47 - 8.08mg/dL) (Mitruka & Rawnsley, 1977). The phytogenic supplement (clove powder) had a significant (p<0.05) effect on the creatinine of the cocks having values ranging from 0.50 – 0.60 mg/dL, this is below 0.90 – 1.85 mg/dL reported by Mitruka and Rawnsley, (1977). Creatinine is used to determine the condition of the kidney. The kidney functions to eliminate waste products resulting from protein metabolism and muscle contraction (Ileke *et al*., 2014). Cholesterol showed a significant effect across the treatment with 1.50g CP having the highest value (135.33mg/dl), while birds on 0.0g CP had the least value (120.66 mg/dL) with birds on 0.50g and1.00g CP had similar value (106.33 and 109.66 mg/dL respectively). However total blood cholesterol was within the reported range (52.00 - 148.00 mg/dL) (Mitruka & Rawnsley, 1977). Glucose values across the treatments showed significant differences (p< 0.05) ranging from 205.33 mg/dL in 0.50g CP and 304.00 mg/dL in 1.5g/dL. The results obtained in this study were above the 152.00 – 182.00 reported by (Mitruka & Rawnsley, 1977).

Table 3: Serum biochemical indices of Cocks drenched with varying levels of Clove Powder (CP) in grams.

Parameters	0.00 CP	0.05 CP	1.00 CP	1.50 CP	SEM
Total protein (g/dl)	4.80^b	4.50^b	4.46^b	5.40^a	0.16
Albumin (g/dl)	0.86^c	1.60^c	4.46^b	5.40^a	0.13
Globulin (g/dl)	3.93^{ab}	3.90^{ab}	3.80^b	4.20^a	0.09
A:G	0.22^{ab}	0.15^{bc}	0.07^c	0.28^a	0.00
Glucose (mg/dl)					
Uric acid (mg/dl)	0.02^b	0.02^b	0.02^b	0.03^a	0.00
Creatinine (mg/dl)	0.53^b	0.50^b	0.50^b	0.60^a	0.00
Cholesterol (mg/ml)	120.66^b	127.33^{ab}	128.33^{ab}	133.66^a	3.04

$^{a, b, c}$ means with different superscript within a row are significantly different

4.0 CONCLUSION

It is concluded that clove could be administered to cocks up to 1.5g without any detrimental effect on their semen characteristics and health status. It is recommended that cocks can be drenched up to 1.5g of clove powder for best performance.

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