Synergistic effects of dietary supplements of brimstone *(Morinda lucida)*, and cloves (*Syzygium aromaticum*) on growth performance and blood profile of broiler chickens

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Abstract

The emergence of antibiotic-resistant pathogens due to use of antibiotics in poultry production has raised global concerns which necessitated the search for safe and effective alternatives to antibiotics. This experiment investigated the effects of dietary supplements of brimstone (Morinda lucida), clove (Syzygium aromaticum) and their combination on performance, haematology and serum parameters of broiler chickens. A total of one hundred and sixty, day old unsexed Ross 308 broiler chickens were used for the study. The broiler chickens were allotted to four dietary treatments formulated for starter (1-4 weeks) and finisher (5-8 weeks) phases. The treatments comprised T1 basal diet without additives, T2 (basal diet with brimstone meal (BM) at 1% (10g/kg)), T3 (basal diet with 1% clove meal (CM)) and T4 (basal diet with 0.5% (5g/kg) each of BM and CM; 1% BCM). Each treatment group has four replicates with ten birds in each replicate. At both phases, performance data was collected, while serum and haematological analysis were also carried out. At day 28, the live weight (LW) (701.20 g) and weight gain (WG) (643.87 g) were higher (P<0.05) for broiler chickens fed BCM supplemented diet and those fed control and BM diets. The feed conversion ratio (FCR) was better (P<0.05) for broiler chickens in the CM (0.98) and BCM (0.92) groups compared to other treatments. Broiler chickens fed BCM supplemented diet had the least (1.07 %) mortality (P<0.05) while feed intake (FI) was not significantly affected by phytogenic supplementation (P>0.05). Packed cell volume (PCV) (33.84 %), haemoglobin (Hb) (12.30 g/dL) and lymphocyte (65.00 %) were higher for broiler chickens in the BCM group compared to other treatments (P<0.05) while low white blood cell (WBC) (17.30 x10^9/L) was obtained in this group compared to other treatments. Total protein (TP) (48.90 g/L), globulin (GLB) (23.40 g/L) and albumin (ALB) (25.50 g/L) were higher while Aspartate aminotransferase (110.21 iU/L) was lower for broiler chickens fed BCM supplemented diet compared to other treatments (P<0.05). At day 56, LW (3642.81 g), WG (2941.61 g) and FCR (1.22) improved for broiler chickens in the BCM group compared to other treatments (P<0.05). The PCV (35.00 %) and Hb (13.21 g/dL) were higher in broiler chickens fed BCM supplemented diet while WBC (18.24 x10^9/L) was lower compared to other treatments. Similarly higher TP, ALB and lower cholesterol was observed for broiler chickens fed BCM supplemented diet compared to other treatments. It was concluded that dietary supplementation of BCM in chicken diet improved performance and health status in broiler chickens.

Keywords: broiler chickens, brimstone, clove, performance, haematology, serum
Running title: Response of broiler chickens to brimstone and clove supplemented diets

Effets synergiques des compléments alimentaires de brimstone (*Morinda lucida*) et de clou de girofle (*Syzygium aromaticum*) sur les performances de croissance et le profil sanguin des poulets de chair

Résumé

L'émergence d'agents pathogènes antibiorésistants due à l'utilisation d'antibiotiques dans la production avicole a soulevé des préoccupations mondiales qui ont nécessité la recherche d'alternatives sûres et efficaces aux antibiotiques. Cette expérience a étudié les effets des compléments alimentaires de brimstone (Morinda lucida), de clou de girofle (Syzygium aromaticum) et de leur combinaison sur les performances, l'hématologie et les paramètres sériques des poulets de chair. Un total de cent soixante poussins de chair Ross 308 d'un jour non sexés a été utilisé pour l'étude. Les poulets de chair ont été répartis en quatre traitements alimentaires formulés pour les phases de démarrage (1-4 semaines) et de finition (5-8 semaines). Les traitements comprenaient T1 régime de base sans additifs, T2 (régime de base avec farine de brimstone (BM) à 1 % (10g/kg)), T3 (régime de base avec 1 % de farine de clou de girofle (CM)) et T4 (régime de base avec 0,5 % (5g/kg) chacun de BM et CM ; 1 % BCM). Chaque groupe de traitement avait quatre répétitions avec dix oiseaux dans chaque répétition. Aux deux phases, les données de performance ont été collectées, tandis que l'analyse sérique et hématologique était également réalisée. Au jour 28, le poids vif (LW) (701,20 g) et le gain de poids (WG) (643,87 g) étaient plus élevés (P<0,05) pour les poulets de chair nourris avec le régime supplémenté en BCM et ceux nourris avec les régimes témoin et BM. L'indice de consommation (FCR) était meilleur (P<0,05) pour les poulets de chair des groupes CM (0,98) et BCM (0,92) par rapport aux autres traitements. Les poulets de chair nourris avec le régime supplémenté en BCM ont eu la mortalité la plus faible (1,07 %) (P<0,05) tandis que la consommation alimentaire (FI) n'était pas significativement affectée par la supplémentation phyto-génique (P>0,05). L'hématocrite (PCV) (33,84 %), l'hémoglobine (Hb) (12,30 g/dL) et les lymphocytes (65,00 %) étaient plus élevés pour les poulets de chair du groupe BCM par rapport aux autres traitements (P<0,05) tandis qu'un faible taux de globules blancs (WBC) (17,30 x109/L) était obtenu dans ce groupe par rapport aux autres traitements. Les protéines totales (TP) (48,90 g/L), la globuline (GLB) (23,40 g/L) et l'albumine (ALB) (25,50 g/L) étaient plus élevées tandis que l'aspartate aminotransférase (110,21 iU/L) était plus faible pour les poulets de chair nourris avec le régime supplémenté en BCM par rapport aux autres traitements (P<0,05). Au jour 56, le LW (3642,81 g), le WG (2941,61 g) et le FCR (1,22) se sont améliorés pour les poulets de chair du groupe BCM par rapport aux autres traitements (P<0,05). Le PCV (35,00 %) et l'Hb (13,21 g/dL) étaient plus élevés chez les poulets de chair nourris avec le régime supplémenté en BCM tandis que le WBC (18,24 x109/L) était plus faible par rapport aux autres traitements. De même, des TP, ALB plus élevées et un cholestérol plus faible ont été observés pour les poulets de chair nourris avec le régime supplémenté en BCM par rapport aux autres traitements. Il a été conclu que la supplémentation alimentaire en BCM dans l'alimentation des poulets améliorait les performances et l'état de santé des poulets de chair.

Mots-clés : poulets de chair, brimstone, clou de girofle, performances, hématologie, sérum

Introduction

Poultry meat plays a significant role in human nutrition, especially broiler chickens which are precisely raised for meat production due to their high feed conversion rate and early maturity (Al-Homidan, 2005). Despite that broiler chickens are good source of meat; their rapid metabolic rate and fragility predispose them to diseases which are often treated with antibiotics. Under the intensive system of production, the use of antibiotics has increased in the last decade due to high demand for poultry meat (Collignon and Voss, 2015). Intestinal infections such as necrotic enteritis, colibacillosis with other diseases are major concern in the poultry industry causing substantial economic losses (USDA, 2015) and antibiotics such as tetracycline, tylosin and bacitracin have been used in treatment and prevention of these diseases as well as exhibiting growth promoting effect (Poole and Sheffield, 2013; Diarra and Malouin, 2014). However, the abuse and misuse of these active compounds have resulted in its ineffectiveness causing untreatable infections arising from antibiotic resistance which has become a public health concern ((Diarra *et al*., 2010; Prescott, 2014). This menace has led to intense search for suitable alternatives to antibiotics capable of exhibiting antimicrobial properties as well as having growth promoting effect without adverse residual impact.

Previous studies have reported that bioactive components in phytogenic plants performs array of functions including antimicrobial (Yang *et al*., 2015), antioxidant (Tanaka *et al*.,

2012) and other therapeutic purposes (Reis *et al*., 2019; Galli *et al*., 2020). The potency of phytogenic plants is associated with their constituent bioactive compounds such as phenolic acids, flavonoids and volatile oils which inhibits the proliferation of harmful pathogens (Tanaka *et al*., 2012; Cheng *et al*., 2023) and are referred to as natural growth promoters (Banerjee *et al*., 2011). Brimstone (*Morinda Lucida*) and cloves (*Syzygium aromaticum*) belongs to this group of plants which possess naturally active biological compounds that can serve as alternatives to in-feed antibiotics. Brimstone (*Morinda lucida*) which belongs to the family Rubiaceae is an evergreen small to medium sized rainforest tree that can grow up to 18 meters high (Lawal *et al*., 2012). It is well documented that brimstone has several medicinal properties with no adverse effect (Lala *et al*., 2018; Adeyemi, 2021; Adeyemi *et al*., 2023). The plant parts including the leaf, stem bark, root and fruit-seed have been utilised in the treatment of different ailments such as fever, trypanosomiasis, diabetes, stomach-ache, ulcers, wounds and microbial infections in rural West Africa which is due to the constituent bioactive compounds (saponins, anthraquinones, cardenolides, alkaloids, sterols and tannin) (Olajide *et al*., 1999; Addy *et al*., 2013). Cloves on the other hand, are known for their therapeutic functions such as protection against internal parasite and it acts as antimicrobial agents against fungi and bacteria (Burt and Reinders, 2003). It has also been reported to exhibit antibacterial and anti-inflammatory properties (Muruganadan *et al*., 2001).

Essentially, cloves contain a potent phenolic compound known as eugenol which is the major active components and it also contain β-caryophyllene and eugenol acetate (Ertas *et al*., 2005; Jimoh *et al*., 2017) which possess antifungal and antiprotozoal properties (Omidbeygi *et al*., 2007; Machado *et al*., 2011). Increased weight gain and better feed conversion ratio (FCR) was achieved with 0.5% clove powder supplementation in the diet of broiler chickens (Mahrous *et al*., 2017). Agostini *et al*. (2012) also observed improved performance and feed efficiency for broiler chickens fed diets supplemented clove powder at 0.1-2.5g/kg. Brimstone have been reported to be effective against salmonella infections and it has been established from in-vitro and in-vivo studies that it inhibits *E. coli* growth (Akinyemi *et al*., 2005; Ogundare and Onifade, 2009). Lala *et al*. (2017) reported increased weight gain and FCR for broiler chickens fed diet supplemented brimstone powder at 0.1 and 0.2g/kg. Considering the enormous benefits of these phytogenic plants arising from the activity of their constituent bioactive compounds, we hypothesised that the combined use of these plants will exert greater impact through synergistic effect of inherent phytobiotics. In addition, recent strategy in enhancing the efficacy of these plants is the utilisation of their combination and therefore this study is designed to investigate the sole effects of dietary supplements of brimstone and clove and their combination on growth performance, and blood profile of broiler chickens

Materials and Methods

Preparation of test ingredients

The fresh leaves of brimstone were plucked from the mature plant; the leaves were air dried for 24 hours and subsequently dried in an oven for 15hrs at temperature of 40^{o}C. The dried leaves were ground to obtain brimstone leaf meal (BM). The properly dried fruit of clove was purchased from Poka Market, Epe, Lagos State, Nigeria and the fruit was ground using electric blender to particle size of 0.2mm to obtain clove fruit meal (CM). These ingredients were stored in plastic containers at room temperatures before the feeding trial.

Determination of chemical composition for test ingredients

4g of ground samples of BM and CM were analysed for chemical composition (Table 1). The proximate analysis was carried out according to standard procedures (AOAC, 2005). The phytochemical composition was also assayed using standard protocols; the flavonoid was determined according to Arvouet-Grand *et al*. (1994), the extractable tannin and saponin content were analysed according to Hoff and Singleton (1977) and

Edeoga *et al*. (2005), respectively while alkaloids and total phenols were determined according to Saddiqui and Ali (1997) and Wright *et al*. (2000) respectively

Table 1: Proximate, fibre and phytochemical content of brimstone (*Morinda lucida),* cloves (*Syzygium aromaticum*) and their combination

	Phytogenic plant		
Constituents	**BM**	**CM**	**BCM**
Proximate (%)			
Moisture	10.62	19.54	17.84
Crude protein	8.45	7.25	7.63
Crude fat	13.76	2.46	12.65
Crude fibre	16.78	7.62	15.76
Ash	3.44	6.31	5.44
Nitrogen free extract	57.54	69.87	65.47
fibre fractions (%)			
NDF	40.44	20.13	38.26
ADF	35.35	17.70	29.55
ADL	12.85	4.91	10.82
Cellulose	5.09	2.43	4.76
Hemicellulose	22.50	12.79	19.64
Phytochemical (mg/100g)			
Alkaloids	1756.00	1879.00	1984.55
Tannins	1120.00	798.89	1243.62
Flavonoids	0.32	0.24	0.29
Phenolics (GAE/g)	32.81	49.98	51.23

BM= brimstone meal, CM= clove meal, BCM= brimstone and clove meal, GAE= Gallic acid equivalents

Management of experimental birds and dietary treatments

One hundred and sixty, day old unsexed Ross 308 broiler chickens used for this study were obtained from a reputable hatchery. The birds were placed in pens cleaned and disinfected two weeks earlier before arrival and they were managed in deep litter with wood shavings as bedding materials. Feed and water were made available to the birds ad-libitum. Pre-heating and heating of the pen was done with the use of lighted charcoal while pen temperature and relative humidity was monitored with thermohygrometer. All vaccinations were administered to the birds at the due periods. No form of antibiotics was given to the birds throughout the experimental period which lasted for eight weeks. The birds were allotted on weight equalisation basis into four treatment groups using completely randomised design. Each group has four replicates with ten birds in each replicate. The treatments are a control group (basal diet without additives), the second group (basal diet with BM at 1% (10g/kg)), the third group (basal diet with 1% CM) and the fourth group (basal diet with 0.5% (5g/kg) each of BM and CM; 1% BCM). The test ingredients were mixed with the basal diet thoroughly for proper distribution. Diets that met the requirement of Ross 308 broiler chickens according to Ross Broiler Nutrition Specifications (2016) were formulated (Table 2) for the starter and finisher phase (4 weeks for each phase).

Table 2: Composition of experimental diet

Ingredients (%)	**Starter**	**Finisher**
Maize	56.00	62.00
Soyabean meal	21.80	17.00
Fish meal	2.00	0.00
Groundnut cake	7.00	6.00
Wheat offal	7.00	9.00
Bone meal	3.00	3.00
Oyster shell	2.00	2.00
Mineral/Vitamin Premix*	0.30	0.25
Salt	0.30	0.25
Lysine	0.30	0.25
Methionine	0.30	0.25
Total	**100**	**100**
Calculated nutrient composition (%)		
Metabolisable Energy (kcal/kg)**	2,845.60	3,098.78
Crude protein	22.91	20.54
Ether extract	3.42	3.25
Crude fibre	3.05	3.59
Calcium	1.49	1.36
Phosphorus	0.74	0.68

* Mineral/vitamin: - Vit. A, 10,000 I.U; Vit. E, 5 mg; Vit. D3, 3000 I.U, Vit. K, 3 mg; Vit. B2, 5.5 mg; Niacin, 25 mg; Vit. B12, 16 mg; Choline chloride, 120 mg; Manganese, 5.2 mg; Zinc, 25 mg; Copper, 2.6 g; Folic acid, 2 mg; Iron, 5g; Pantothenic acid, 10 mg; Biotin, 30.5 g; Antioxidant, 56 mg (Starter diet)

* Mineral/vitamin: Vit. A, 8,500 I.U; Vit. E, 10 mg; Vit. D3, 1500 I.U, Vit. K, 3.8 mg; Vit. B2, 10 mg; Niacin, 15 mg; Vit. B12, 10 mg; Choline chloride, 250 mg; Manganese, 5.0 mg; Zinc, 56 mg; Copper, 1.6 g; Folic acid, 2.8 mg; Iron, 5.1 g; Pantothenic acid, 10 mg; Biotin, 30.5 g; Antioxidant, 56 mg (Finisher diet)

**Metabolisable energy (ME) calculated using the formulae. ME= (37 × CP % + 81.8 × CF % + 35.5 × NFE %) by Pauzenga, (1985).

Note: mineral/vitamin premix included per kilogram diet

Growth performance

The live weight of the birds per pen (n = 10) was measured after arrival of the birds and subsequent weight was measured weekly while the weight gain was computed by determining the difference between the final weight at the end of each week and the initial weight at the beginning of that week. Daily feed intake was calculated as the difference between the feed offered and leftover. The feed conversion ratio (FCR) was calculated as the ratio of feed consumed to weight gain. Record of mortality was taken as it occurred during the trial and it was expressed in percentage.

Blood parameters

Blood collection

At 28th and 56th days of the study, blood samples (2.5 mL each) were collected from the brachial wing vein of four birds per treatment (1 bird per replicate) into vials containing ethylene diamine tetra-acetate (EDTA) for the determination of haematological indices. Another 2.5 mL was collected into plain bottles

(without EDTA) which were centrifuged (2,500 × *g* for 15 min) and it was used for serum chemistry analysis.

Haematological indices

Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan, 1958). Packed cell volume (PCV), red blood cell (RBC), and white blood cell count (WBC) were determined with Wintrobe haematocrit tube according to the method of Schalm *et al*. (1975). Differential leucocyte counts (heterophils, lymphocytes, basophils, eosinophils) were carried out on blood smears stained with May-Grunwald-Giemsa stain and calculations done to obtain the values.

Serum chemistry

The total serum protein (TP), albumin (ALB) and globulin (GLB) were determined using bromocresol purple method (Varley *et al*., 1980). Serum creatinine and serum uric acid concentration were determined according to Bousnes and Taussky (1945) and Wootton (1964) respectively. The serum enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were spectrophotometrically analysed with the aid of Randox® diagnostic cholesterol kit. The Alkaline phosphatase (ALP) activity was determined colorimetrically by utilising commercial clinical diagnostics kits (ELItech, France). The serum cholesterol was estimated using the commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04)

Statistical analysis

Data were subjected to one way analysis of variance using SAS (1999). The significant means among the treatments were separated using Tukey HSD test in the statistical package. The significance was considered at $P < 0.05$

Results

Performance

Table 3 shows the performance of broiler chickens fed diets supplemented with BM, CM, and BCM. At 28th day, the live weight (LW), weight gain (WG), FCR and mortality were significantly ($P<0.05$) affected while feed intake (FI) was not affected ($P>0.05$). Broiler chickens fed diet supplemented with BCM had the highest LW (701.20 g), followed by those fed CM diet LW (654.21 g) ($P<0.05$). while those fed control (580.34 g) and BM diets (570.42 g) had the lowest LW. The WG (643.87 g) was higher for broiler chickens fed BCM diet than those fed the control and BM diet but statistically similar to those fed CM diet ($P<0.05$). The FCR (0.85) was better for broiler chickens fed CM and BCM diet compared to those fed BM and control diet ($P<0.05$). The lowest mortality was recorded in broiler chicks fed BCM (1.07%) diet followed by chickens fed CM supplemented diet (3.22 %) while significantly higher ($P<0.05$) mortality was recorded in chicks fed the control (4.78 %) and BM diet (5.12 %). At 56th day, all parameters were significantly affected ($P<0.05$). The LW and WG followed the same trend as the starter phase with chicks fed BCM having the highest values ($P<0.05$) while the lowest LW and WG values were recorded in chicks fed BM diet. The FI was higher ($P<0.05$) for broiler chickens fed BCM diet (3601.23 g) than those fed control and BM diet but similar to those fed CM diet. Broiler chickens fed BCM diet had better FCR (1.22) compared to other treatments ($P<0.05$). Mortality was higher ($P<0.05$) for broiler chickens fed control diet (4.15 %) than those fed BM (2.28 %) and CM diets (2.43 %) but mortality did not occur for those fed BCM diet.

Table 3: Effect of brimstone and cloves dietary supplementation on the performance of broiler chickens

Parameters	Control	BM	CM	BCM	SEM	P-value
Initial weight (g)	57.42	57.11	56.98	57.21	1.87	0.28
At 28 days						
Live weight (g)	580.34^{c}	570.42^{c}	654.21^{b}	701.20^{a}	38.46	0.02
Body weight gain (g)	522.92^{b}	513.32^{b}	597.23^{ab}	643.87^{a}	39.85	0.04
Feed intake (g)	598.44	576.38	587.25	590.42	43.21	0.31
Feed conversion ratio	1.14^{a}	1.13^{a}	0.98^{b}	0.92^{b}	0.85	0.03
Mortality (%)	5.12^{a}	4.78^{a}	3.22^{b}	1.07^{c}	1.43	0.03
At 56 days						
Live weight (g)	3142.32^{b}	2875.49^{c}	3131.62^{b}	3642.81^{a}	86.43	0.01
Body weight gain (g)	2491.98^{b}	2305.07^{c}	2457.41^{b}	2941.61^{a}	82.25	0.01
Feed intake (g)	3301.32^{b}	3299.91^{b}	3498.29^{ab}	3601.23^{a}	154.23	0.03
Feed conversion ratio	1.32^{a}	1.43^{a}	1.42^{a}	1.22^{b}	0.10	0.01
Mortality (%)	4.15^{a}	2.28^{b}	2.43^{b}	0.00^{c}	1.52	0.02

^{abc}Means on same row having dissimilar superscripts indicates significant difference ($P<0.05$)

BM= brimstone meal, CM= clove meal, BCM= brimstone and clove meal, SEM= standard error of mean

Haematology

The haematological parameters of broiler chickens fed diets supplemented brimstone and clove is presented in Table 4. At 28th day, broiler chickens fed diet supplemented BCM had the highest (P<0.05) PCV (33.84 %). Chicks fed control and BM diets had the lowest PCV while those fed CM diet had lower PCV values compared to chicks on BCM diet. Highest Hb (12.30 g/dL) was observed for broiler chickens fed BCM diet, those fed control diet had the lowest Hb (7.40 g/dL) while those fed BM and CM diets had similar Hb (P<0.05). The RBC (2.41 $x10^{12}$/L) of broiler chickens fed the control diet was lower compared to other treatments (P<0.05). Lower WBC (17.30 $x10^{9}$/L) was obtained for broiler chickens fed BCM diet compared to other treatments (P<0.05). Broiler chickens fed BCM diet had higher lymphocyte count (65.00 %) compared to other treatments (P<0.05). Basophil, heterophil and eosinophil counts were not affected (P>0.05). At 56th day, PCV (35.00 %) was highest for broiler chickens fed BCM diet and those fed BM diet had the lowest (24.42 %) while those fed control and CM diets were intermediate (P<0.05). Haemoglobin (13.21 g/dL) was higher for broiler chickens in the BCM group compared to other treatments (P<0.05). Broiler chickens fed BM and BCM diets had higher RBC than those fed control and CM diets (P<0.05). The WBC count (18.24 $x10^{12}$/L) was lower for broiler chickens fed BCM diet than other treatments (P<0.05). Basophil, lymphocyte, heterophil and eosinophil counts were not significantly influenced (P>0.05).

Table 4: Effect of brimstone and cloves dietary supplementation on the haematological indices of broiler

Parameters	Control	BM	CM	BCM	SEM	P-value
At 28 days						
Packed cell volume (%)	23.26^{c}	22.43^{c}	27.87^{b}	33.84^{a}	8.96	0.00
Haemoglobin (g/dL)	7.40^{c}	8.90^{b}	10.00^{b}	12.30^{a}	7.01	0.01
Red blood cell ($x10^{12}$/L)	2.41^{b}	3.10^{a}	2.95^{a}	3.25^{a}	1.09	0.00
White blood cell ($x10^{9}$/L)	21.50^{a}	21.60^{a}	22.00^{a}	17.30^{b}	7.43	0.02
Basophil (%)	0.00	0.00	0.00	0.10	0.01	0.21
Lymphocyte (%)	55.00^{b}	56.10^{b}	54.82^{b}	65.00^{a}	19.70	0.01
Heterophil (%)	20.00	19.98	19.57	21.00	6.70	0.23
Eosinophil (%)	1.00	0.20	0.00	1.00	0.01	0.10
At 56 days						
Packed cell volume (%)	27.85^{b}	24.42^{c}	28.65^{b}	35.00^{a}	9.97	0.01
Haemoglobin (g/dL)	10.10^{b}	9.87^{b}	10.20^{b}	13.21^{a}	6.02	0.03
Red blood cell ($x10^{12}$/L)	2.56^{b}	3.01^{a}	2.53^{b}	3.14^{a}	0.98	0.02
White blood cell ($x10^{9}$/L)	20.83^{a}	20.54^{a}	21.10^{a}	18.24^{b}	8.21	0.02
Basophil (%)	1.00	1.00	0.10	0.00	0.01	0.13
Lymphocyte (%)	60.13	59.86	61.10	60.22	21.10	0.76
Heterophil (%)	20.12	19.84	19.98	21.02	7.52	0.32
Eosinophil (%)	1.00	1.00	0.00	1.00	0.01	0.13

^{abc}Means on same row having dissimilar superscripts indicates significant difference (P<0.05)

BM= brimstone meal, CM= clove meal, BCM= brimstone and clove meal, SEM= standard error of mean

Serum parameters

Table 5 presents the serum parameters of broiler chickens fed diets supplemented brimstone and clove. At 28 days, the TP (48.90 g/L) and ALB (25.50 g/L) was highest for broiler chickens fed BCM diet and it was lowest for broiler chickens fed BM diet while it was intermediate for those fed control diet and CM diet (P<0.05). The GLB (23.40 g/L) was higher for broiler chickens fed BCM diet compared to other treatments (P<0.05). Serum AST was highest for broiler chickens fed control diet (148.34 iU/L) and BM diet (144.67 iU/L) and it was lowest for those fed BCM diet (110.21 iU/L) while it was intermediate for those fed CM diet (125.40 iU/L) (P<0.05). Broiler chickens fed control diet and those fed BCM diet had higher uric acid than other treatments (P<0.05). The total cholesterol (TC), creatinine, ALT and ALP were not affected significantly (P>0.05). At 56 days, broiler chickens fed BCM diet had the highest TP (44.86 g/L) and those fed control diet had the lowest TP (36.84 g/L) while those fed BM (39.59 g/L) and CM diet (38.45 iU/L) had intermediate TP (P<0.05). The ALB (23.86 g/L) was highest for broiler chickens fed BCM diet but lowest for those fed control (16.83 g/L) and CM diets (17.33 g/L) (P<0.05). The TC was lowest for those fed BCM diet (9.86 mmol/L) and highest for those fed control diet (12.32 mmol/L) while those fed BM (11.81 mmol/L) and CM diet (11.97 mmol/L) had intermediate TC. The serum GLB, creatinine, AST, ALT, ALP and uric acid were not significantly influenced (P>0.05).

Table 5: Effect of brimstone and cloves dietary supplementation on the serum parameters of broiler chickens

Parameters	Control	BM	CM	BCM	SEM	P-value
At 28 days						
Total protein (g/L)	42.76^{b}	37.40^{c}	43.50^{b}	48.90^{a}	8.44	0.02
Globulin (g/L)	21.43^{b}	20.53^{b}	22.01^{b}	23.40^{a}	6.53	0.04
Albumin (g/L)	22.33^{b}	16.87^{c}	21.49^{b}	25.50^{a}	4.25	0.01
Total cholesterol (mmol/L)	7.23	7.40	6.97	6.89	1.10	0.08
Creatinine (mmol/L)	0.05	0.15	0.06	0.05	0.04	0.12
AST (iU/L)	148.34^{a}	144.67^{a}	125.40^{b}	110.21^{c}	14.21	0.02
ALT (iU/L	20.49	21.00	20.91	22.21	7.30	0.14
ALP (iU/L	200.25	201.12	202.00	199.87	10.91	0.67
Uric acid (mmol/L)	0.21^{a}	0.11^{b}	0.12^{b}	0.19^{a}	0.22	0.01
At 56 days						
Total protein (g/L)	36.84^{c}	39.59^{b}	38.45^{b}	44.86^{a}	8.45	0.04
Globulin (g/L)	20.01	19.59	21.12	21.00	2.10	0.32
Albumin (g/L)	16.83^{c}	20.00^{b}	17.33^{c}	23.86^{a}	4.41	0.03
Total cholesterol (mmol/L)	12.32^{a}	11.81^{b}	11.97^{b}	9.86^{c}	3.41	0.03
Creatinine (mmol/L)	0.05	0.06	0.06	0.05	0.15	0.13
AST (iU/L)	159.41	160.32	158.45	161.42	15.53	0.42
ALT (iU/L	19.43	20.10	19.85	20.20	6.42	0.40
ALP (iU/L	210.11	212.32	211.31	215.24	12.30	0.86
Uric acid (mmol/L)	0.13	0.14	0.13	0.15	0.03	0.12

^{abc}Means on same row having dissimilar superscripts indicates significant difference (P<0.05)

BM= Brimstone meal, CM= Clove meal, BCM= Brimstone and clove meal, SEM= Standard error of mean, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALT= Alkaline phosphatase

Discussion

Performance

At day 28, although chicks on CM diet had higher LW than those fed control and BM diets, result showed that broiler chicks fed BCM diet had improved LW compared to other treatments. This improvement in LW could be attributed to the combined effect of brimstone and clove supplementation due to the impact of the bioactive constituents. This observation agrees with the report of Petrovic *et al*. (2012), that improvement in body weight of broiler chickens fed diet supplemented with 1.0% clove buds and 0.2% lemon balm extract. Although Agostini *et al*. (2012) observed no significant improvement in performance of broiler chickens fed diet supplemented clove at 2500 mg/kg of diet. Mahrous *et al*. (2017) also reported no significant effect of clove bud dietary supplementation at 0.5-1.5% on final body weight of broiler chickens. This further suggests that the efficacy of these phytogenic plants can be enhanced if used in combination. However, Al-Mufarrej *et al*. (2019) reported a gradual decrease in LW at 21 days for broiler chickens fed diet supplemented clove powder at 30-60 g/kg diet. This discrepancy could be due to the high dose of clove inclusion used in the study by Al-Mufarrej *et al*. (2019) which is known to contain tannin which may affect nutrient utilisation (Keshavarzi *et al*., 2017). The increased WG recorded for broiler chickens fed BCM diet is also associated with the benefit of combining the two additives. This is similar to the report of Brenes and Roura (2010), the authors reported positive synergistic effect of dietary essential oils supplementation on WG of broiler chickens. The feed intake was not significantly affected by the treatments, however, the FCR improved for broiler chickens fed CM and BCM diets which indicates better nutrient utilisation for these groups of broiler chickens. Phytogenic additives including spices have been reported to enhance digestion through enzyme stimulation in the intestinal mucosa (Hernández *et al*., 2004). Lowest mortality was observed for broiler chickens fed BCM supplemented diet and this could be attributed to the antimicrobial and antiparasitic properties exhibited by brimstone and clove (Kammon *et al*., 2019; Ola-Fadunsin and Ademola, 2014).

At day 56, significant higher LW and WG than other treatments were recorded for broiler chickens fed diet supplemented BCM. The increased LW and WG observed is associated with the growth promoting properties of brimstone and clove which through inherent secondary metabolites such as saponin and alkaloids enhance digestion and nutrient utilisation by suppressing the growth of pathogenic bacteria and reducing toxins of intestinal microflora (Cross *et al*., 2007). This is similar to the report of Ola-Fadunsin and Ademola (2014) on increase in body weight of Eimeria species infected broiler chickens administered brimstone acetone extract at 2.0-6.0 g/kg body weight compared to the negative control without any treatment. Higher FI observed in broiler chickens fed BCM diet may be due to increased nutrient uptake in the intestine stimulated by bioactive constituents of the additives which are known to exhibit digestive stimulatory properties (Alkassie *et al*., 2009). This was contrary to the report of Adesina (2020) that no significant difference was observed in FI of broiler chickens administered brimstone leaf extract at 0.5-1.5g/L at the finisher phase. The FCR was better in broiler chickens fed BCM supplemented diet relative to other treatments. This is however similar to the report of Adesina (2020) on improved FCR and WG in broiler chickens offered brimstone leaf extract at 1.5g/L despite recording no impact on FI. It is important to state that the FCR for the group of broiler chickens fed CM diet and those fed BM diet were similar with the control group which suggests that the individual effect of the additive was not sufficient to elicit a better FCR. Mortality did not occur in the group of broiler chickens fed BCM diet while the highest mortality was recorded for broiler chickens in the control group. This negates the report of Othman *et al*. (2022) that no significant

reduction in mortality was recorded in broiler chickens fed diets supplemented 0.5-1% clove bud powder compared with the control. However, Lala *et al*. (2017) reported significantly reduced mortality for broiler chickens fed diet supplemented with 0.1 and 0.2g/kg brimstone leaf meal compared to the control. This discrepancy indicates that brimstone could be more potent in supporting immunity through the activity of bioactive components as it has been reported to contain phenolic compounds which acts as antioxidant preventing oxidative stress and altered metabolic process resulting in death (Grashorn, 2010)

Haematology

Results showed significantly higher PCV and Hb in broiler chickens fed BCM diet compared to other treatments at day 28. This increase is due to improved nutrient uptake which is associated with the digestive stimulatory properties of brimstone and clove. Haematological parameters are important indicators of the physiological and nutritional state of the animal as they are used in interpreting nutritional quality of the diet (Ologhobo *et al*., 2008). This is in agreement with the report of Suliman *et al*. (2023) who observed improvement in crude protein digestibility for broiler chickens fed diet supplemented clove powder at 1.5%. The RBC of broiler chickens fed control diet was significantly lower compared to other treatments. This observation is tied to the beneficial effect of the phytogenic plants and this suggests increased oxygen and nutrient transport to the cells (Isaac *et al*., 2013). The reduced WBC count observed for the group of broiler chickens fed BCM diet implies good health status of the birds as increased WBC in circulating blood is indicative of infection ((Maroufyan *et al*., 2010). Lymphocyte count was observed to be higher for the broiler chickens fed BCM diet which however, as a WBC differential does not suggest disease state but increased resistance to disease as it is known as one of the major defences against infection (Masoudi *et al*., 2011). Similar to what was observed at day 28, the PCV and Hb of broiler chickens fed BCM diet was higher compared to other treatments at day 56. This suggests good health status of the birds and adequacy of blood constituents and components. Adu *et al*. (2022) reported no significant effect of clove leaf meal inclusion at 0.25 and 0.50% in diet of laying hens on PCV and Hb. Ola-Fadunsin and Ademola (2014) observed positive effect of brimstone extract at 2-6 g/kg on Hb of broiler chickens infected with Emeria species though no significant difference in PCV. The improved PCV count observed in this study with broiler chickens fed BCM diet indicates the preponderance of the synergistic effect of combined brimstone and clove over their individual use. Increased RBC was evident for the groups of broiler chickens fed BM and BCM diets. This indicates an improved haematopoiesis which resulted due to the influence of bioactive components of the additives. Phytogenic plants such as cumin, anise, cloves and black pepper are known for their beneficial effects on health of poultry birds and precisely cloves contain the active compound eugenol and β-caryophyllene which are known to enhance digestion for improved nutrient absorption (Reddy *et al*., 2004). The reduced WBC count recorded for broiler chickens fed BCM diet indicates the inhibitory impact of the bioactive components of brimstone and clove against disease invasion. Brimstone and clove as aromatic plants have been reported to play antibacterial and antiparasitic role by exhibiting immune stimulatory functions (Chowdhury *et al*., 2018).

Serum

The increase in serum TP, GLB and ALB observed in broiler chickens fed BCM diets at day 28 indicated adequate protein intake due to enhanced nutrient utilisation. Improved dietary utilisation of nutrients particularly protein as direct positive influence on serum TP, GLB and ALB resulting in good health status which reflected in improved weight gain and reduced mortality in this group of broiler chickens. The serum proteins of the blood play an essential role in the immune and health condition of

poultry birds due to their significant roles in metabolic and physiological processes (Liu *et al*., 2015). The finding in the current study is in agreement with Olayemi *et al*. (2016) who reported higher serum TP and GLB in broiler chickens fed diets supplemented with mix of *Morinda lucida* (0.5 g/kg) and *syzygium aromaticum* (0.5 g/kg) compared to other treatments. However, Saeid *et al*. (2010) reported no significant effect of aqueous ginger extract administration at 0.4 and 0.6% on TP, ALB and GLB of broiler chickens compared to the control. Hassan and Awad (2017) also observed no significant influence of dietary thyme powder supplementation at 0.2%, 0.5%, and 0.8% on TP, ALB and GLB of broiler chickens compared to the control. From our study, it was observed that broiler chickens fed CM diet had similar TP, GLB and ALB to those in the control group. In comparison with the report of Saeid *et al*. (2010) and Hassan and Awad (2017), this suggests that when phytogenic plants are included in combination it enhances their potency to exhibit superior function. The serum AST was significantly lower for broiler chickens fed BCM diet which indicates good liver health while increased AST in those fed BM and control diets suggest defect in the liver as high concentration of AST is implicated in liver damage (Lu, 2007). However, the non-significant difference observed for ALT and ALP is in consonance with the report of Mustafa (2016) that no significant difference was observed in ALT and ALP of broiler chickens fed diets containing mix of essential oils (cloves, anise, and caraway) included at a rate of 200, 400, and 600 mg/kg. The increased uric acid observed for broiler chickens in the control group and those in the BCM group may be due to inefficient protein assimilation and rapid protein degradation respectively. Increased uric acid is associated with defect in protein utilisation and increased deamination due to high amino acid in circulation (Wang *et al*., 2020). Serum TP and ALB were significantly higher for the group of broiler chickens fed BCM supplemented diet at day 56. This is similar to the observation at day 28 and this implies improved nutrient digestion and utilisation resulting in better health status. This is contrary to the report of Tariq *et al*. (2014) who observed no significant difference in serum TP, ALB and GLB of broiler chickens fed diet containing 0.5% clove powder compared to the control group. Chowdhury *et al*. (2018) also observed no significant influence of 0.6% dietary clove bud oil supplementation on serum TP of broiler chickens. The increased serum TP and ALB observed in the current study could be due to the combined effect of brimstone and clove inclusion in the diet. The TC was lower for broiler chickens in the treated groups compared to the control, however, those in the BCM group had the lowest TC. This is similar to the report of Mahrous *et al*. (2017) who reported decrease in TC of broiler chickens fed diet supplemented clove bud at 0.5-1.5 g/kg. The lower cholesterol is achieved as a result of the activity of the bioactive constituents such as saponins which combines with cholesterol and impedes intestinal uptake. It also limits enzymatic activity in the biosynthetic pathway of lipids (Konjufca *et al*., 1997).

Conclusion

The outcome of this study reveals that dietary supplementation of brimstone and clove in combination resulted in improved performance, serum and haematological parameters of broiler chickens compared to their supplementation in isolation which indicates superior impact arising from synergistic effect of the bioactive components. This strategy can be utilised to enhance the efficacy of phytogenic plants as replacement for antibiotics.

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