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EFFECT OF FEEDING BROILERS WITH PHYTOGENIC FEED ADDITIVES CONTAINING DIETS ON BLOOD BIOCHEMICAL AND HAEMATOLOGICAL CONSTITUENTS

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ABSTRACT. The intense pressure on the poultry sector to promote safe chicken meat and egg has sparked interest in the use of natural and consumer-accepted phytogenic feed additives. The majority of literature on the benefits of turmeric (*Curcuma longa*) and Negro pepper (*Xylopia aethiopica*) is focused on layers and there is no evidence of turmeric and Negro pepper interactive effects. Therefore, this study was conducted to evaluate the effect of turmeric and Negro pepper mixture on serum biochemical and haematological constituents of broiler chicks fed for 48 days. A total of 96 one-day-old Arbor acres chicks of mixed sexes were used for the experiment. The birds were randomly assigned to four treatment groups and replicated three times in a completely randomized design. The milled turmeric and Negro pepper were mixed in the ratio of 50:50 (50%) and used in the formulation of turmeric and Negro pepper mixture (TNPM) diets. The four experimental diets were formulated to contain 0.00, 0.50, 1.00, and 1.50% dietary levels of inclusion of TNPM at the starter phase and 0.00, 1.50, 3.00, and 4.50% inclusion levels at the finishing phase respectively. Blood samples were collected from the brachial wing vein on the 24th and 48th days of the experiment and were evaluated for serum biochemical and haematological constituents. At the starter phase, red blood cells (RBC) was significantly ($P < 0.05$) improved at a 1.50% dietary level of TNPM. Haemoglobin (Hb) was better ($P < 0.05$) in 0.50 and 1.50% inclusion levels. Mean cell haemoglobin concentration (MCHC) and White blood cells were enhanced ($P < 0.05$) among the treatment groups compared to the control. Total protein, albumin, uric acid and cholesterol concentrations were reduced significantly ($P < 0.05$) in 1.50% inclusion. Alkaline phosphatase value was significantly ($P < 0.05$) lower in birds fed 1.00% TNPM. At the finishing phase, packed cell volume was significantly ($P < 0.05$) higher in 1.50 and 3.00%. RBC was significantly ($P < 0.05$) higher in birds fed 1.50 and 4.50% TNPM. Hb and mean cell volume values were significantly ($P < 0.05$) higher in 1.50% when compared to 0.00%. MCHC was better ($P < 0.05$) among birds fed 1.50% in comparison with those fed 0.00 and 4.50%. WBC was generally improved ($P < 0.05$) among the treatment groups. Total protein and cholesterol values were higher ($P < 0.05$) in the control. Albumin was higher ($P < 0.05$) in 0.00 and 1.50%. Globulin produced a significantly ($P < 0.05$) lower value in 1.50%. Aspartate aminotransferase produced significantly ($P < 0.05$) higher value in T_2. It was concluded that up to 1.50 and 4.50% of TNPM could be included in starting and finishing broiler diets without any detrimental effect on serum biochemical and haematological constituents.

Introduction

Phytogenic products have been used for many decades as spices, herbs, in ethnoveterinary interventions, and products derived thereof, but their use in the feed industry is gaining ground in recent times. Following the European Union ban on antibiotic growth promoters in the animal feed industry in 2006 (Castanon, 2007) phytogenic plant parts have gained much interest as feed additives. Sequel to the ban in Europe, growing pressure on livestock and poultry farmers in other parts of the world to produce safe food for human consumption, alternative ingredients and methods for the promotion of animal growth and the prevention of diseases were explored, with increased attention being paid to phytogenic and herbal products as they have gained greater market acceptance as natural additives. Jin (2010) characterized phytogenic feed additives to have natural properties, multiple functions, side effect reduction, nonresistance and leaving no residues in livestock production. These may have enhanced their wide acceptability as a new alternative to age-long synthetic antibiotics and growth promoters for poultry and livestock. Among the phytogenic products commonly used in Nigeria are turmeric (*Curcuma longa*), Negro pepper (*Xylopia aethiopica*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), Moringa (*Moringa oleifera*), soursop (*Annona muricata*), Pawpaw (*Carica papaya*), Never die (*Bryophyllum pinnatum*), African peach (*Nauclea latifolia*), Gmelina (*Gmelina arborea*) *etc*.

Turmeric (*Curcuma longa*), belongs to the family *Zingiberaceae* and is of Indian origin, but is highly cultivated in East and West Africa. Turmeric is known with diverse names in Nigeria such as Ukpo by Igbos, Akika by Yorubas, Kafi-nama-zaki by Hausas and Utantan by Edos. Turmeric is a perennial herbaceous plant that is planted majorly because of its tuberous rhizomes or underground stems. Turmeric serves as a major source of curcumin, turmerone, germacrone, atlantone and zingliberene. The antioxidant, anti-inflammatory, anticarcinogenic, anti-viral, anti-bacterial, anti-fungal, anti-protozoal, nematocidal and insecticidal, antivenom, anti-HIV, anti-tumour, anti-allergic and immunological activating properties of the rhizome have been reported (Ali *et al.*, 2016). Beta-carotene, ascorbic acid (vitamin C), calcium, flavonoids, fibre, iron, niacin, potassium, zinc and other nutrients, and over 300 naturally occurring components are present in turmeric.

Negro pepper (*Xylopia aethiopica*) belongs to the family *Annonaceae* and is of African origin. Negro pepper is commonly known as Uda by Igbos, as Eeru Alamo by Yoruba and as Chimba by Hausas speaking parts of Nigeria. Negro pepper is an evergreen, aromatic tree that can grow up to 15–30 m high and about 60–70 cm in diameter, which thrives in lowland rainforest and moist fringe forests in the savanna zones of Africa. Every part of *Xylopia aethiopica* such as the bark, seeds, stem, fruit and leaves are of great importance in medicine for therapeutic purposes (Ikrang, Anyanwu, 2019). *Xylopia aethiopica* is known for its high-quality wood, food spices, medicinal and pharmaceutical properties. The dried seeds of *Xylopia aethiopica* have been reported (Jirovetz *et al.*, 1997; Erhirhie, Moke, 2014) to have antioxidant, anti-inflammatory, anti-carcinogenic, insecticidal, anti-microbial, anti-malaria, anti-respiratory, natural contraceptive, pain relief, treatment of cough, cold, flu and gastric ulcer, promotes weight loss and preservative properties. It contains copper, zinc, protein, camphene, manganese, alkaloids, diter penic, limonene, folic acid, flavonoid, vitamins: A, B1, B2, C and E. The plant contains annonacin, which is an alkaloid resembling morphine in action (Alagawany *et al.*, 2015). The fruit contains volatile aromatic oil, fixed oil and rutin. However, there is a paucity of information on the effect of *Curcuma longa* and *Xylopia aethiopica* mixture on broilers. This study, therefore, aimed to determine the effect of diets with turmeric and Negro pepper mixture on the blood profile of broiler chickens. We hence assume that inclusion of 0.00 to 1.5% and 0.00 to 4.50% of *Curcuma longa* and *Xylopia aethiopica* mixture in starting and finishing broilers respectively will have a positive effect on the blood profile of broiler, due to their high phytobiotic properties.

Material and Methods

The experiment was carried out at the Poultry Unit, Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria. The College is situated at a latitude of 5.56° N and longitude of 7.31° E, with an average rainfall of 1653 mm and a prevailing temperature of 28.50 °C and relative humidity of about 80% (FCAI, Meteorological Centre Data, 2017).

Fresh turmeric (*Curcuma longa*) tubers were sourced from the National Root Crop Research Institute (NRCRI), Umudike, Ikwuano Local Government Area of Abia State, Nigeria. The turmeric tubers were washed properly and sundried to about 15% moisture, while the ripped Negro pepper (*Xylopia aethiopica*) fruits were procured from Akwete market Enugu, Enugu State, Nigeria and were also washed properly and air-dried to about 15% moisture. These materials were subsequently milled into flour differently using the harmer mill. The milled turmeric and Negro pepper were mixed in the ratio 50:50 each to have turmeric and Negro pepper mixture (TNPM). The 50:50 mixing ratio was based on the equal addition of the phytobiotics.

Four experimental diets were formulated to contain 0.00, 0.50, 1.00, and 1.50 levels of TNPM at the starter phase and 0.00, 1.50, 3.00, and 4.50 levels at the finishing phase respectively. The experimental diets were formulated according to the standards of NRC. The compositions of the experimental diets are given in Table 1 and Table 2.

Table 1. Composition of the experimental diets for starting broilers

Ingredient	Diet composition, %			
Maize	50.00			
Soya bean meal	16.00			
Groundnut cake	20.00			
Wheat offal	8.00	7.50	7.00	6.50
Negro pepper/turmeric mix	0.00	0.50	1.00	1.50
Fish meal	2.00			
Limestone	1.50			
Bone meal	1.50			
Salt	0.25			
Premix	0.25			
Methionine	0.25			
Lysine	0.25			
Total	100			

Table 2. Composition of the experimental diets for finishing broilers

Ingredient	Diet composition, %			
Maize	50.00			
Soya bean meal	16.00			
Groundnut cake	12.00			
Wheat offal	16.00	14.50	13.00	11.50
Negro pepper/turmeric mix	0.00	1.50	3.00	4.50
Fish meal	2.00			
Limestone	1.50			
Bone meal	1.50			
Salt	0.25			
Premix	0.25			
Methionine	0.25			
Lysine	0.25			
Total	100			

96 one-day-old Arbor Acres broiler chicks of mixed sexes were sourced from Chi Farms® in Ibadan, Oyo State, Nigeria. They were randomly assigned to four (4) experimental treatment groups, each replicated three times with eight (8) birds constituting a replicate. The experimental birds were managed following the permission and stipulated guidelines of the Federal College of Agriculture, Ishiagu (FCAI) Animal Ethics Committee. The birds were assigned to the four experimental diets in a Completely Randomized Experimental Design (CRD) and fed the experimental diets in two phases respectively for 24 days to have a total of 48-day feeding trials. The experimental diets were introduced from the beginning of the study (one day old). Each replicate group was housed in a poultry pen that was demarcated and covered with a wire net. Pen concrete floors were covered with wood shavings as litter material. Before the arrival of the one-day-old chicks, the brooding house was washed and disinfected and allowed to dry. The brooder house was pre-heated for 6–12 hrs to enable it to reach the normal temperature (32 ºC) required by day-old chicks. Fresh feed and water were provided *ad libitum* in the mornings on daily basis. Heat and light were provided for the first 14 days using stoves and electric bulbs. The birds were vaccinated against infectious bursal (Gumboro) disease at days 10 and 24 respectively, while the NDV – Lasota vaccine was given on days 1, 14 and 28 to protect against New Castle Disease.

Blood samples (5 ml) were drawn from one bird per replicate on the 24th (24 days old) and 48th day (48 days old) of the experiment. The birds were bled through the brachial wing vein. The samples were respectively collected in two parts for biochemical and haematological studies: 2.5 ml was collected into the labelled sterile universal bottle containing 1.0 mg ml^{-1} ethyldiamine tetraacetic acid for haematological analysis and another 2.5 ml was also collected into the anti-coagulant free bottle for biochemical analyses. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean cells haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentrations (MCHC) were calculated.

Chemical analyses (dry matter, crude protein, crude fibre, ash, and ether extract) of experimental diets and that of the test ingredients were carried out according to the methods of AOAC (2000). The nitrogen-free extract was derived by subtracting the sum of other components (crude protein, ether extract, ash, crude fibre) from 100 on a dry matter basis. Metabolizable energy was calculated using the formula:

$$ME = (3.5 \times CP) + (8.5 \times CF) + (3.5 \times NFE) \times 10, \quad (1)$$

where
ME – metabolizable energy, kcal kg^{-1};
CP – crude protein, %;
CF – crude fat, %;
NFE – nitrogen-free extract, %.

Data obtained were analysed using analysis of variance (ANOVA) as described by SAS (2008). Significant means were separated using the Duncan Multiple New Range Test.

Results and Discussion

The chemical compositions of the experimental diets for broiler starter, turmeric and Negro pepper meals are presented in Table 3. The dry matter (DM) range of 92.15–93.08% obtained in this study for starting broilers compared well with 91.81–92.62 9% reported by Jiwuba *et al.* (2016b) for broiler starters. The crude protein (CP) range of 22.12–23.39 reported in this study is higher than 20.70–21.90%, reported by Jiwuba *et al.* (2016b).The values reported in this study nevertheless fall within the recommended values of 21–24% reported by Livestocking (2020) for broiler starters and compared well with 23% recommended by NRC (1994) for broilers within the age of 0–3 weeks. The crude fibre (CF) range of 3.32–3.47% reported in this study failed to follow a particular trend but is lower than 9.0–0.3% reported by Abu *et al.* (2015), but compared well with 3.04–3.63% reported by Jiwuba *et al.* (2017). The energy value of 2940.70–3060.35 kcal kg^{-1} reported in this study is comparable with 2959.90–3106.45 kcal kg^{-1} reported by Jiwuba *et al.* (2016a) for starter broilers and in agreement with the recommended values of 2900–3100 kcal kg^{-1} reported by Livestocking (2020) for broiler starter.

The turmeric meal showed a high DM value of 92.56% reported in this study and compared with 91.00% reported by Ikpeama *et al.* (2014). The high DM reported for turmeric in this present study may indicate a high amount of nutrient availability to the animal since DM is an indicator of the amount of nutrients that are available to the animal in a feed sample. The CP value of 9.76% obtained in this present study is higher than 1.83 and 5.8% reported by Asagwara *et al.* (2018) and Attiaa *et al.* (2017) respectively but compared well with 9.40% reported by Ikpeama *et al.* (2014) for the same phytobiotic rhizome meal. This result may imply that turmeric can be substituted for maize in terms of crude protein and hence makes it an important feedstuff due to its relatively appreciable CP value as well as its ethnoveterinary properties. However, the high tannin content of turmeric which hinders protein digestion and utilization and the relative higher cost of turmeric rhizome may still constitute a problem. The CF value of 4.22% is higher than 1.95% but compared with 3.5 and 4.60% reported by Attiaa *et al.* (2017) and Ikpeama *et al.* (2014) respectively for turmeric meal. The percentage of fibre reported in this study make it well suited for broiler feeding since it is below 7% as recommended as ideal for broilers (Salah, 2012) and also compared favourably with 3.0–4.0% CF, depending on the age of the bird as reported by Swennen *et al.* (2010). The 6.45% ether extract (EE) reported may be attributed to a high level of curcumin, fat-soluble vitamins (A, D, E, K) and fat, hence EE, is the amount of fat and fat-soluble components in a feed. Furthermore, it includes plant pigments (chlorophyll, xanthophylls, carotene) and is fat-soluble, but it does not tell us how much of each vitamin or fatty acid is present. The result of the present study compared well with 6.85% reported by Ikpeama *et al.* (2014), but higher than 4.7% reported by Attiaa *et al.* (2017). Ash value of 6.45% observed in this study was higher than 2.85, 3.04 and 4.2% reported by Ikpeama *et al.* (2014), Asagwara *et al.* (2018) and Attiaa *et al.* (2017) respectively for Turmeric meal (TM). The differences in ash values may be attributed to varieties, soil mineral/nutrient and maturity/date of harvesting. The high ash value is an indication of a large amount of minerals. Minerals function to enhance growth, development, bone development, nerve impulse transmission, hormone synthesis, immunity and general well-being of the animals. The NFE value of 69.30% reported in this study entails the ability of TM to provide readily available energy since NFE is considered to provide an estimation of water-soluble polysaccharides in the form of sugars or starch. The value reported in this present study is lower than 71.5% reported by Attiaa *et al.* (2017) for TM. The Metabolizable energy value of 3315.35 kcal kg^{-1} reported in this study further indicated the potentials of TM in providing the required energy for broilers.

The 91.78% DM reported in this present study compared with 93.83% reported by Muhammad *et al.* (2016) for Negro pepper meal (NPM). This high DM entails high nutrient availability to the animals fed the supposed diet. The 9.99% CP reported in this study for NPM is lower than 10.59% reported by Muhammad *et al.* (2016) but higher than 8.33% reported by Ndelekwute and Enyenihi (2018). The 18.66% CF is lower than 9.23% reported by Ndelekwute and Enyenihi (2018) but higher than 3.33% reported by Muhammad *et al.* (2016) for NPM. The differences may be attributed to level of maturity before harvesting and possible variety differences. However, the CF of NPM reported in this study is above 3.0–4.0% CF reported by Swennen *et al.* (2010) but below 7% CF recommended by Salah (2012) but falls within 5 and 8% CF recommended for starter and finisher broilers respectively by Livestocking (2020). This may still indicate the relevance of NPM in broiler feeding. The 5.45% EE reported in this study reveals high level of fat and fat-soluble components of NPM. The result of this study is compared to 32.96 and 12.17% reported by Ndelekwute and Enyenihi (2018) and Muhammad *et al.* (2016) respectively for same phytogenic feed additive. The ash value (6.33%) is higher than 3.83% reported by Muhammad *et al.* (2016) but compared well with 6.05% reported by Ndelekwute and Enyenihi (2018) for same product. The high metabolizable energy of 2610.15 kcal kg^{-1} reported in this this study reveals that it can as well be used as energy source in broiler feeding.

The chemical composition of the experimental diets for broiler finishers is presented in Table 4. The nutrient values reported in this present study for broiler finisher is in agreement with the recommendations of NRC (1994) and Livestocking (2020).

Table 3. Chemical composition of the experimental diets for broiler starter, turmeric and Negro pepper mixtures and meals

Nutrient, %	Dietary levels, %					
	0.00*	0.50*	1.00*	1.50*	TM	NPM
Dry matter	92.15	93.08	92.44	92.99	92.56	91.78
Crude protein	23.39	22.39	22.25	22.12	9.76	9.99
Crude fibre	3.33	3.32	3.43	3.47	4.22	6.66
Ether extract	3.45	3.65	3.44	3.01	6.45	5.45
Ash	6.31	8.69	8.33	8.81	2.83	6.33
NFE	55.67	55.03	54.99	54.59	69.30	53.35
ME, kcal kg^{-1}	3060.35	3019.95	2995.80	2940.70	3315.35	2610.15

* Turmeric and Negro pepper mixture 50:50 ratio; TRM – turmeric rhizome meal; NPM – Negro pepper meal; NFE – nitrogen-free extract; ME – metabolizable energy

Table 4. Chemical composition of experimental diets for broiler finisher

Nutrient, %	Dietary levels, %			
	0.00*	1.50*	3.00*	4.50*
Dry matter	93.49	92.96	93.24	92.21
Crude protein	20.39	20.21	20.38	20.27
Crude fibre	3.76	3.82	4.03	3.32
Ether extract	4.02	3.65	3.44	4.01
Ash	4.31	6.34	6.77	7.34
NFE	61.01	58.94	58.62	57.27
ME, kcal kg^{-1}	3190.70	3080.50	3057.40	3054.75

* Turmeric and Negro pepper mixture 50:50 ratio; NFE – nitrogen-free extract; ME – metabolizable energy

The effect of turmeric and Negro pepper mixture containing diets on haematological indices of broiler starter is presented in Table 5. Packed cell volume (PCV), Mean cell volume (MCV) and Mean cell haemoglobin (MCH) showed no significant (P >0.05) difference across the groups. Red blood cell (RBC), haemoglobin, mean cell haemoglobin concentration (MCHC) and white blood cells (WBC) differed significantly (P <0.5). The haemoglobin of the broiler starter ranged from 11.13–12.88 g dl^{-1} for 1.00 and 0.50, respectively. The haemoglobin values of the starter birds fed TNPM in their diets were within the normal physiological range of 7.5–13.1 g dl^{-1} for broilers (Mitruka, Rawnsley, 1977), an indication that the diets supported high oxygen-carrying capacity of blood in the birds. The result of the present study is in agreement with 8.65–11.80 g dl^{-1} for broiler starters fed turmeric (*Curcuma longa*) powder and cayenne pepper (*Capsicum frutescens*) reported by Adegoke *et al.* (2018) and 10.24–11.31 g dl^{-1} for broiler chickens fed diets containing ginger and black pepper reported by Aikpitanyi and Egweh (2020). The RBC range of 2.27–3.81×10^{12} L^{-1} reported in this study for broiler starter birds fell within the values of 1.5–3.9×10^{12} L^{-1} reported for apparently healthy broilers by Mitruka and Rawnsley (1977). This may indicate that the utilization of the experimental diets ensured effective transport of haemoglobin through the red blood cells of the broilers. This further gave a clear indication of adequate oxygen transportation within the tissues of the birds for oxidation of digested feeds. The significant (P <0.05) effect obtained in this study for starter broilers fed turmeric and Negro pepper mixture agreed with the results of Adegoke *et al.* (2018) and Aikpitanyi and Egweh (2020) for broiler starter birds fed turmeric (*Curcuma longa*) powder and cayenne pepper (*Capsicum frutescens*) and broiler chickens fed diets containing ginger and black pepper respectively. The mean cell haemoglobin concentration (MCHC) values of 26.38–33.04% reported in this study were within the normal range of 25.3–32.5% reported by Mitruka and Rawnsley (1977). The normal physiological range for the MCHC reported in this study gave a clear indication that the birds were not anaemic. The range of values (9.72–12.74×10^{9} L) reported in this present study for WBC are within 9.7–31.0×10^{9} L^{-1} reported normal physiological range for apparently healthy birds by Mitruka and Rawnsley (1977). This indicated that there were no microbial infections or presence of foreign bodies or parasites in the circulatory system of the experimental birds which further indicates that the feeding of turmeric and Negro pepper mixture in the diets of broilers do not affect the immune system negatively. This corroborates the finding of Adegoke *et al.* (2018) and Aikpitanyi and Egweh (2020) for broiler starter birds fed turmeric (*Curcuma longa*) powder and cayenne pepper (*Capsicum frutescens*) and broiler chickens fed diets containing ginger and black pepper respectively. Jiwuba *et al.* (2017) noted that the white blood cell function is to fight infections, defend the body through phagocytosis against invasion by foreign organisms and produce or transport antibodies in the immune response. The normal physiological values of WBC obtained in this study may suggest a well-developed immune system of the broilers at the starter phase. The highest WBC values recorded for birds fed 1.00 and 1.50% turmeric and Negro pepper mixture groups possibly suggests that Negro pepper and turmeric at this level facilitated immunity of the birds. Earlier studies on the antimicrobial activity of *X. aethiopica* have been reported. Mono- and sesqui-terpene hydrocarbons from *X. aethiopica* have been shown to have antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria, and *Candida albicans* (Tatsadjieu *et al.*, 2003; Asekun, Adeniyi, 2004). Turmeric, however, has been reported to promote brain health, courtesy of its potent antioxidant and anti-inflammatory properties (Adegoke *et al.*, 2018). Curcumin has been reported to (Subash *et al.*, 2011) affect signalling molecules and has been demonstrated to influence about 700 genes. Akram *et al.* (2010) described curcumin exertion on anti-inflammatory activity by inhibiting several different molecules that play an important role in inflammation.

Table 5. Effect of turmeric and Negro pepper mixture containing diets on haematological indices of broiler starter

Parameters	Dietary levels, %				SEM
	0.00*	0.50*	1.00*	1.50*	
Packed cell volume, %	35.67	38.10	37.28	38.78	2.61
Red blood cell, ×10^{12} L^{-1}	2.66^{b}	2.27^{c}	2.32^{bc}	3.81^{a}	0.28
Haemoglobin, g dl^{-1}	11.17^{b}	12.88^{a}	11.13^{b}	12.30^{a}	0.79
Mean cell volume, f	104.61	106.40	104.70	105.16	13.55
Mean cell haemoglobin, pg	28.61	28.29	29.55	30.17	2.41
Mean cell haemoglobin concentration, %	26.38^{b}	33.04^{a}	32.58^{a}	32.58^{a}	4.65
White blood cells, ×10^{9} L^{-1}	9.72^{c}	11.11^{b}	12.07^{a}	12.74^{a}	1.20

$^{a–c}$ means on the same row with different subscript are significantly different (P <0.05); * turmeric and Negro pepper mixture 50:50 ratio

The effect of turmeric and Negro pepper mixture containing-diets on serum biochemistry of broiler starter is presented in Table 6. Total protein, albumin, uric acid, cholesterol and alkaline phosphatase (ALP) showed significant differences across the treatments. 1.50% showed significantly (P <0.05) lower total protein value. The lower total protein in 1.50% may indicate poor absorption of dietary protein. The values are perhaps in agreement with 2.38–5.22 g dl^{-1} recorded by Meluzzi *et al.* (1992) for apparently healthy broilers. Albumin was significantly improved (P <0.05) at 0.00 and 1.50% A range of 1.18 to 2.69 g dl^{-1} recorded in this present study is in agreement with 1.17 to 2.74 g dl^{-1} reported by Meluzzi *et al.* (1992) for apparently healthy broilers. The importance of albumin in blood clotting and transporting of insoluble substances in the blood was enumerated by Fischbach and Dunning (2009).

Table 6. Effect of turmeric and Negro pepper mixture containing diets on serum chemistry of broiler starter chickens

Parameters	Dietary levels, %				SEM
	0.00*	0.50*	1.00*	1.50*	
Total protein, g dl^{-1}	4.53^a	4.17^{ab}	3.66^b	3.05^c	0.21
Albumin, g dl^{-1}	2.69^a	2.56^{ab}	2.11^b	1.18^c	0.37
Globulin, g dl^{-1}	1.84	1.61	1.55	1.87	0.45
Urea, mg dl^{-1}	9.52^a	6.83^{ab}	4.76^b	2.31^c	2.74
Creatinine, mmol L^{-1}	48.98	49.07	47.65	48.87	1.15
Cholesterol, mg dl^{-1}	153.3^a	144.3^{ab}	124.5^b	117.36^c	3.84
AST, U L^{-1}	123.09	120.94	121.85	122.54	3.63
ALT, U L^{-1}	26.84	28.37	28.04	29.51	0.98
ALP, U L^{-1}	48.18^{ab}	49.72^a	47.29^c	47.65^b	1.14

$^{a-c}$ means on the same row with different subscript are significantly different (P <0.05); AST – aspartate aminotransferase; ALP – alkaline phosphatase; ALT – alanine aminotransferase; * turmeric and Negro pepper mixture 50:50 ratio

No significant difference (P >0.05) was observed for globulin but the highest value was recorded for 1.50% (1.87 g dl^{-1}). This implies that the immunity of the broilers was not compromised and the values were numerically improved at 1.50%. From this study, broilers fed a 0.00% diet had better total protein and albumin than the other dietary groups at the chick phase, this is in agreement with the findings of Adegoke *et al.* (2018) but disagrees with the findings of Aikpitanyi and Egweh (2020). The serum uric acid concentration differed significantly (P <0.05) and fell within the physiological value of 1.9–12.5 mg dl^{-1} reported by Clinical Diagnostic Division (1990). Serum uric acid is the main end-product of protein metabolism in poultry and Eggum (1970) associated high blood urea concentration with poor protein quality in the animal. The significant lower uric acid value reported for the treatment groups (0.50, 1.00 and 1.50%) may suggest better utilization of protein and amino acid digestibility. The cholesterol values were significantly different (P <0.05), and decreased linearly with incremental levels of TNPM in the diets. The findings of this study reveal that the TNPM caused a significant reduction in the levels of serum cholesterol, which is in agreement with the reports of Aikpitanyi and Egweh (2020). The significant reduction in serum cholesterol concentration of broilers fed TNPM diets may suggest a general decline in lipid mobilization. In addition, the progressive lower cholesterol values reported in this study with incremental levels of TNPM indicated the ability of the test ingredients in reducing heart-related diseases associated with high cholesterol in the blood. The activities of the liver enzymes; aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in the blood are bio-indicators of liver function and damage (Yildirim *et al.*, 2011). Lumeij (2008) attributed an increase in the concentration of liver enzymes to liver or muscle damage, resulting from the body's response to stress. The non-significant effect reported in this study for AST and ALT and the significantly (P <0.05) lower concentration of ALP for 1.00 and 1.50% may indicate better liver function among the birds.

The effect of turmeric and Negro pepper mixture containing diets on haematological indices of finishing broilers is presented in Table 7. Birds fed 1.50 and 3.00% had higher (P <0.05) packed cell volume values in comparison with those fed 0.00 and 4.5% turmeric and Negro pepper mixture. The packed cell volume concentration (31.86 to 38.00%) obtained in this study however was within the normal physiological range of 25–41% reported by Mitruka and Rawnsley (1977). The result of this present study is similar to the findings of Shivappa-Nayaka *et al.* (2013) who reported that the inclusion of turmeric and combination with neem and vitamin E in the diet of broilers had a significant effect on PCV values. PCV is the quickest and reliable indirect way of evaluating values of red blood cells in a circulating medium and is often used as a modest screening test for anaemia. Birds fed 0.00 and 3.00% turmeric and Negro pepper mixture recorded similar (P >0.05) RBC concentration values while birds fed 1.50 and 4.5% were (P >0.05) similar, but higher (P <0.05) than those fed 0.00 and 3.00% turmeric and Negro pepper mixture. The significant effect recorded in this study for RBC for finisher broilers fed phytogenic mixture agreed with the findings of Adegoke *et al.* (2018). The RBC values (3.00–3.96×10^6 mm$^{3\,-1}$) obtained in this study were slightly higher than the normal physiological range of 1.5–3.9 reported by Mitruka and Rawnsley (1977). Haemoglobin was highest (P <0.05) in birds fed 1.50% when compared with the control diet. The Hb values (11.88–13.02 g dl^{-1}) obtained in this study however were within the normal physiological range of 7.5–13.1 g dl^{-1} reported by Mitruka and Rawnsley (1977). The result of this present study is similar to the findings of Shivappa-Nayaka *et al.* (2013) who reported that the inclusion of turmeric and combination with neem and vitamin E in the diet of broilers had a significant effect on Hb values. The increase in the PCV, Hb and RBC of broiler finisher fed turmeric and Negro pepper mixture indicates an improvement in the health status. This can be attributed to the antioxidant capacities of turmeric and Negro pepper. Red blood cell is formed in the long bones of the body, and sufficient production is dependent on the amount of iron absorbed from food digested. Furthermore, Reece (2009) disclosed the key constituent of erythrocytes as haemoglobin, as it forms about one-third red blood cell content, which, according to Sugiharto *et al.* (2011) generated increased haemoglobin concentration by possibly signalling production of haemoglobin. 1.5% had the highest value for these parameters (PCV, RBC and Hb) which may be in consonance with the adaptation of chickens to antioxidants fed, as well as proper absorption of iron from feed at the finisher phase may explain high values for circulating red blood cells among the treatment group mentioned above. The mean corpuscular haemoglobin (27.40–32.69 pg) and mean corpuscular haemoglobin concentration (28.60–31.78%) values reported in the finisher phase of the present study were within the physiological range of 25.3–33.4 pg and 25.3–32.5% respectively for broiler birds. The within normal physiological range for the MCH and MCHC reported in this study gave a clear indication that the birds were not anaemic, hence, the diets were nourishing and supported erythropoiesis among the experimental birds. The WBC aids to protect the body from pathogens; turmeric and Negro pepper build up immunity (Ali *et al.*, 2016). The turmeric and Negro pepper mixture containing diets increased the WBC of the birds in the current study. This is similar to an earlier report that supplementation of a mixture of phytogenic materials increases WBC concentration in broilers (Adegoke *et al.*, 2018; Aikpitanyi, Egweh, 2020).

Table 7. Effect of turmeric and Negro pepper mixture containing diets on haematological indices of finishing broilers

Parameters	Dietary levels				SEM
	0.00*	1.50*	3.00*	4.50*	
Packed cell volume, %	31.86^{b}	38.00^{a}	37.87^{a}	32.59^{b}	1.54
Red blood cell, $\times 10^{6}$ mm$^{3\ -1}$	3.00^{b}	3.96^{a}	3.20^{b}	3.75^{a}	0.15
Haemoglobin, g dl^{-1}	11.88^{b}	13.02^{a}	12.06^{ab}	12.30^{ab}	0.53
Mean cell volume, f	109.16	107.52	104.01	104.21	2.28
Mean cell haemoglobin, pg	27.40^{c}	32.69^{a}	30.64^{b}	31.10^{ab}	0.76
Mean cell haemoglobin concentration, %	28.60^{b}	31.78^{a}	29.48^{ab}	30.65^{b}	0.54
White blood cells, $\times 10^{9}$ L^{-1}	10.59^{c}	14.41^{b}	16.47^{ab}	18.35^{a}	1.13

$^{a–c}$ means on the same row with different subscript are significantly different (P <0.05); * turmeric and Negro pepper mixture 50:50 ratio

The effect of turmeric and Negro pepper mixture containing diets on serum chemistry of finishing broiler is presented in Table 8. The total serum protein, albumin, globulin, cholesterol and aspartate transaminase (AST) were significantly (P <0.05) influenced by the experimental diets. It was found that the 0.00% group had a significant (P <0.05) increase in total protein in comparison to the other groups. Total serum protein is made up of albumin and globulin. The within normal physiological range of 3.0–4.9 mg dl^{-1} reported by Clinical Diagnostic Division (1990) for apparently healthy birds is an indication that the turmeric and Negro pepper mixture supported good protein digestibility, as high protein in blood is an indicator of protein adequacy. According to Melillo (2013), serum albumin aids in the movement of molecules and the maintenance of blood pressure. A high albumin concentration usually indicates dehydration, whereas a low concentration indicates poor liver function owing to malnutrition or infection. The results of this investigation, which ranged from 2.03 to 2.62 g dl^{-1}, were similar to those published by Meluzzi *et al.* (1992) for apparently healthy broilers, which ranged from 1.17 to 2.74 g dl^{-1}. The fact that albumin concentrations are within normal physiological ranges suggests that the liver and other extrahepatic organs involved in protein synthesis are in good condition. Serum albumin and globulin depend on the availability of dietary protein. Globulin values of 1.40 to 1.81 mg dl^{-1} fell within the reference range of 0.5 to 1.8 mg dl^{-1} reported by Thrall (2007) for *Gallus gallus* species. Since the liver is the site of serum protein production, this could indicate a stronger immune system and better hepatic function. The cholesterol of finishing broilers fed turmeric and Negro pepper mixture containing diets showed a significant decrease with increasing levels of the phytogenic feed additives. However, the cholesterol was within the reported range of 52.00–148.00 mg dl^{-1} by Mitruka and Rawnsley, (1977). This suggests that turmeric and Negro pepper in the treatment diets may promote liver and vascular function by increasing cholesterol transfer to the liver. Ademola *et al.* (2009) found that having an ideal cholesterol level is beneficial to the welfare of animals. The liver enzymes are vital in determining the liver's healthy functioning (Jiwuba *et al.*, 2017). The damaged or diseased liver may cause a rise in the levels of these enzymes. The decreasing AST concentration with increasing levels of inclusion indicates that the liver of birds fed phytogenic feed additives were functioning efficiently. The AST levels obtained in this experiment, however, are within the normal range of 70–220 U L^{-1} reported by Meluzzi *et al.* (1992).

Table 8. Effect of turmeric and Negro pepper mixture containing diets on the serum biochemistry of finishing broiler chickens

Parameters	Dietary levels				SEM
	0.00	1.50	3.00	4.50	
Total protein, mg dl^{-1}	4.49^{a}	4.02^{b}	3.90^{b}	3.87^{b}	0.89
Albumin, mg dl^{-1}	2.61^{a}	2.62^{a}	2.03^{b}	2.06^{b}	0.66
Globulin, mg dl^{-1}	1.88^{a}	1.40^{b}	1.87^{a}	1.81^{a}	0.40
Uric acid, mmol L^{-1}	20.47	20.61	20.39	20.10	0.13
Creatinine, mmol L^{-1}	49.81	50.39	49.19	51.11	0.01
Cholesterol, mg dl^{-1}	124.92^{a}	93.53^{b}	88.56^{bc}	85.18^{c}	6.01
AST, U L^{-1}	48.77^{b}	51.37^{a}	47.55^{bc}	46.59^{c}	0.70
ALT, U L^{-1}	61.40	62.94	61.45	66.46	1.10
ALP, U L^{-1}	78.58	77.25	78.39	77.88	1.03

$^{a–c}$ means on the same row with different subscript are significantly different (P <0.05); Aspartate aminotransferase (AST); Alkaline phosphatase (ALP); Alanine aminotransferase (ALT)

Conclusion

Blood can be used to determine the response of chickens to hazardous substances as well as organ function. It is well known that birds with a healthy (normal) blood composition are more likely to perform well. The results showed that inclusion of turmeric and Negro pepper up to 1.5 and 4.5% levels at starting and finishing phases respectively produced no deleterious effect on haematology and serum constituents of the broilers. In conclusion, the research shows that all dietary additives can have a favourable impact on blood profile at demonstrated levels of inclusion.

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Conflict of interest
The authors have declared that no competing interest exists.

Author contributions
NLA – wrote the first draft of the manuscript, reviewed the experimental design, managed the analyses of the study and performed the statistical analysis
PCJ – guidance and monitoring of experiment, designed the study, wrote the protocol, critical revision on the initial draft and approval of the final manuscript
NTM and FCE – sourced the turmeric and Negro pepper, performed the chemical analyses and carried out literature search.
All authors read and approved the final manuscript.

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