International Journal of Veterinary Sciences and Animal Husbandry 2020; 5(1): 108-114

ISSN: 2456-2912
VET 2020; 5(1): 108-114

www.veterinarypaper.com
Received: 22-11-2019
Accepted: 24-12-2019

Yusuf HU
Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

Aliyu-Paiko M
Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

Correspondence
Yusuf HU
Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

Evaluation of cashew nut meal as phytobiotics in diet of broiler chickens and effects on feed efficiency, growth performance, blood metabolic and antioxidant profiles

Yusuf HU and Aliyu-Paiko M

Abstract
This study evaluated the effects of including cashew nut meal (CNM) in feed on feed efficiency, growth performance and other parameters in broiler chickens during feeding trial. Sixty birds were grouped into three treatments, where T_1 (0% CNM) was fed commercial diet only. T_2 and T_3 were experimental groups maintained on diets containing 2% and 4% CNM, respectively. Growth performance, and feed consumption were monitored weekly. Results revealed that T_2 differed significantly ($P<0.05$) in growth compared toT_1 and T_3. However, no significant ($p>0.05$) difference was noted in blood biochemical parameters. Similarly, the values for serum metabolite were also not significantly affected ($P>0.05$) by the dietary treatments. However, the values of antioxidant enzymes such as superoxide dismutase, and catalase were significantly ($p<0.05$) higher in T_2 and T_3 compare to T_1 while the values of lipid peroxidation significantly ($p<0.05$) decreased with increase in CNM in the diets. Based on results in this study, it may be concluded that CNM supplementation in diets could improve immune parameters.

Keywords: Phytobiotics, cashew nut meal, antioxidant enzymes, haematological parameters

1. Introduction
The economic development of countries is usually accompanied by improvement in their food supply and the gradual elimination of dietary deficiencies [1]. Therefore, the global demand for animal products increases as global population continues to grow and offers potential opportunity to animal producer's worldwide [2]. This potential could be satisfied by increasing production of poultry [3].

Unfortunately, commercial farming systems for broiler chickens to meet increasing population and demand for cheap protein/meat encounter numerous problems. These problems include low capital base, inefficient management, technical and economic inefficiencies, infectious diseases, high costs of feeds, poor quality of day-old chicks, and inadequate extension and training facilities [4]. However, the most prominent among these challenges include outbreak of diseases, high cost of quality feeds and poor quality seeds [5].

Overcoming these challenges and attaining higher body weight with better feed conversion ratio in a short period of time is the main objective of efficient broiler production[6]. This has resulted in excess use of vaccines and antibiotics as growth promoters [7]. Growth promoters are added to feeds of farm animals, which when ingested, improve feed utilization and efficiency. It also enhances growth performance, stimulates the immune system to prevent outbreak of poultry diseases, promote good health, and increase animal performance and overall production cost [8]. However, extensive use of antimicrobials has also resulted in induction of resistant strains of pathogenic microorganisms [9]. Consequently, the use of AGPs in the production of farm animals has been banned since 2006 by the European Union (EU) [10]. This led to the search for natural alternative to antibiotic growth promoters such as phytobiotics [11].

Phytobiotics are well known for their pharmacological effects and are therefore commonly used in conventional and alternative medicine for human use. Phytobiotics also play also an important roles in human nutrition as flavouring and food preservatives [12]. As alternative to antibiotics, phytobiotics are plant derived products added to feed in order to improve

performance of agricultural livestock [13]. Many plants have potentials as phytobiotics and have been used as feed additives in poultry. These have been shown to include Ginger (*Zingiber officinale*), Cloves (*Syzygium aromaticum*), Rosemary, Garlic, and Thyme [14]. Most of these plants contain bioactive compound which is also found in cashew nut [15].

Cashew (*Anacardium occidentale* L) nut is a major industrial and export crops that ranks third in the global production of edible nuts traded worldwide [16]. In 2013, the world production of cashew nut was estimated at 4.39 million tons with Nigeria ranked as second, with about 0.97 million tons [17]. In general, cashew nut kernels are found to possess appreciable levels of vital bioactive compounds that could elicit many health benefits in poultry. Such compounds include carotenoids, lutein, zeaxanthin, thiamin, and fatty acids like stearic acid, oleic acid, and linoleic acid [15].

The present study was therefore, aimed to evaluate the beneficial effects of cashew-nut kernel as additive in broiler feeds to improve feed efficiency, growth performance and immune response in commercially farmed poultry.

2. Materials and Methods

2.1 Source and preparation of cashew nut meal

The cashew nuts were purchased from Lapai central Market, Niger State. The collected nuts were cleaned and processed by hand picking of stones and debris and dried for two weeks under ambient temperature. The kernels were obtained by cracking the shell with a wooden mallet. The kernels were placed on clean trays and dried at ambient temperature for one week. The dried kernels were placed in a thermostatic drying oven (DHG-9202, Gulfex, England) maintained at 50 °C for 48 hours to dry to constant weight. The dried kernels were pulverized with a grinding mill (GX 390-13HP, Guangdong, China) and stored in an air tight plastic container at ambient temperature prior to analysis. The dried meal was labeled as cashew nut meal (CNM).

2.2 Experimental Design and Schedules

A total of sixty-five (65) day-old, broiler chicks were purchased from a commercial hatchery in Minna, Niger State Nigeria and transported to the research facility house of IBB University, Lapai, Nigeria. The birds were acclimated for three days. During the acclimation, the birds were fed commercial starter feed and water was supplied ad libitum under strict biosecurity control, according to previously published protocol [18].

Sixty (60) birds (mean initial weight 75.5g) were randomly distributed into three treatments. Each treatment was fed in two replicates with ten (10) individual chicks per replicate. Treatment T1 was fed with the control diet (commercial feed with 0% cashew nut meal). Treatment T2 and T3 were administered with experimental diets containing 2% and 4% cashew nut meal respectively as additives to the commercial feed. The birds were fed for the six weeks trial period. Growth performance, feed consumption and water intake were monitored weekly, when also cages were cleaned.

2.3 Proximate composition analysis of experimental feeds and cashew nut meal

Experimental diets and cashew nut meal (CNM) were analyzed for Moisture content, crude fat, crude fiber, crude protein, ash content and carbohydrate following standard Association of Official Analytical Chemists AOAC [19] methods

2.4 Feed Efficiency and growth Parameters

Feed efficiency and growth parameters were calculated by applying the appropriate formulae where necessary, from the following:

Feed intake (FI) = total feed intake/number of live chicks

Feed conversion ratio (FCR) = wet weight gain (g)/feed intake (g)

Weight gain (WG %) = $[(W_f - W_i)/W_i] \times 100$

Specific growth rate (SGR %) = $[(\ln W_f - \ln W_i)/T] \times 100$

Where W_f refers to the mean final weight, W_i is the mean initial weight and T is the feeding trial period in days.

2.5 Collection of blood from experimental birds

At the end of the feeding trial (6 weeks), six birds were randomly selected from each treatment. The blood sample was obtained by venipuncture the branchial veins (wing veins) of the experimental birds (broilers), according to method described by Lisa and Leanne, (2013). This was done by plucking a few feathers from the ventral surface of the humeral region of the right wing to expose the veins beneath the skin. The skin was then sterilized with methylated spirit, and was afterwards punctured with 2mm syringe to obtain 2ml of blood sample. The blood sample was transferred into an anticoagulant free test-tube to clot at room temperature. The serum was obtained by centrifugation using an electric desktop lab centrifuge, (EU Plug 220V, Jersey, UK) for 10 minutes at 3,000 rpm. The serum was collected by the use of micro pipette and transferred into anticoagulant free test-tube and stored in refrigerator (1.6) for subsequent analyses.

2.6 Analysis of Liver Biomarker Enzymes

The activities of ALT and AST in the chicken serum were assayed using enzymatic method of Reitman and Frankel[20] and Karmen *et al*. [21] respectively. ALP was assayed using enzymatic procedure of Klein *et al*. [22]

2.7 Determination of Serum metabolic parameters

The serum concentration of sodium was estimated by the method of Maruna [23]. Serum potassium was determined by the method of Terri and Sesin [24]. Total protein, Albumin, creatinine, chloride and urea were determined by method of Bartel and Bohmer [25].

2.8 Assay of Antioxidant Enzyme Activities

Antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) and lipid peroxidation were assayed spectrophotometrically according to the method described by Health and Parker [26].

2.9 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and the means were compared using Tukey test. Statistical significance was set at $P<0.05$. Statistical analyses were performed using SPSS software Version 20.0.

3. Results

3.1 Proximate composition of CNM and experimental feeds

Proximate compositions of CNM and experimental feeds containing graded levels of CNM are presented in Table 1. From the Table, CNM is a good source of protein, fat and fibre. Moreover, no significant difference ($P>0.05$) was recorded in moisture, fat, protein, fiber, ash and carbohydrate content across all the treatments.

Table 1: Proximate composition of CNM and experimental feeds.

Treatments				
Parameters	**CNM**	**0%CNM**	**2% CNM**	**4% CNM**
Moisture %	4.05±0.48	8.44±0.34[a]	8.34±0.37[a]	8.12±0.03[a]
Crude fibre%	2.91±0.49	4.90±0.45[a]	5.50±0.34[a]	5.60±0.19[a]
Ash %	3.10±0.07	4.30±0.35[a]	4.20±0.56[a]	4.09±0.34[a]
Fat %	38.90±0.98	14.70±0.99[a]	14.20±0.68[a]	14.20±0.43[a]
Protein %	28.00±0.05	23.62±0.87[a]	25.12±0.34[a]	27.75±0.95[a]
Carbohydrate%	23.05±0.45	44.04±1.57[a]	42.64±0.43[a]	40.24±0.57[a]

Data are expressed as Mean ±SEM; where mean is average of three replicate measurements.

Data in the same row with different superscript lower case letter are significantly different (p<0.05).

3.2 Weekly growth performance of broiler chickens fed graded levels of CNM

Result of weekly growth performance of broiler chickens fed experimental diets for 6 weeks starter period is summarized in Figure 2. The result clearly indicates no significant difference (P>0.05) in the first 3 weeks of feeding trial across all the treatments. However, we observe a significant (P<0.05) higher weight gain from week 4 to week 6 in broiler chickens supplemented with 2% CNM diet when compared to 4% CNM and 0% free CNM

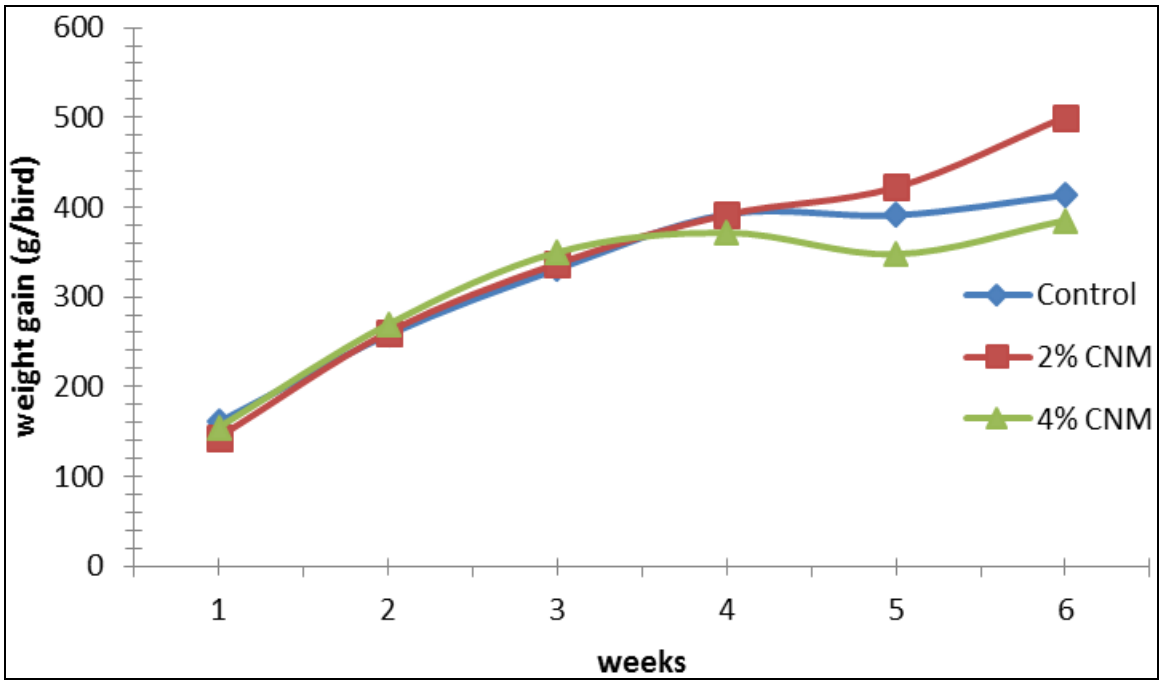

Fig 1: Line graph showing weekly weight gain (g) of broiler chickens reared on graded levels of CNM for 6 weeks

3.3 Growth performance and feed efficiency parameters of broiler chickens fed graded level of CNM

Table 2 presents the result of feed efficiency and growth performance of broiler chickens fed graded level of CNM for 6 weeks of starter period. The data shows better growth performance in broiler chickens fed diet supplemented with 2% CNM than in broiler chickens fed 4% CNM and 0% free CNM with regard to weight gain and relative growth rate (RGR). However no significant (p<0.05) difference was recorded in feed conversion ratio, although 2% CNM supplementation has a better feed conversion ratio than 4%CNM and 0% free CNM.

Table 2: Feed efficiency and growth performance of broiler chickens fed graded level or CNM for 6 weeks starter period

Parameters	**Dietary treatments**		
	0% CNM	**2%CNM**	**4%CNM**
Mean initial weight (g)	77.54[a]	75.53[a]	73.51[a]
Mean final weight (g)	2023.52[b]	2126.53[b]	1944.52[a]
Weight gain (g/bird)	1945.85[b]	2055.93[c]	1878.40[a]
Relative growth rate (%)	2510.77[a]	2721.28[b]	2550.44[a]
Specific growth rate (%)	7.77[a]	7.95[a]	7.80[a]
Feed intake (g)	3614.12[a]	3805.81[b]	3707.29[ab]
Feed conversion ratio	1.86[a]	1.85[a]	1.97[a]

Data are expressed as Mean ±SEM. Data in the same column with different superscript lower case letter are significantly different (p<0.05).

3.4 Liver biomarkers enzymes of broiler chickens fed graded level of CNM

Results of liver biomarker enzymes of broiler chickens fed graded level of CNM for 6 weeks starter period are shown in Table 4 The results of serum concentration of ALT, AST and ALP indicates no significant (P>0.05) differences across all the treatments group when compared.

Table 3: Liver Biomarker Enzymes of broiler chickens fed experimental diets for 6 weeks starter period

	Treatments		
Parameters	**0% CNM**	**2% CNM**	**4% CNM**
ALT (µL)	17.530.14[a]	15.00±0.40[a]	26.70±1.60[a]
AST (µL)	19.13±0.98[a]	14.83±0.32[a]	27.83±2.01[a]
ALP(µL)	16.60±0.79[a]	15.43±1.61[a]	29.36±0.33[a]

Data are expressed as Mean ±SEM; where mean is average of three replicate measurements.

Data in the same column with different superscript lower case letter are significantly different (p<0.05).
AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase.

3.5 Serum metabolic parameters of broiler chickens fed graded level of CNM

Table 4.7 shows the results for serum metabolic parameters of broiler chickens reared on diet either supplemented with or

without CNM. The result of serum metabolic parameters proves that no significant (P>0.05) differences were observed in the serum concentrations of total bilirubin, direct bilirubin, albumin, creatinine, proteins, potassium, chloride, sodium and urea across all the treatments.

Table 4: Serum metabolic parameters of broiler chickens fed graded level of CNM for 6 weeks starter period.

Parameters	Treatments		
	0% CNM	2% CNM	4% CNM
Potassium (mmol/l)	4.26±0.16 [a]	4.06±0.03 [a]	3.70±0.55 [a]
Chloride (meq/l)	60.66±2.60 [a]	57.66±0.90 [a]	59.86±1.81 [a]
Sodium (meq/l)	136.60±13.25 [a]	112.63±6.78 [a]	111.70±4.44 [a]
Urea (mg/dl)	46.90±0.32 [a]	46.50±0.10 [a]	45.43±0.17 [a]
Creatinine (mg/dl)	1.433±0.08 [a]	1.56±0.16 [a]	1.46±0.24 [a]
Total Protein (g/gl)	1.70±0.15 [a]	1.36±0.06 [a]	2.50±0.79 [a]
Albumin(µl)	1.63±0.03 [a]	1.63±0.08 [a]	1.66±0.18 [a]
Total Bilirubin (mg/dl)	1.90±0.45 [a]	2.23±0.46 [a]	1.63±0.28 [a]
Direct Bilirubin (mg/dl)	2.26±0.38 [a]	1.46±0.21 [a]	1.70±0.05 [a]

Data are expressed as Mean ±SEM; where mean is average of three replicate measurements. Data with different superscript are significantly different (p<0.05).

3.6 Antioxidant enzyme of broiler chickens fed graded level of CNM

The serum antioxidant enzyme (SOD and CAT) and LPO in serum of broiler chickens fed graded level of CNM after the 6 weeks of starter period, are summarized in Table 6 From the Table, the activities of serum SOD and CAT were significantly (p<0.05) higher with increased level of CNM in the diet from 2% to 4%. However, LPO profile decreased significantly (P<0.05) with increased level of CNM in the diet when compared to 0% free CNM diets.

Table 5: Antioxidant Enzymes of broiler chickens fed graded level of CNM

Treatments	Treatments		
	0% CNM	2% CNM	4% CNM
SOD (µ/l)	3.30±0.98[a]	4.70±0.30 [b]	5.66±0.69 [b]
CAT (µ/l)	3.10±0.50 [a]	4.60±0.36 [b]	4.26±0.28 [b]
LPO (nmol/ml)	4.70±0.51 [b]	2.60±0.37 [a]	2.00±0.30 [a]

Data are expressed as Mean ± SEM; where mean is average of three replicate measurements.

Data with different superscript are significantly different (p<0.05).
SOD, Superoxide dismutase; CAT, catalase; LPO, Lipid peroxidation assay.

4. Discussion

In the present study, moisture content of CNM is lower than earlier reported by Akinhanmi *et al.* [27]. Little moisture contents usually indicates high shelf life especially for foods that are accurately packaged against external condition[28]. The moistness of cashew nut has been found to vary due to location and environmental reasons [29].
Crude fibre helps to maintain normal peristaltic movement of the intestinal tract, hence diets having lower fibre could cause constipation and may consequently lead to colon disease, piles, cancer and appendicitis [30]. According to Eromosele[31], fibre is useful in maintaining animal health and has been proven to reduce cholesterol level in the body. The crude fibre gotten in this study was in line with the finding of Ogungbenle and Afolayan [32]. This observation suggests that the CNM would offer good dietary fibre in the diet.
The crude protein content for CNM is high at 28% making it a very good source of protein. Protein is a vital component of diet required for the survival of both animal and human of which basic function is to supply suitable amount necessary [33]. Crude protein and metabolizable energy of the experimental diets are sufficient for broiler production in the tropics [34].
Fat content of CNM was recorded to be high indicating that CNM is a good source of fat which can serve as source of energy. According to Lim [35], fat is the principal nutritional component of cashew nuts, as fats act as an energy reserve for the seeds of plants. Although it has been noted that fats are the principal component of all edible nuts, cashews have lower levels of total fats [36].
The Ash content offers an idea of the inorganic content of the sample from where the mineral content could be gotten [37]. The Ash content in this study agrees with earlier finding by Sogunle *et al.* [38], but however higher than the values obtain by Ogungbenle and Afolayan [32].
The determined nutrient content of cashew nut meal gotten in this trial was slightly different from the findings of Fetuga *et al.* [39]. Several factors ranging from the processing method, length of storage and storage facility, the type of soil on which the crop was grown and specie differences could be liable for such variations [40]. The result obtained from this study is thus most likely due to the quantity added since according to Windisch *et al.* [41] addition levels of phytobiotics to poultry diets differ between 0.1 and 40 g/kg for dried products and plant extracts. Therefore the inclusion of CNM up to 4% did not significantly change the nutrient composition of all dietary treatments compared to the control
The significant (p<0.05) higher weight gain observed for broiler chickens fed 2% CNM in Table 4.3 could be due to adequate proportion of CNM in the diet. This could have provided a helpful balance of amino acid for the birds[42]. Also higher weight gain in 2% CNM could be due to improved palatability while the 4.0% CNM could have slightly depressed appetite on the broilers.
Generally, better feed conversion ratio were gotten in all treatments, but the group supplemented with 2% CNM had the best nutrient utilization, although no significant difference (p>0.05) was observed across all treatements. Better feed conversion ratio of the broiler chicks fed with diet containing CNM additive could be ascribed to the antibacterial properties of these additive, which may have resulted in better absorption of the nutrients in the gut and excellently leading to improvement in feed conversion ratio of the treatments group [43].
AST and ALT are biomarkers of hepatic integrity, and to a certain level can be used to assess the extent of hepatocellular damage [44]. The ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST[45]. According to Hansen *et al.* [46], ALT and AST are two major enzymes which are quantitatively important in transamination of amino acids in the liver and kidney. ALP are often used to assess the integrity of plasma membrane and endoplasmic reticulum [47]. In the present study, the serum AST, ALT and ALP activities reported were not significantly affected (P>0.05) and was in line with the finding of Ekpenyong and Biobaku [48], who stated that the values of AST, ALP and ALT are normally low in blood but become high when there is occurrence of liver damage by toxic substances. Therefore, the activity reported in this study followed the reported pattern signifying that the broilers were not subjected to any environmental challenges.

Sodium, chloride and potassium are major anions which are important in maintaining cation/anion balance between intra- and extracellular fluids. These electrolytes are therefore essential to the maintenance of proper hydration, osmotic pressure, acid/base equilibrium, bone formation and integrity [49]. Ions are very essential for any organism because they are involved in most biological processes [50], and responsible for the maintenance of osmotic pressure in blood [51]. The values of sodium, potassium and chloride obtained in this study did not differ significantly, indicating that addition of CNM up to 4% did not damage the kidney.

Creatinine is very important in determining the synthetic and excretory roles of the kidney and liver [52]. According to Kaptan and Szabo [53], about 50% of kidney function must be lost before a rise in the serum creatinine can be detected. Creatinine levels in the serum were however, not significantly (p>0.05) influenced by dietary treatments; thus indicating that there was no renal damage or muscle wastage that is attributed to inclusion of CNM in the diets of broiler chickens used in this study.

The levels of metabolic waste product are considered to be a significant factor in assessing the state of the kidney and some other organs in animals [54]. The level of total protein, electrolytes and creatinine plays important roles in determining the synthetic and excretory roles of the kidney and liver [52]. It is noted that the quantity of total protein is dependent on the rate of protein synthesis and its rate of degradation; the quantity of protein may also be affected by impaired incorporation of amino acid in the polypeptide chains [55]. The total protein reported was within the range reported by Abu *et al*. [56].

The antioxidant enzymes, Superoxide Dismutase, Catalase, and Glutathione Peroxidase are considered the first line of antioxidant defense mechanism against oxidative stress by reactive oxygen species, to protect cells from oxidative damage by the free radical process [57]. The SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite [58]. Catalase is a ubiquitous enzyme that catalyzes the breakdown of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production [59, 60, 61]. In the present study, the antioxidant assay revealed that the inclusion of CNM in the diet of broilers increased the activity of serum superoxide dismutase (SOD) and catalase. The increased serum activities of catalase and SOD as observed in this study suggest that CNM has an *in vivo* antioxidant activity that is capable of ameliorating the effect of ROS in biologic system [61, 62]. Furthermore, the rise in the activities of the antioxidant enzymes is a sign of enhanced scavenging of free radicals in the blood. The interplay between free radicals, antioxidants, and diseases in cells, tissues and organisms is important in maintaining health, aging and age-related diseases [63].

Also, ROS react with all biological substances; however, the most vulnerable ones are polyunsaturated fatty acids. Reactions with these cell membranes constituents lead to lipid peroxidation (LPO) [62]. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptor [64]. In the present study, the level of LPO in the CNM treated groups decreased in a dose dependent manner when compared to the control. This decrease in the TBARS levels may indicate increase in the activities of glutathione peroxidase and hence inactivation of LPO reactions [65]. Some of the phytochemical constituents of the CNM may be responsible for the antioxidant activity as demonstrated in the study.

5. Conclusion

Inclusion of CNM up to 4% in starter feeds of broiler chickens did not affect feed consumption and growth performance of the broilers. CNM supplementation significantly improved antioxidant enzyme activity of broiler chickens whereas serum biochemical analysis and serum metabolic parameters were not significantly affected across all the treatment.

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