GROWTH RESPONSE, HAEMATOLOGICAL INDICES AND SERUM BIOCHEMICAL PROFILES OF FINISHER BROILERS FED VARYING LEVELS OF *CALOCYBE INDICA* SUPPLEMENT (CIS)

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ABSTRACT

This study aimed to assess the impact of different levels of *Calocybe indica* supplement on the growth traits, haematological indices, and serum biochemical profiles of finisher broilers. A total of 120 Cobb 500 day-old chicks were utilized and randomly distributed into four dietary groups: T1 (control), T2 (0.5% *Calocybe indica* supplement), T3 (1.0% *Calocybe indica* supplement), and T4 (1.5% *Calocybe indica* supplement). Each treatment had five replicates, with six birds per replicate. Feed and water were provided ad libitum throughout the experimental period. Data on body weight, feed intake, feed conversion ratio, hematological, and serum biochemical indices were analyzed using analysis of variance in a completely randomized design. The results indicated that birds in T_3 group (1.0% *Calocybe indica* supplement) exhibited the highest weight gain of 1.66±0.04 kg, which significantly ($p < 0.05$) surpasses the other treatment groups. Hematological analysis revealed that dietary CIS significantly ($p < 0.05$) improved Packed Cell Volume, Red Blood Cell count, and hemoglobin levels. In terms of serum biochemical indices, *Calocybe indica* supplement significantly ($p < 0.05$) reduced cholesterol, low-density lipoprotein, triglycerides, aspartate aminotransferase, and alanine aminotransferase levels, while significantly ($p < 0.05$) increasing high-density lipoprotein and total protein levels. The findings revealed that *Calocybe indica* supplement improved growth traits and enhanced hematological and serum biochemical profiles in broiler chickens. In conclusion, incorporating *Calocybe indica* supplement at an optimal level of 1.0% in broiler diets improves weight gain, supports erythropoiesis, and enhances overall meat quality.

Key: Broiler, *Calocybe indica*, Growth, Haematology, Serum biochemistry

1.0 INTRODUCTION

Poultry industry has experienced significant growth and industrialization worldwide (FAO, 2022). Broiler production, in particular, has expanded substantially, driving global meat consumption and contributing to the industry's profitability, a trend expected to continue into the next century (Makonnen *et al*., 2019; Newswire Globe, 2019). This growth can be attributed to various factors, including high nutritional value of broiler meat, appealing taste, low fat content, short production cycles, cost-efficiency, and affordability, which make it accessible even to economically disadvantaged populations (Petracci *et al*., 2015). Poultry industry has thrived so well recently though not without challenges. Thus, report has it that feed additives such as antibiotics are widely used in the poultry industry to enhance production traits and maintain animal health (Ahiwe *et al.,* 2018). However, the sub-therapeutic use of antibiotics as growth promoters (booster) has raised concerns about drug resistance and residues in meat. These issues have led to restrictions on antibiotic use in many countries of the world. This has driven the search for alternative strategies to improve broiler performance and immunity using phyto (natural) additives. One promising approach involves incorporating *Calocybe indica* (milky mushroom) as a natural supplement to enhance productivity and improve the immunity of broiler chickens.

Blood indices as we know, have been widely used in nutritional research to analyze biochemical constituents and hematological profiles of livestock animals. The examination of blood is a well-established method for assessing the physiological, pathological, nutritional, and overall health status of farm animals (Adedokun *et al*., 2017). Routine blood sample collection and analysis facilitate the evaluation of haematological responses to nutrition and disease. Variations in blood composition, when compared to standard values, serve as indicators of an animal's metabolic status and the quality of feed provided (Nara *et al*., 2018). Thus, it is a valuable tool in detecting nutritional disorder as it provides insights into an animal's health, nutrient deficiencies, and growth patterns over time. Researchers have extensively examined these profiles to facilitate clinical assessments of an animal's health. Given its diverse constituents, blood serves as a crucial medium for clinical diagnostics and nutritional evaluations (Charles *et al.,* 2019).

Milky Mushrooms (*C. indica*) are epigeous macro fungi characterized by an umbrella-like structure where spores develop. Their growth occurs in two phases: the vegetative stage (mycelia) and the reproductive stage (fruiting bodies) (Sánchez *et al.,* 2017). Across the world, more than 14,000 mushroom species exist, but only around 2,000 are classified as edible. Consequently, approximately 200 species are cultivated commercially for human consumption and therapeutic applications (Mleczek *et al.,* 2018; Garofalo *et al*., 2017). Moreover, edible mushrooms are in high demand as a staple food due to their desirable texture, flavor, medicinal benefits, and tonic properties. C. *indica* is Rich in protein, vitamins, and minerals. It is a cholesterol-free food source with appealing flavors. It is particularly abundant in thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, and ascorbic acid (Sobita *et al*., 2019). Despite its nutritional and therapeutic potential, limited research has been conducted on its use as a dietary supplement for broilers. This study was designed to evaluate the growth response, hematological profiles and serum biochemical indices of broiler chickens fed diets supplemented with varying levels of CIS.

2.0 MATERIALS AND METHODS

2.1 Animal welfare statement: The authors affirm that the research adhered strictly to the ethical guidelines governing studies of this nature. This study was conducted in accordance with the guidelines for the care and use of animals in research, as approved by the Institutional Animal Care and Use Committee of the University of Nigeria, Nsukka (Approval number: UNN/IACUC/2023/001).

2.2 Research trial location: The research trial was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research farm University of Nigeria Nsukka. Its greatest plateau altitude is 443 m, while its minimum elevation is 207 m above sea level. Its annual rainfall ranges from 1680 mm to 1700 mm, and its relative humidity is from 73.1% to 76.6% (Energy Centre, UNN 2018). Eight weeks were allotted to the trial.

2.3 Substrate Preparation and *C. indica* Production

The substrate for milky mushroom (*C. indica)* production was done using palm bunch, the palm bunches were properly composted, pasteurized, or sterilized to eliminate contamination. Thereafter, they were spawned and the substrate bags were incubated in a controlled environment until complete mycelia colonization in a dark room. The substrates were exposed to air, light and humidity after colonization to induce fruiting body formation. The mature fruiting bodies were harvested by cutting near the base.

2.4 Preparation of *Calocybe indica* Supplements (CIS)

Milky mushrooms *(C. indica)* and their substrates were obtained from the Department of Crop Science Research Farm, University of Nigeria, Nsukka. The substrate, which composed of fermented palm bunch and *C. indica* were dried and blended together at the Greenhouse unit of the Department of Crop Science for two days. Following the drying process, they were milled into fine particles using a milling machine within the same Department, resulting in the production of *calocybe indica* supplement (CIS).

2.5 Experimental Diets: For the research trial, iso-nitrogenous and iso-caloric experimental rations were formulated. The finisher feeds were fed to the animals from day 28–to- day 56. The feeds were formulated (as detailed in Tables 1) to meet the dietary nutrient requirements of the birds (NRC 1994). Four dietary treatments were prepared by incorporating the CIS at four different levels.

2.6 Research Chickens and Management Procedures: A total of 120 day-old Cobb 500 chickens were used for the research trial. These chickens were randomly assigned to four dietary groups T1 (Control), T2 (0.5%), T3 (1.0%) and T4 (1.5%). Each dietary group was further divided into five replicates, with each replicate consisting of 6 birds. The brooding pen and equipment's were washed thoroughly with detergent and disinfectant few days before the arrival of the day old chicks (DOC). Dry wood shavings were spread on the pen floor as bedding material, and the pen was preheated with a charcoal heater a day before the arrival of the DOC. The chicks were provided with vitamins and anti-stress in fresh drinking water. Vaccination against Gumboro and Newcastle diseases were administered at specified age intervals. High hygiene and strict biosecurity measures were thoroughly observed. Feed and water was provided *ad-libitum* through-out the experimental period

2.7 Growth Performance: The growth traits assessed were the body weight, feed intake, and feed conversion ratio. Daily feed intake was calculated by subtracting the feed refusal from the provided feed and dividing the result by the number of birds in each pen/replicate. Live weight of all birds was measured weekly. Feed and live weight measurements were conducted using a 10,100 g (10.1 kg) capacity precision weighing balance (A and D Weighing GF-10 K industrial balance, Japan). The feed conversion ratio was calculated by dividing the feed consumed by the weight gain.

2.8 Slaughter Procedure: At the end of the feeding trial (day 56), the chickens were sent to the abattoir at the Animal Science Teaching and Research Farm, UNN, for slaughter. The chickens were fasted for 12 hours prior to slaughter and were gas stunned. The gas stunning process involved initially exposing the chickens to relatively low concentrations of carbon dioxide (< 40% by volume in air) to render them unconscious, followed by exposure to a higher concentration (approximately 80% to 90% by volume in air). At the abattoir, the chickens were hung upside down by their feet on a movable metal rack. They were then sacrificed by cutting the jugular vein with a sharp knife and allowed to hang until bleeding ceased.

2.9 Haematological and serum Biochemical Analysis

Blood collection for hematological and biochemical analysis were done at the end of the feeding trial, blood samples were collected for the analysis. The blood samples were taken from the wing vein of the bird using disposable needle and syringe.

2.9.1 Hematological Indices: At the conclusion of the trial, three birds from each group had an auxiliary vein piercing to provide blood samples for an anticoagulant tube containing EDTA. After collection, the following parameters were examined: PCV, RBC and WBC counts were performed using the upgraded Neuberger hemocytometer and hemoglobin concentration (Hb) was determined according to the guidelines provided by Baker and Silverton (1985). A compound microscope was used to count the platelets. While the differential Leucocyte count were prepared using the method as described by Barbara (1975).

2.9.2 Biochemical Analysis:

In serum biochemical analysis, 3mls of blood samples were taken from five birds in each treatment from the wing vein of the birds using disposable needle and syringe. The samples thereafter were frozen for processing and centrifuged for 15 minutes at 1500 rpm to fix the blood. The following liver enzymes and lipid profiles were determined using the methods as described by Bahman, *et al*. (2011); alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol, triglycerides (TAG), Glucose and Total protein

2.12 Data collection

Data collected were subjected to analysis of variance (ANOVA) in a Completely Randomized Design (CRD) using SPSS version 17 while statistically different means were separated using Duncan's new multiple range test as found in the statistical package/ software (Duncan, 1955).

Table 1: Proximate Composition of the Experimental Diet

Ingredients	T1 (Control)	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)
CIS	0	0.5	1.0	1.5
Cassava Chips	30	30	30	30
Maize	13	13	13	13
Soybean Cake	25	24.5	24	23.5
Palm Kernel Cake	22	22	22	22
Fish Meal	5	5	5	5
Bone Meal	4	4	4	4
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vit. Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Crude protein %	18.46	18.37	18.27	18.18
Energy kcal/kg	2852.61	2850.20	2848.39	2845.99
Crude fibre	3.95	3.94	3.90	3.92

Provided the following per kg of feed: vitamin A, 10,000iµ; vitamin D2, 2000iµ; vitamin E, 6iµ; vitamin K, 2mg; riboflavin, 4.2 mg; vitamin B12, 0.01mg; pantothenic acid, 5mg; nicotinic acid, 20mg; folic acid, 0.5mg; choline, 3mg; Fe, 20mg; Mg, 56mg; Cu, 1.0mg; Zn, 5.0mg; Co, 1.25mg; Iodine, 0.8mg.

Table 2: Proximate Composition of *Calocybe indica* Supplement (CIS)

Parameters	Values
Dry matter	94.60
Crude protein	17.60
Ether extract	5.90
Ash	12.83
Crude fibre	11.73
Nitrogen free extract	46.54

3.1 RESULTS

Table 3: Growth Response of Finisher Broiler Chickens fed Varying Levels of Dietary *Calocybe indica* Supplement (CIS)

Parameters	T1 (Control)	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)	P Value
IW (g)	1.18 ±1.89	1.18±1.06	1.19±1.62	1.19±1.75	0.72
FWT(kg)	2.40^{c}± 1.76	2.76^{b}± 0.46	2.85^{a}± 1.66	2.31^{d}± 1.77	0.00
FI(kg)	4.39± 0.95	4.13± 1.79	4.32± 1.10	4.21± 0.76	0.78
WTG(g)	1.22^{c}± 0.63	1.57^{b}± 1.66	1.66^{a}±0.04	1.18^{d}± 1.18	0.00
FCR	3.59^{a}± 0.04	2.63^{b}± 0.15	2.60^{b}± 0.25	3.57^{a}± 0.94	0.00
FC/KG(₦)	500± 0.00	520± 0.00	512± 0.00	528± 0.00	0.56
FC/KG WTG(₦)	1795.33^{a}± 1.40	1367.60^{b}± 1.23	1337.20^{b}± 1.60	1884.96^{a}± 1.78	0.00
Survival %	94.67± 0.00	91.67±.00	100±1.33	100± 0.00	0.44

a, b, c, d means on the same row with different superscripts are significantly ($p<0.05$) different.

IW - Initial weight, FWT - Final weight, FI - Feed intake, WTG - Weight gain, FCR - Feed conversion ratio, FC/kg - Feed cost per kg, FC/kg WTG - Feed cost per kg weight gain, Dressed % - Carcass percentage weight

RESULTS

The result showed that there were significant ($p<0.05$) effects on the following parameters; FWT, WTG, FCR, FC/KG WTG and Dressed %. Meanwhile, there was no significant effect on IW, FI, FC/KG, and Survival Percentage.

The results for FWT showed that T_3 (2.85 ± 1.66kg) was significantly ($p<0.05$) higher than the other treatment means while T_4 (2.31 ± 1.77kg) had the lowest value which was significantly different from the other treatment groups. The results for WTG followed the same trend as FWT. T_3 (1.66 ±0.04kg) was significantly ($p<0.05$) higher than the other treatment means while T_4 (1.13 ± 1.18kg) had the lowest value which was significantly different from the other treatment groups. The results for FCR showed that T_4 (3.75± 0.94) and T_1 (3.59 ± 0.04) were similar and significantly ($p<0.05$) higher than T_3 (2.60 ± 0.25) and T_2 (2.63 ± 0.15) which were statistically the same. The results for FC/KG WT showed that T_4 (₦1971.60) and T_1 (₦1793.33) were themselves similar but significantly ($p<0.05$) higher than T_3 (₦1337.20 ± 1.60) and T_2 (₦1367.60 ± 1.23) which were statistically the same.

Table 4: Haematological Profiles of Finisher Broiler Chickens fed Varying Levels of Dietary *Calocybe indica* Supplement (CIS)

Parameters	T1(Control)	T2(5g/kg)	T3(10g/kg)	T4(15g/kg)	P. Value
PCV (%)	34.50^{c}±0.29	38.50^{b}±0.29	41.0^{a}±0.58	40.50^{a}±0.29	0.000
RBC($x10^{12}/mm^3$)	8.72^{c}±0.07	9.89^{b}±0.19	10.79^{a}±0.08	10.74^{a}±0.06	0.000
WBC ($10^{12}/mm^3$)	8228±82.65	8300±57.74	8550±44.34	8600±44.34	0.460
HB (g/dl)	9.60^{d}±0.12	9.25^{c}±0.09	10.30^{b}±0.06	10.70^{a}±0.06	0.000
Platelet (%)	14400^{b}±75.47	15000^{a}±57.74	1445^{b}±86.60	15050^{a}±86.60	0.001
Lymphocyte (%)	79.50^{a}±0.29	72.50^{b}±1.44	77.50^{a}±1.44	76.50^{a}±0.87	0.013
Monocyte (%)	2.00^{b}±0.00	3.00^{a}±0.00	1.00^{c}±0.58	2.00^{b}±0.00	0.009
Eosinophil (%)	1.00±0.58	1.50±0.29	0.67±0.33	1.00±0.00	0.485
Basophil (%)	0.67^{ab}±0.33	1.00^{a}±0.00	0.67^{ab}±0.33	0.00^{b}±0.00	0.008

a, b, c, d means on the same row with different superscripts are significantly ($p<0.05$) different.

KEY: PCV= Pack cell volume, RBC= Red blood cell, WBC= White blood cell, HB= Hemoglobin, Platelet, Lymphocyte, Monocyte, Eosinophil, Basophil.

The result showed that there were a significant ($p<0.05$) effect on the following parameters; PCV, RBC, HB, Platelet, Lymphocyte, Monocyte and Basophil. The treatment however had no significant ($p>0.05$) effect on the WBC and Eosinophil. Though, the treatment groups were better than the control, the results showed that the values were within the normal range of the broilers of their age and size. The results for PCV showed that T_3 (41.0±0.58%) and T_4 (40.50±0.29%) were statistically similar and had the highest value which were significantly ($p<0.05$) higher than T_2 (38.50±0.29%). T_1 had the lowest value of 34.50±0.29% which was significantly lower than the other treatment groups. The RBC followed similar trend with PCV. The result for Hb showed that T_4 (10.70±0.06 g/dl) was significantly ($p<0.05$) higher than the other treatment means while T_1 (9.60 g/dl) had the lowest value which was significantly different from the other treatment groups. The result for Platelet showed that T_2 (15000±57.74%) and T_4 (15050±86.60%) were themselves similar but differed ($p<0.05$) significantly from T_1 (14400±75.47%) and T_3 (1445±86.60%) which were themselves similar. The result for

Lymphocyte showed that T_1 (79.50±0.29%), T_3 (77.50±1.44%) and T_4 (76.50±0.87%) were statistically similar but differed ($p<0.05$) significantly from T_2 (72.50±1.44%) which had the lowest value across the treatment groups.

Table 5: Serum Biochemical Indices of Finisher Broiler Chickens fed Varying Levels of Dietary *Calocybe indica* Supplement (CIS)

Parameters	T1(Control)	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)	P Value
ALT (IU/L)	24.50^{a}±0.29	21.00^{b}±0.58	20.50^{ab}±0.29	19.50^{c}±0.29	0.00
AST(IU/L)	39.50^{a}±0.29	37.00^{b}±0.58	35.00^{c}±0.57	35.00^{c}±0.28	0.00
ALP(IU/L)	59.00±0.58	55.00±0.57	56.50±0.87	55.00±0.58	0.08
T. Cholesterol (mg/dl)	152.00^{a}±2.31	98.00^{bc}±2.31	103.00^{b}±2.89	92.00^{c}±1.16	0.00
HDL(mg/dl))	26.50^{c}±0.87	74.50^{a}±4.33	60.50^{b}±0.87	62.00^{b}±1.16	0.00
LDL (mg/dl)	51.50^{a}±1.44	26.00^{b}±1.56	23.50^{b}±0.86	27.00^{b}±1.15	0.00
TAG (mg/dl)	161.00^{a}±1.73	101.50^{b}±0.21	102.00^{b}±2.31	102.50^{b}±0.87	0.00
T- Protein (mg/dl)	3.76^{c}±0.10	6.89^{a}±0.03	6.29^{b}±0.21	6.64^{ab}±0.17	0.00
Glucose (mg/dl)	101.50^{a}±1.44	67.00^{b}±1.73	71.50^{b}±5.49	72.00^{b}±1.15	0.00

a, b, c, d means on the same row with different superscripts are significantly ($p<0.05$) different KEY: ALT= Alanine transaminase, AST= Aspartate transaminase, ALP= Alkaline phosphatase, Total cholesterol HDL= High density lipoprotein, LDL= Low density lipoprotein, TAG= Triacylglyceride, T-Protein = Total protein.

The treatment showed that there were significant ($p<0.05$) effect on the following parameters; ALT, AST, T-Cholesterol, HDL, LDL, TAG, T-Protein, and Glucose. The treatment however had no significant ($p>0.05$) effect on ALP. The results of ALT showed that T_1 had the highest value of 24.50±0.29 IU/L which was significantly ($p<0.05$) higher than the values of T_2 (21.00±0.58 IU/L), T_3 (20.50±0.29 IU/L) and T_4 (19.50±0.29 IU/L). The results of AST showed that T_1 had the highest value of 39.50±0.29 IU/L which was significantly ($p<0.05$) higher than the values of 37.00±0.58 IU/L, 35.00±0.57IU/L and 35.00±0.28 IU/L recorded for T_2, T_3 and T_4 respectively. The results for total cholesterol showed than T_1 had the highest value of 152.00±2.31 mg/dl which was significantly ($p<0.05$) different from the value of 103.00±2.89 mg/dl and 98.00±2.31 mg/dl recorded for T_3 and T_2 respectively that were themselves similar. While T_4 had the least value of 92.00±1.16 mg/dl which was lower than other treatment groups. The results for HDL showed that T_2 had the highest value 74.50±4.33 mg/dl which was significantly ($p<0.05$) higher than T_4 (62.00±1.16 mg/dl) and T_3 (60.50±0.87 mg/dl) which were themselves similar but differed significantly from the value of T_1 (26.50±0.87 mg/dl). The result for LDL showed that T_1 (51.50±1.44 mg/dl) had the highest value which was significantly ($p<0.05$) different from T_2 (26.00±1.56 mg/dl), T_3 (23.50±0.86 mg/dl),) and T_4 (27.00±1.15 mg/dl) which were themselves similar.

3.2 DISCUSSION

3.2.1 Growth Response of finisher broilers fed diets containing varying levels of CIS

The study investigates the impact of different inclusion levels of CIS in broiler chicken diets on growth traits, haematological indices and serum biochemical profiles of finisher broilers. The findings revealed that incorporating CIS into broiler diets improved growth performance when compared with the control diet and this could be attributed to rich nutritional and phytochemical content of CIS. These results align with prior research by Rathore *et al*. (2021) which reported enhanced weight gain in broilers fed *C. indica*-supplemented diets. The study suggests that an optimal inclusion level of approximately 0.1% of CIS in broiler diets enhanced growth and weight gain, while higher level of 1.5% resulted in lower body weight gain and lower feed conversion efficiency. This underscores the importance of determining the precise inclusion level to achieve the best results. While the findings support the potential benefits of *C. indica*, they should be interpreted in the context of other studies on mushroom-based feed additives. Some studies reported positive outcomes, while others show mixed or inconsistent results, likely due to variations in mushroom species, type of substrates and inclusion levels.

Additionally, the study corroborates research by Fouad *et al.* (2022), who observed improved broiler performance with dietary supplementation of oyster mushroom (*Pleurotus ostreatus)*. However, it is in contrast with the findings by Dooley *et al.* (2022) and Ebrahimi *et al.* (2021) that reported no significant effects of some mushroom species on broiler performance. These discrepancies highlight the need to consider multiple factors, such as mushroom type and dosage, when evaluating mushroom-based feed additives.

3.2.2 Hematology of broiler birds fed diets containing varying levels of CIS

The results showed that increasing the level of CIS in the diet had a significant impact on several hematological parameters. Specifically, the packed cell volume (PCV), red blood cell (RBC) count and hemoglobin (HB) concentration improved significantly with higher levels of CIS compared to the control group. These findings were consistent with recent work of Gao, *et al.* (2018) who reported that supplementing broiler diets with oyster mushroom (*Pleurotus ostreatus*) improved the hematological profile, including PCV and RBC count. Similarly, Chitsaz *et al*., (2018) found that adding shiitake mushroom (*Lentinula edodes*) to broiler diets enhanced the hemoglobin levels and immune response. The observed increase in PCV, RBC and HB in this study suggests that CIS may have compounds that support erythropoiesis. These hematological improvements could potentially enhance the overall health and performance of broiler chickens. The observed increase in packed cell volume (PCV), red blood cell (RBC) count, and hemoglobin (HB) levels in the broilers fed varying levels of CIS suggest an enhancement in their oxygen-carrying capacity and consequently, their overall physiological performance. Improved oxygen transport can lead to better nutrient utilization, growth, and productivity in broiler chickens. The elevated platelet counts in the groups receiving CIS could be indicative of enhanced blood clotting and wound healing capabilities. This finding is particularly important in the context of commercial broiler production, where birds may be susceptible to injuries or stress-related conditions that could impair their overall health.

Furthermore, the changes in the differential leukocyte counts (lymphocytes, monocytes, eosinophils, and basophils) observed in the study suggest that CIS may have immunomodulatory properties. While the overall white blood cell (WBC) count did not differ significantly, the shift in the proportions of specific leukocyte types could indicate an influence on the immune system's response to pathogens or inflammatory stimuli. These findings align with the current trend of research in exploring the potential health-promoting properties of CIS and their bioactive compounds, such as polysaccharides, phenolic compounds, and terpenoids. Several studies have reported antioxidant, immunomodulatory, anti-inflammatory, and antimicrobial activities of various mushroom (Mallick, *et la*., 2019; Olusegun and Olutope, 2020). It is important to note that the observed hematological effects may be dose-dependent, as the study showed varying degrees of impact across the different levels of CIS.

3.2.3 Serum biochemistry of finisher broilers fed diets containing varying levels of CIS

This research compares the serum biochemical parameters of finisher broiler birds fed diets containing varying levels of CIS. The results suggest that dietary CIS significantly influenced various serum biochemical indices such as liver enzymes (ALT, AST, ALP), lipid profile (total cholesterol, HDL, LDL, triglycerides), total protein, and glucose levels. This study aligns with the growing interest in exploring an alternative feed supplement that will serve as functional feed additives in poultry diets. The findings of this study contribute to the understanding of how *C. indica* supplement influences broiler health and metabolism. The observed changes in liver enzyme levels, lipid profile, and glucose levels suggest potential hepatoprotective, hypolipidemic, and hypoglycemic effects of CIS. These effects could be attributed to the bioactive compounds present in CIS such as polysaccharides, phenolic compounds, and antioxidants (Chakraborty *et al.,* 2016; Rana *et al*., 2021). The decreased levels of liver enzymes like ALT, AST, and ALP in the treatment groups (T_2, T_3, and T_4) compared to the control group (T_1) suggest a potential hepatoprotective effect of CIS. Elevated levels of these enzymes are generally associated with liver damage or dysfunction. The reduction in enzyme levels observed in this study could indicate that CIS may help protect the liver from oxidative stress or other harmful factors. This finding is consistent with the earlier work of Wasser, 2017 who observed the hepatoprotective properties of various mushroom species and their antioxidant and anti-inflammatory activities.

Lipid Profile: The significant reduction in total cholesterol, LDL and triglyceride levels, coupled with an increase in HDL cholesterol levels in the treatment groups, suggest that CIS has beneficial effect on the lipid profile of broiler chickens. This hypolipidemic effect could be attributed to the presence of bioactive compounds in CIS, such as polysaccharides and phenolic compounds, which are known to have cholesterol-lowering and anti-hyperlipidemic properties (Rana *et al.,* 2021). This finding is particularly relevant as high cholesterol and lipid levels in poultry can negatively impact on meat quality and human health. More so, this study also observed a reduction in glucose levels and an increase in total protein levels in the treatment groups compared to the control group. The hypoglycemic effect of CIS could be beneficial in regulating blood sugar levels and preventing metabolic disorders in broiler birds. The increased total protein levels may indicate improved protein synthesis and utilization, which could positively impact on growth and muscle development. These findings suggest that *C. indica* could potentially be used as a functional feed additive in broiler diets, offering various health benefits and improving the overall quality of broiler meat.

4.0 CONCLUSION

This study has revealed the potential benefits of incorporating CIS to broiler finisher diets. Moderate inclusion level of 1.0% CIS significantly improved body weight gain, feed conversion efficiency and reduced feed cost/kg weight gain when compared to the control group. More so, CIS positively impacted the hematological indices and serum biochemical profiles of the broiler chickens, suggesting its possible immunomodulatory and hepatoprotective effects. It is therefore, recommended to include CIS at the rate of 1.0% in finisher broiler diets for enhanced growth traits, better health conditions and increased profit margins.

Acknowledgment

The authors wish to acknowledge the Department of Animal Science, University of Nigeria, Nsukka for granting them access to her facilities for this experiment.

We want to also acknowledge Ojinnaka, Darlington Chidubem for assisting in feeding the animals and in data collection.

Conflict of interest

The authors declare that there is no conflict of interest

Author's contribution

OEO: Design and Statistical analysis, UCM: Drafting of the manuscript, NCC: Supervision and Writing of the Manuscript, ANW: Review Editing and Experimentation, NOC: Assisted Data Collection, OMC: Assisted in review and data analysis. OSE: Review and editing of the manuscript, JET: Assisted in feeding of the birds and data collection

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