Haematology and Serum Biochemistry of Broiler Chicks Fed Mushroom Meal at Varying Levels

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DOI: https://doi.org/10.51244/IJRSI.2025.1210000304

Received: 27 October 2025; Accepted: 03 November 2025; Published: 20 November 2025

ABSTRACT

An eight (8) weeks study was conducted to investigate the effect of varying inclusion levels of mushroom (Pleurotus ostreatus) meal on the haematology and serum biochemistry of broiler chicks. One-hundred-and twenty-Ross 308-day-old broiler chicks were randomly assigned to four dietary treatments in a completely randomized design, with 30 birds per treatment, and three replicates having 10 birds per replicate. Mushroom meal was included at varying levels (0, 1, 1.5, 2%) in the formulated starter and finisher diets fed to the birds. The birds were fed a starter diet for the first 4 weeks and a finisher diet for the last 4 weeks, twice daily (8:00 am and 5:00 pm), with free access to water. Five hundred grams (500g) of each dried sample of Oyster mushroom and experimental diets were taken to the laboratory for the determination of their proximate composition. At the end of the experiment, ten (10mls) of blood was collected from three birds from each treatment groups at the end of the feeding trial via venipuncture of pinea vein using a syringe into EDTA and plain containers and taken to the biotechnology laboratory centre for determination of haematology and serum profile, respectively. The data collected were subjected to analysis using SAS 2023, and means were compared at a 5% probability level using Fisher's least Significant difference (FLSD). The crude protein, ether extract, crude fibre, ash, and metabolizable energy contents were found to be 24.70, 1.41, 8.00, 7.50 % and 2454.21kcal/kg respectively. The haemoglobin, lymphocytes, and mean corpuscular heamoglobin concentration were significantly ($P< 0.05$) influenced by the experimental diet, with the birds in T2 recording the highest haemoglobin and mean corpuscular haemoglobin concentration (15.70 and 38.12g/dl, respectively). The levels of urea and creatinine was significantly ($P<0.05$) highest (14.26, 0.51 mg/dl, respectively) in birds fed 2% of mushroom meal. The cholesterol and calcium levels were significantly reduced ($P < 0.05$) as the inclusion level of mushroom increased, with the lowest levels (240.7, 1.16 mg/dl respectively) observed at the 2% inclusion level. The haematological profile of the experimental birds showed that the animals were in good physiological condition throughout the period of study. Mushroom meal can therefore be included in broiler diets at a 2% inclusion level for optimal performance.

Keywords: Biochemistry, Broiler-chicks, Haematology, Mushroom meal, Serum.

INTRODUCTION

In recent years, there has been a growing focus on developing sustainable, functional feed-solution that can enhance the output, health, and product quality of poultry production (Suberu *et al.,* 2024). More recently, interest has grown in novel feed resources such as black soldier fly (*Hermetia illucens*) larvae, mealworms (*Tenebrio molitor*), plant leaf proteins, algae, insect-based products and mushroom meal due to their dual roles as nutrient sources and functional additives. Edible mushrooms and their by-products (whole powder, stem residues, spent substrate) have become promising feed additives because they are nutrient-dense and rich in bioactive compounds, such as β-glucans, other polysaccharides, phenolic antioxidants, trace minerals, and when added to poultry diets, these compounds have various physiological effects (Suberu *et al.,* 2024; Bormon *et al.,* 2024).

A growing body of research indicates that mushroom supplementation can positively modulate several haematological and biochemical traits in poultry. Bormon *et al.* (2024); Suberu *et al.* (2024) reported increases in haemoglobin and erythrocyte indices, reduced total cholesterol, and improved antioxidant status in broilers

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

and layers fed dietary inclusion of *Pleurotus ostreatus* residues. These effects have been attributed to the immunomodulatory and "trained immunity" actions of mushroom β-glucans and polysaccharides, which can alter leukocyte proportions, functional responsiveness, antioxidant polyphenols and micronutrients that protect cellular membranes (including erythrocytes and hepatocytes) from oxidative damage (Bar-Dagan *et al.,* 2023; Suberu *et al.,* 2024). While weight gain and FCR appear to be major parameters for the assessment of the usefulness of a feed ingredient, blood parameters further authenticate nutrient absorption and utilization by the animal, so blood parameters also show the merits of a feed ingredient and at the same time showcase the enhancement of health status of animals that consume the feed.

Blood is a medium through which nutrients are conveyed to various parts of the body system of an animal and a readily available and fast means of assessing the health and nutritional status of an animal on feeding trial (Oloche *et al.,* 2018). According to Kumar *et al.* (2021) haematological indices (e.g., haemoglobin concentration, packed cell volume, red blood cell counts, leucocyte differentials) and serum biochemical markers (e.g., total protein, albumin, urea, creatinine, aminotransferases, cholesterol, triglycerides, and mineral levels) are sensitive indicators of metabolic status, organ function, immune competence, and nutritional adequacy in broiler chickens. Monitoring these parameters provides insight into whether a dietary intervention is physiologically beneficial, neutral, or deleterious. Therefore, haematology and serum biochemistry constitute essential endpoints when evaluating novel feed ingredients or functional supplements (Karageorgou *et al.,* 2024). It is against this background that this study was conducted to evaluate the haematology and serum biochemistry of broiler chicks fed mushroom meal at various inclusion levels.

MATERIALS AND METHODS

Experimental Site and Location

The study was conducted at the Poultry Section of the Animal Science Teaching and Research Farm, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Awka lies within the Guinea Savanna zone (latitude 6.25°–6.28′ N and longitude 7.00°–7.08′ E) with an elevation of about 300 m above sea level, annual rainfall of 1,000–1,500 mm, and a mean daily maximum temperature of 27 °C (Abajue and Ewuim, 2018).

Oyster Mushroom Processing

Oyster mushroom (*Pleurotus ostreatus*) was cultured on sorghum grains enriched with rice bran and $CaCO_3$ sterilized at 121 °C for 30 minutes, and inoculated with pure mycelium obtained from a local farm. Fruiting substrates were prepared with sawdust, rice bran, and $CaCO_3$, sterilized at 100 °C for 4 hours, inoculated with mushroom spawn, and incubated under 80% relative humidity for 6 weeks and sprinkled with water daily as the mushroom shoots out towards by the sixth week. Mature mushrooms were harvested, oven-dried at 80 °C for 3 h, milled, and stored for diet formulation.

Experimental Animals, Management, Design and Treatment

A total of 120-day-old Ross 308 broiler chicks were purchased from Amo Hatchery, Ibadan. On arrival, the chicks were weighed and randomly allotted to the four dietary treatments: T1 (0%), T2 (1.0%), T3 (1.5%), and T4 (2.0%) inclusion levels of mushroom meal. Each treatment was replicated three times with 10 chicks per replicate in a completely randomized design. Birds were brooded for two weeks and reared under standard management with water and feed supplied *ad libitum*. The experimental diets were formulated to contain 23% crude protein (starter) and 20% crude protein (finisher) with mushroom meal inclusions as specified (T1 (0%), T2 (1.0%), T3 (1.5%), and T4 (2.0%), the trial lasted for 8 weeks. The ingredient and chemical composition of experimental diet is presented in Table 1.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

	Starter				Finisher			
	T1	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
Maize	55.00	54.00	53.50	53.00	60.00	60.00	59.50	59.00
Soybeans Meal	24.00	24.00	24.00	24.00	18.00	18.00	18.00	18.00
Fishmeal	4.00	4.00	4.00	4.00	3.00	3.00	3.00	3.00
Mushroom Meal	0.00	1.00	1.50	2.00	0.00	1.00	1.50	2.00
Wheat Bran	3.00	3.00	3.00	3.00	5.00	4.00	4.00	4.00
Bone Meal	2.00	2.00	2.00	2.00.	2.00	2.00	2.00	2.00
Groundnut Cake	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Toxin Binder	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Calculated Analysis								
Crude Protein (%)	23.36	23.32	23.29	23.27	20.57	20.54	20.52	20.50
Metabolizable Energy	2921	2918	2915	2913	2940	2937	2935	2931
Chemical Composition of Experimental Diet								
Total carbohydrate	58.23	58.30	54.02	54.25	59.19	58.51	55.51	58.10
Dry Matter	92.25	90.25	88.75	87.50	90.25	91.75	86.75	91.00
Crude Protein	23.06	22.97	22.95	22.93	20.44	20.36	20.36	20.27
Crude Fibre	3.60	2.60	2.90	2.60	3.55	4.50	3.95	4.50
Ether Extract	4.61	4.38	6.13	5.25	4.47	5.50	4.13	5.38
Moisture	7.75	9.75	11.25	12.50	9.75	8.25	13.25	9.00
Ash	2.75	2.00	2.75	2.47	2.60	2.88	2.80	2.75
ME(Kcal/kg)	2879.25	2864.48	2849.03	2977.85	2973.23	2965.50	2963.83	2945.88

Table 1. Ingredient and Chemical Composition of Starter and Finisher Diet Ingredient (kg)

Haematology and Serum Biochemistry and Analytical Methods

About ten (10mls) of blood was collected at the end of the feeding trial via venipuncture of pinea vein of three birds randomly selected from each of the treatment groups using a syringe into EDTA and plain containers and taken to the Biotechnology Centre at Nnamdi Azikiwe University Awka, for determination of haematology and serum profile respectively. Packed cell volume was determined using the standard technique described by (Coles, 1986), haemoglobin concentration was assayed colourimetrically using the cyanomethaemoglobin method (Drabkin, 1945). Red blood cell and white blood cell was measured with the aid of Neubaur counter

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

(haemocytometer) (Schalm,1976). Total protein determination was carried out by the biuret method of (Lubran, 1978), serum albumin concentration was determined using bromocresol green method (Doumas and Peters, 1997), determination of serum cholesterol concentration was done using the method as described by (Abel *et al.,* 1952) and serum creatinine concentration was determined by the modified Jaffe method (Blass *et al.,* 1974). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated from Hb, PCV and erythrocyte concentration of blood (RBC) (Jain, 1986). The indices were derived using the following formulae:

$$MCV\ (fL) = \frac{(PCV \times 10)}{RBC\ count\ (\times 10^6/\mu L}$$

$$MCH\ (pg) = \frac{(Hb \times 10)}{RBC\ count\ (\times 10^6/\mu L)}$$

$$MCHC\ (\frac{g}{dL}) = \frac{(HB \times 100)}{PCV}$$

Chemical and Statistical Analysis

Proximate composition of mushroom meal and diets were analyzed according to AOAC (2012), and metabolizable energy was calculated using the Pauzenga (1985) equation, and NFE by difference. Data collected were subjected to analysis of variance (ANOVA) using SAS (2023), and treatment means were separated using Fisher's Least Significant Difference (LSD) test at a 5% level of significance.

RESULTS AND DISCUSSION

Proximate Composition of Mushroom Meal

The proximate composition of Mushroom meal is presented in Table 2.

Table 2 Proximate Composition of Mushroom Meal

Parameters (%)	Mushroom Meal
Moisture	18.25 ± 0.25
Crude Protein	24.70 ± 3.51
Ether Extract (Fat)	1.41 ± 0.90
Crude Fibre	8.00 ± 0.60
Ash	7.50 ± 0.00
Nitrogen-Free Extract (Carbohydrate)	40.14 ± 3.21
*Metabolisable Energy (kcal/kg)	2454.21 ± 2.69

*ME = (37 × Crude Protein) + (81.8 × Crude fat) + (35.5 × NFE)

The crude protein level (24.70 %) confirms that mushroom meal is a valuable alternative protein source. Comparable protein contents have been documented for *Pleurotus ostreatus* (21–28 %), *Agaricus bisporus* (20–27 %), and *Lentinus edodes* (22–25 %) (Suberu *et al.,* 2024; Bormon *et al.,* 2024). These variation in protein values among mushroom species and substrates reflects differences in cultivation media, harvest age, and drying

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

methods (Rathore *et al.,* 2017). Adebayo *et al.* (2021) in their study poibted out that mushroom protein is highly digestible and rich in essential amino acids such as lysine, methionine, threonine, and valine, which are often deficient in cereal-based broiler diets, and therefore its inclusion in poultry feed can help balance amino-acid profiles and reduce reliance on costly soybean meal.

The ether extract value (1.41 %) indicates a low lipid content, consistent with the lean nature of mushrooms. Most edible mushrooms contain 1–3 % crude fat, which is composed largely of unsaturated fatty acids such as linoleic and oleic acids (Barros *et al.,* 2008). Low lipid levels are advantageous for feed formulation because they reduce oxidation risks and improve product stability. Nevertheless, Yildiz *et al*. (2017) documented that the presence of essential fatty acids in mushroom lipids contributes to antioxidant potential and enhances flavour acceptability in feed.

The crude fibre (8.00 %) observed is relatively high compared with conventional plant proteins but within the range (6-10%) reported by Suberu *et al*. (2024) for mushroom by-products. Mushroom cell walls are rich in chitin, β-glucans, and other non-starch polysaccharides that contribute to fibre content and functional benefits as dietary β-glucans can act as prebiotics, improving gut health and modulating the immune response in poultry (Bar-Dagan *et al.,* 2023). However, Mthana and Mthiyane, (2024) pointed out that excessive fibre levels may reduce nutrient digestibility if inclusion levels are not well balanced. Therefore, while mushroom meal provides functional fibre, its dietary proportion must be optimized to avoid compromising energy utilization.

The ash content (7.50 %) indicates considerable mineral density. Mushrooms are recognised for their high mineral content particularly potassium, phosphorus, magnesium, calcium, iron, and zinc (Rathore *et al.,* 2017). The ash content in the current study is within the range of 6-10% reported by Suberu *et al.* (2024), and Adebayo *et al.* (2021) for *Pleurotus* and *Agaricus* species. Such mineral richness enhances the feed's potential to support bone development and metabolic processes in poultry.

The NFE value (40.14 %) reflects the carbohydrate fraction, mainly comprising glycogen-like polysaccharides, hemicellulose, and soluble sugars. These carbohydrates provide an accessible energy source and may also include prebiotic polysaccharides that promote beneficial gut microflora (Adebayo *et al.,* 2021; Suberu *et al.,* 2024). The calculated metabolisable energy (2454 kcal/kg) lies within the range reported for mushroom meals (2300–2600 kcal/kg) and is adequate to support moderate inclusion in broiler diets without adversely affecting energy balance (Bormon *et al.,* 2024). Although mushrooms are less energy-dense than cereal grains, their functional components justify partial substitution for conventional energy and protein ingredients.

Haematological Parameters of Broiler Chicks Fed Varying Levels of Mushroom Meal

Table 3 shows the haematological parameters of broiler chicks fed varying levels of mushroom meal.

Table 3 Haematological Parameters of Broilers fed Varying Levels of Mushroom Meal

Parameter	T1	T2	T3	T4	SEM	LOS	Ref Value
PCV (%)	41.00	42.33	37.33	41.67	7.98	NS	25.20 - 45.20
Hb (g/dl)	15.30^{a}	15.70^{a}	14.20^{b}	15.50^{a}	0.66	*	10.02 - 15.1
RBC (10^{12}/L)	4.17	4.40	4.13	4.33	0.30	NS	2.0 – 3.0
TWBC (10^{9}/L)	91.50	90.57	89.63	89.33	1.22	NS	20.0– 30.0
Lymphocytes (%)	61.20^{b}	60.47^{b}	63.97^{a}	59.03^{b}	1.17	*	50.0 - 75.0
Platelet (10^{9}/L)	40.30	40.00	39.33	39.23	1.01	NS	20.0– 30.0
MPV (fL)	13.07	13.07	12.87	12.90	0.19	NS	10.0 – 20.0
MCV (fL)	99.28	102.88	87.82	96.89	10.75	NS	90.0-130.0

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

MCH (pg)	37.05	38.12	33.40	35.96	3.66	NS	28.0 - 34.0
MCHC (g/dl)	37.33^{b}	38.12^{a}	38.04^{a}	35.96^{c}	0.36	*	26.0 – 35.0
Granulocytes (%)	14.33	14.67	14.33	14.37	0.64	NS	20.0 – 40.0

^{abc} Mean values with different superscripts within a row differed significantly; PCV= Packed Cell Volume; Hb= Haemoglobin; RBC= Red Blood Cell; TWBC= Total White Blood Cell; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration; SEM= Standard Error of Mean; LOS= Level of Significance; * Significant at 0.05; NS= Not Significant.

Feeding varying levels of mushroom meal to broiler chicks significantly (P< 0.05) influenced the Hb, Lymphocytes and MCHC of broiler chickens. The other measured parameter numerically varied among the treatment groups, but no significant differences were observed. The values of PCV, lymphocytes, MPV, and MCV recorded by all the groups were, however, within the normal range for healthy chicken (Aeangwanich *et al.,* 2004; Ahmed, 2018), while the other parameters were outside the reference range reported by Aiello and Mays (1998), and Okeudo *et al.* (2003) for broiler chicken.

Haematology is available in determining the physiological responses of chickens as the blood constituents are the biochemical medium of transportation in all animals, and thus their profile shows the health status of the birds (Akintomide *et al.,* 2021; Oyebode, 2015). The two significant parameters used in assessing the health status of broiler chickens are the PCV and Hb. Packed Cell Volume (PCV) is involved in the transport of oxygen and absorbed nutrients round the body, delivering it to target cells or tissues (Onunkwo *et al.,* 2022). The PCV value ranged between 37.33 - 42.33% and were within the normal range described by Ahmed, (2018) for a healthy chicken. Feeding varied levels of mushroom recorded no significant effect on the PCV values of broiler chickens in the present study.

Haemoglobin is the oxygen carrying protein in the RBC. Hb levels is a direct reflection of the amount of oxygen in the blood. The Hb values were significantly (P <0.05) affected by levels of mushroom meal inclusion and its values were slightly above the normal range described by Aeangwanich *et al.* (2004) and Ahmed, (2018), for healthy birds. This elevated haemoglobin concentration suggests an improvement in oxygen-carrying capacity and general erythropoietic efficiency. Similar findings were reported by Bormon *et al.* (2024), who observed enhanced haemoglobin levels in broilers supplemented with *Pleurotus ostreatus* stem residue. The authors attributed this to improved mineral bioavailability, particularly iron, and reduced oxidative degradation of red blood cells due to antioxidant compounds in mushrooms. Suberu *et al.* (2024) also confirmed that mushroom polysaccharides, phenolics, and vitamins protect erythrocyte membranes from oxidative stress, thereby sustaining haemoglobin integrity.

The Hb values in these chickens correspond with their PCV values as chickens with higher PCV had higher Haemoglobin concentrations (Jubril *et al.,* 2022). These findings are in line with the report by Kumar *et al.* (2021), who also observed a corresponding higher Haemoglobin concentration in broiler birds with higher PCV. Lakurbe *et al.* (2018) documented that the range of values obtained in the present study could indicate that the birds were adequately nourished and thus not anaemic or showing any sign of disease infection or parasite infestation.

The lymphocytes were significantly (P<0.05) influenced by varied levels of mushroom meal and the values were within the normal range of 50.0-75.0 documented by Ahmed, (2018) for a healthy chicken. According to Guyton and Hall (2006), an increase in lymphocytes in the blood indicates enhanced immunological well-being of the body.

The MPV and MCV were not influenced by mushroom meal, but their values were within the normal range reported for a healthy chicken. MCH indicates the blood-carrying ability of RBC. The study reveals that the mushroom increased the blood-carrying ability of the RBC, with the highest value recorded, which is slightly above the reported normal range for a healthy chicken (Ahmed, 2018).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue X October 2025

Serum Metabolites of Broiler Chicks Fed Varying Levels of Mushroom Meal

The serum metabolites of broiler chicks fed varying levels of mushroom meal is shown in Table 4.

Table 4. Serum Metabolites of Broilers fed Varying Levels of Mushroom Meal

Parameter	T1	T2	T3	T4	SEM	LOS	Ref Value
Total Protein (g/dl)	3.10	3.15	3.16	3.24	0.32	NS	3.0 -5.5
Globulin (g/dl)	1.83	2.14	1.92	2.20	0.25	NS	1.5 – 3.5
Albumin (g/dl)	1.27	1.01	1.24	1.04	0.16	NS	1.0 – 3.0
Urea (mg/dl)	13.06^{b}	12.04^{b}	12.67^{b}	14.26^{a}	0.60	*	2.0 – 8.0
Creatinine (mg/dl)	0.35^{b}	0.45^{a}	0.46^{a}	0.51^{a}	0.07	*	0.2 – 0.5
ALT (U/L)	3.33	4.67	3.00	4.33	1.42	NS	5.0 – 20.0
AST (U/L)	110.67	112.83	119.67	122.17	9.61	NS	100 – 300
Triglycerides (mg/dl)	191.56	193.56	191.11	178.22	9.31	NS	30 -150
Cholesterol (mg/dl)	455.3^{a}	440.7^{a}	309.3^{a}	240.7^{b}	80.16	*	100 – 200
Calcium (mg/dl)	8.48^{a}	6.15^{b}	1.99^{c}	1.16^{c}	0.54	*	8.0 – 12.0

^{abc} Mean values with different superscripts within a row differed significantly; AST = Aspartate Amino Transferase; ALT = Alanine Amino Transferase; SEM= Standard Error of Mean; LOS= Level of Significance; * Significant at 0.05; NS= Not Significant.

The results of the serum metabolite analysis show that most of the parameters tested in this study were not influenced by dietary treatments; only a significant ($P<0.05$) increase in the level of urea, creatinine and a reduction of cholesterol and calcium concentration in blood serum was observed in response to inclusion of mushroom in the diet. The levels of total protein, Albumin, and globulin in this study fell within the normal range reported by Mitruka and Rawnsley, (1977).

The use of biochemical indices as a pointer to conditions that may not be readily noticed by performance indices cannot be overemphasised (Oloche *et al.,* 2018). According to Upah *et al*. (2024), the plane of nutrition is known to affect these values. Total protein, globulin, and albumin are critical for maintaining physiological functions and immunity, and their levels in this study did not show significant differences across the treatments (T1-T4), but their values are within the reference ranges reported by Mitruka and Rawnsley, (1977) for a healthy chicken. Similar findings have been reported by Suberu *et al.* (2024), who found that dietary supplements, including mushroom extracts, did not significantly alter serum protein levels in broilers. This stability suggests that mushroom meal, within the levels tested, does not adversely affect protein synthesis or liver function. The total protein (TP) content numerically increased in the treated group of broiler chicken as the inclusion level increased in this study. The reason for the higher plasma protein accretion in blood vessels might be a result of mushroom supplementation in broiler diets. Shang *et al.* (2014) reported that mushrooms can increase the availability of amino acids (methionine and cysteine) for protein formation in broiler chickens.

Significant ($P<0.05$) differences were observed in urea and creatinine levels. The urea level was significantly ($P<0.05$) higher in T4 compared to other treatments, while the inclusion of mushroom meal significantly ($P<0.05$) increased creatinine levels in the birds than those on the control diet. Their levels exceeded the reference ranges reported by Meluzzi *et al*. (1992) for a healthy chicken. Elevated urea and creatinine levels can indicate dehydration, renal stress or impaired kidney function. Research by Olanrewaju *et al*. (2022) suggests that highprotein diets can increase urea and creatinine levels due to higher nitrogenous waste production. The increase in urea and creatinine in the treatment groups might be a result of higher levels of mushroom meal.

Alterations in the activities of serum enzymes, such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), are often utilized as markers of toxicity for the assessment of biochemical and physiological health of vital organs and tissues, such as liver and heart (Akinsulie *et al.,* 2021). Upah *et al.* (2024) established that damage to the cellular membrane in tissues where these enzymes are normally localized results in their leakage into the bloodstream, giving an index of cellular integrity. The results of this study showed that mushroom meals had no significant ($P > 0.05$) effect on the levels of AST and ALT, but the ALT value falls within the normal range documented by (Meluzzi *et al*., 1992), while the AST levels fall below the normal range reported by (Meluzzi *et al.,* 1992). The values of the liver enzymes in this study showed that they were not influenced by the anti-inflammatory activities of the phytochemicals, flavonoids and terpenes present in mushroom meal.

Cholesterol levels were significantly ($P<0.05$) lower in T4 compared to the other treatment groups, and their levels in the present study was higher than the range reported by Abdi-Hachesco *et al.* (2011) for the broiler strain. Calcium levels were significantly ($P<0.05$) lower in T3 and T4 compared to T1 and T2, with T4 showing the lowest levels. Lower cholesterol levels in broilers fed higher mushroom meal levels could be related to the high fiber content or specific bioactive compounds found in mushrooms that affect lipid metabolism. This aligns with findings by Duda *et al.* (2025), who reported that mushroom supplementation can lower serum cholesterol levels due to their bioactive components. The drop in calcium levels in higher mushroom meal groups might be attributed to the high phosphorus content of mushroom meal, which, according to the study of Mahfuz *et al.* (2019), can affect calcium absorption.

CONCLUSION

From the results obtained, it can be concluded that the inclusion of mushroom meal at 2% in broiler diet resulted in optimum performance of broiler chicks without any negative effect on the health status of the chicks.

ACKNOWLEDGEMENTS

The authors acknowledge the academic staff of the Department of Animal Science, Nnamdi Azikiwe University, Awka, for providing constructive criticism, which improved the quality of the study.

Ethical Statement

This study adhered to ethical research standards as approved by the Ethical Committee of the Nnamdi Azikiwe University, Nigeria, ensuring responsible data use, informed community participation, non-invasive environmental practices, transparency and adhering to the Animal handling protocols.

Funding Information

The authors declare that this study has received no financial support.

Conflict Of Interest

The authors have no conflicts of interest to declare.

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