SSRG International Journal of Veterinary Science
ISSN: 2455–0868 / https://doi:10.14445/24550868/IJVS-V12I2P105

Volume 12 Issue 2, 36-43, May-Aug 2026

Original Article

Effects of Supplementation of *Pleurotus tuber-regium* (King tuber) on Haematology and Serum Biochemistry of Broilers in Humid Tropical Environment

Iso Ekong Iso

Department of Animal Science, University of Cross River State, Okuku Campus, Cross River State, Nigeria.

Corresponding Author : imoke.etorti@uee-akamkpa.edu.ng

Received: 02 June 2025 Revised: 04 July 2026 Accepted: 25 July 2026 Published: 17 August 2026

Abstract - *This research investigated and analysed effect of oral supplementation of the Nigerian giant mushroom (Pleurotus tuber-regium) on some hematological and serum biochemical profiles in broiler chickens and their ability to use it as a natural supplement to promote their health. A total of 160 of day-old Arbor Acre broilers (40.00 + 0.55 g) were randomly distributed among four groups in a completely randomized design of ten broiler chicks per replicate and four replicates per treatment for a period of 56 days in a deep litter system of housing under natural ambient temperature. Chicks were fed with the four experimental diets as treatment (0, 10, 20 and 30% supplement of P. Tuber-regium), blood collected on day 56 was subjected to hematological and biochemical analyses in the laboratory. Blood parameters and some selected biochemical components (in the serum) were analysed at the end of feeding trial by six birds per replicate, and the results were analyzed using SAS (2004), and treatment means were separated using Duncan Multiple range test. The Haematological profile revealed a significant ($P < 0.05$) increase in Packed cell Volume, Hb, RBC and WBC level of broiler birds supplemented with a diet contain P. Tuber-regium than the control diet, which was within the range of biological value for healthy broilers chicken. There was no significant difference ($P > 0.05$) on the studied biochemical parameter, and it was conclude that feeding with 10%, 20% and 30% supplement did not interfere with major biochemical function of birds, from where the Nigerian giant mushroom (Pleurotus tuber-regium) was adjudged to be good enough to be used as a supplement in broiler diets.*

Keywords - *Arbor Acre broilers, Dietary supplementation, Deep litter housing, Haemoglobin concentration, Pleurotus tuber-regium.*

1. Introduction

Poultry and Ruminant agriculture is experiencing enormous growth in Nigeria as a strategy to reduce and cover shortfalls in national dietary protein, and as a supplement to plant protein sources (Hoque et al., 2022). The escalating feed costs and unavailability of conventional feed ingredient options are the factors that continue to pose barriers to effective production, in addition to climatic constraints like the extreme heat stresses and subsequent change in physiological and metabolic parameters of animals under humid conditions (Sejian et al., 2013). As is evident, animals exhibit several means of acquiring tolerance via adaptation to the demands of such stringent environmental challenges, and this adaptation directly results in alterations to behavioural, metabolic responses (Ikhimioya et al., 2000; Shitu et al., 2023).

In many instances, this ability relies on adaptation processes related to internal physiology in the body including the functioning of internal organs and internal blood chemistry (Figuier et al., 2015), thereby justifying the study of haematological and serum biochemical constituents, that offer reasonable assessment of general health status that in turn, can indicate adaptation/stress responses, and effect of dietary ingredients, environment and stress (Faraz et al., 2023; Mohammed et al., 2016; Schalm et al., 1975), under various environmental parameters, feeding systems and levels of inclusions as well as other influencing conditions like genetics, age and management practices (Rakib et al., 2022).

Finding the cheapest possible, conventional feed supplements is a major factor challenge facing farmers in developing regions (Amata, 2014; NRC, 1994). As a result, increased attention has shifted to Phytogenic feed supplements, such as plant-based mushroom extract, which have potential as non-antibiotic-alternatives due to health-promoting and anti-biotic activities (Erb et al., 2024). Some recent study demonstrated that extracts from different types of mushrooms improved the immune system and health of animal in poultry (Fadugba et al., 2023; Liu et al., 2024; Seresn Trs et al., 2024). The use of mushroom supplementation has demonstrated an improvement in different blood parameters and growth performance of different type of animal (Akomah et al., 2021; Mthana & Mthiyane, 2024). Studies, in addition to this, investigating

the usage of other mushroom type in temperate area are documented, but less research explored the usage of the Tropical fungus *Pleurotus tuber-regium* singer for broiler performance and Physiological adaptation under hot and humid climate conditions, Furthermore, to cover this research gap, this study investigated the usage of *P. Tuber-regium* at high levels of inclusion(s) up to 30%) to reveal if this tropical fungus can successfully supplement feeding and enhance the haematological and biochemical robustness of broiler chicken under humid tropical conditions.

1.1. The Research Gap

The application of edible mushrooms as dietary natural feed supplements in poultry has been gained greater concerns owing to the presence of nutrients and bioactive compounds that possess antimycotic, antioxidative, immunomodulatory and growth promotion attributes.

Reports on the inclusion of numerous edible mushroom species such as *Pleurotus ostreatus, Lentinula edodes, Ganoderma lucidum and Agaricus bisporus* have already demonstrated a great impact on the performance of broiler chickens, including their performance, immunity, gut health and antioxidative status, as well as carcass traits and meat characteristics. However, the majority of these studies have focused on mushroom powders, extracts, polysaccharides, or fermented mushroom residues rather than *Pleurotus tuber-regium.*

Despite a few studies that have demonstrated the nutritive value of *P. Tuber-regium* and its potential in poultry production, available research papers mainly focused on growth, feed utilization and carcass characteristics.

Information on its effect on the haematological and serum biochemical parameters is still scanty and inconclusive, especially in tropical production system. In addition, these researchers used mainly one or two levels of inclusion, and this makes it difficult to determine the range of dietary inclusion to be used in poultry production.

In addition to the shortcomings outlined above is the fact that most previous studies only report on the conventional performance parameters and lacked comprehensive physiological evaluation to attest the safety of long-term dietary supplementation. Information on the level of inclusion that causes a detrimental effect to blood parameters, immunity status and organ function in broiler chickens is lacking with *P. Tuber-regium.*

The present study, therefore, investigates the effect of inclusion levels of *Pleurotus tuber-regium* (10%, 20% and 30%) on haematological and serum biochemical parameters of broiler chickens over 56 days of production. The present study evaluates physiological parameters of birds at varied levels of dietary inclusion of *Pleurotus tuber-regium,* simultaneously to evaluate efficacy and safe level of its use as a natural feed additive to a sustainable poultry production.

2. Review on Mushroom Feed Additives and Broiler Health

Growing interest and demand to tackle antimicrobial resistance and to withdraw or strictly limit non-veterinary use of antibacterial growth promoters spurred the interest to find economical and sustainable natural feed supplements that might ensure efficient production and a healthy animal while increasing food security.

Edible mushrooms have revealed great potential due to their rich bioactive components like polysaccharides, beta - glucans, and terpenoids, as well as phenolic compounds, flavonoids, dietary fiber, essential amino acids, minerals, and vitamins. These compounds exhibit antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and prebiotic properties that positively influence poultry performance and health (El-Hack et al., 2024; Khursheed et al., 2024).

Recent studies have demonstrated that mushroom supplementation enhances growth performance, feed conversion efficiency, antioxidant status, immune competence, gut microbial balance, and disease resistance in broiler chickens. The principal bioactive constituents, particularly β-glucans and polysaccharides, activate macrophages, stimulate cytokine production, enhance antibody synthesis, and strengthen both innate and adaptive immune responses, thereby improving the birds' resistance to pathogenic microorganisms and environmental stressors (Khursheed et al., 2024; Gao et al., 2024).

Among edible mushrooms, Pleurotusostreatus has received considerable attention as a functional feed additive in poultry nutrition. A comprehensive review by Khan et al. (2024) reported that *P. ostreatus* supplementation improves nutrient digestibility, feed conversion ratio, growth performance, intestinal morphology, antioxidant capacity, and immune responses while reducing dependence on antibiotic growth promoters. The authors attributed these improvements to the mushroom's high concentrations of β-glucans, polysaccharides, and phenolic compounds that promote intestinal health and beneficial microbial populations.

Similarly, a controlled feeding experiment by Elbaz et al. (2024) demonstrated that dietary supplementation with oyster mushroom *(Pleurotus ostreatus)* stem residue significantly improved growth performance, meat quality, blood characteristics, and caecal microbial balance in Arbor Acres broiler chickens. Birds fed a supplement added mushroom products indicated a healthier physiological status with no health disturbances. This implies that products that remain of mushroom can be used as functional feed ingredients.

In poultry nutrition, medicinal mushrooms such as Ganoderma lucidum also proved successful in improving overall animal performance and animal health in young animals (poultry). According to Gao et al. (2024), Ganoderma lucidum supplement improved growth, immune

responses, antioxidant activity, intestinal morphology and gut microbial composition of broilers, while the findings of Elbaz et al. (2024) proved that supplementing broiler feeds with Ganoderma lucidum enhanced antioxidants, immune status, blood, meat quality, while reducing the mortality of slow-growing broilers. These studies further strengthen the current understanding about growing roles of medicinal mushrooms as alternatives to the well-known antibacterial growth promoters.

Additionally, other studies also suggest that mushroom-derived polysaccharides may preserve integrity and balance in the intestinal tract through the promotion of some Lactobacillus species while inhibitory some negative microbes such as E. Coli and Salmonella spp. Thus providing more useful substances that will lead to the absorption of other necessary dietary substances, as well as the improvement of villus development, physiological efficiencies of body and improved immunity (Gao et al. 2024, Khursheed et al. 2024).

Blood analysis provides the most reliable information on the body's status and general physiological condition in poultry. The latest results of the researches about supplementing mushrooms indicate a satisfactory immune system in the poultry, an increase of Packed Cell Volume (PCV), Hemoglobin, Erytrocyte Number (RBC) and leukocyte levels in the body, and also show the body's good health without significant negative changes in the biochemical blood markers (Blood Urea Nitrogen-BU N, Creatinine, AST, ALT) under various levels of supplement at optimum physiological conditions(Elbaz et al. 2024, Khursheed et al. 2024). Unfortunately, only limited research has been conducted on Pleurotus tuber-regium (imbe) as a functional feed additive. Although Pleurotus tuber-regium has been documented to contain relatively high levels of protein and essential amino acids, as well as a remarkable profile of other nutritional and phytochemical compounds, including Dietary Fibre, Vitamins, Minerals, β-glucans and Antioxidant compounds, Most of these studies have not evaluated comprehensively its effects on Haematological and serum biochemisty. Moreover, an optimized level for *P. Tuber-regium* in broiler feed so far, to maximizing its beneficial physiological effects with no detrimental consequences on metabolism is not determined.

Therefore, this work was done to evaluate graded level supplementation (10%, 20% and 30% diet) of Pleurotus tuber-regium on broilers' haematological and biochemical blood constituents.

3. Materials and Methods

3.1. Study Location and Climate

A 56-day feeding experiment was carried out at the Teaching and Research Farm poultry unit, University of Cross River State, Nigeria. The location is within the tropical rainforest belt of Nigeria (Latitude 6°-7° N; Longitude 8°- 9° E). Daily temperatures ranged from 21°C to 30°C, and rainfall annually was between 500 mm and 1007 mm.

3.2. Statement of Ethical Approval

The experiment was approved by the institutional ethics committee before initiation. All handling procedures of the experimental study met with guideline requirements on humane handling for the care of experimental poultry to reduce stress as defined by the National Institutes of Health Guide, a report of the NRC (2011).

3.3. Collection and Preparation of Pleurotus Tuber-Regium

The mushrooms used for this research work was sourced from the forest of the Central Senatorial district of Akamkpa, Cross River State. The tubers harvested were cleansed with clean water and then sun-dried to a constant weight for a period of seven days. The dried tubers were further sliced into 3 mm pieces and then pulverized using 2 mm sieve hammer mill. The pulverized mushroom meals were then mixed at desired proportions with the starter and finisher feeds. Proximate analysis of the mushroom was carried out using the method of AOAC (2000) and presented in table 1.

3.4. Experimental Birds, Randomization, and Management

160-day-old Arbor Acre broiler chickens were procured from a commercial hatchery. The sample size of the 160 broiler chickens (40 broiler chickens/treatment) was selected based on prior experimental results for similar studies and on an ethical restriction on the number of animals. The broiler chickens were weighed, individually given a time of 4 to 7 days to acclimatisation, and later pen randomly allocated using a table of random number for ensuring equal distribution in all treatment. The broiler chickens were pens based on grouping them four replicates of 10 broilers. Each pen is deen litter and provision of water and feed were in freely available (*ad libitum*) as of the usual practice in the case of deep litter house housing systems.

3.5. Experimental Diets and Inclusion Rationale

Four basal diets were prepared for the starter (days 1-28) and the finisher phase (days 29-56). Diet 1 was used as the control diets (0% Supplement). The levels of inclusion in diets 2, 3 and 4 was 10, 20% *P. Tuber-regium,* respectively.

This level of addition in the experimental trial was derived from trials preliminary palatability and available information indicating likely inhibition of the intake if level of addition is increased beyond 30 due to fibre present in this supplement. All experimental diets prepared isocaloric and isonitrogenous and have satisfied standard composition as outlined by NRC (1994) for broiler chickens. Tables 2 & 3 provide the composition of both experimental diets.

3.6. Justification for Selecting 10%, 20%, and 30% Dietary Inclusion Levels of Pleurotus Tuber-Regium

Inclusion levels of 10, 20 and 30% of Pleurotus tuber-regium were used in formulated diets in order to assess dose-dependent physiological effects on broiler chicks on one hand, and on the other hand, to ensure that diets are nutritive

and can be practically and affordably implemented in a commercial broiler farm. Graded levels of inclusion help determine a response threshold beyond which the positive effects of mushrooms in diets are enhanced while nutrient utilization, feed intake and metabolic function remain normal. Edible mushrooms are abundant in nutrients such as protein, essential amino acids, fiber, vitamins and minerals, as well as β-glucans and antioxidant phytonutrients.

With higher incorporation levels, increasing amounts of these active compounds will be consumed, with resulting improvements in immune function, antioxidant capacity, gastrointestinal tract condition and haematopoiesis. The doses are set in order to estimate low (10%), intermediate (20%) and high (30%) dosage in a way that will also determine an optimal level for diet formulation in order to obtain optimal physiological responses without detrimental effects on normal serum biochemical characteristics. These results also offer some insights into practical feeding guidelines and safe incorporation limits of the *Pleurotus tuber-regium* into broiler diets. Different levels of inclusion help investigate the dose-response relationships, which are relatively absent in past research involving *Pleurotus tuber-regium.*

3.7. Data Collection

On day 56, six broiler chickens per replicate were randomly selected for blood sampling. Blood (5 mL) was drawn via brachial vein puncture using a scalp vein needle. A 2 mL portion was transferred to sterilised tubes containing EDTA for haematological estimation, while the remaining 3 mL was collected in plain bottles for serum biochemical analysis. Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin (Hb), and total white blood cell (WBC) counts were determined following standard laboratory procedures (Benerjee, 2016). Differential WBC counts were also recorded.

3.8. Statistical Analysis

The collected data were analysed for normality of distributions and homogeneity of variances using the Shapiro-Wilktestand Levene's test, respectively, before statistical analyses was performed using one way ANOVA a completely randomized design (SAS, 2004) on the raw collected data. Differences in treatment means were discriminated with Duncan multiple range test (Duncan, 1955) at 5% levels of significant (P< 0.05).

The data obtained from the experimental procedure were analyse using General Linear Model (GLM) available in SAS software. Assumptions of the analysis of variance were checked on the raw data before hypothesis tests were conducted. Normality of the residual was evaluated using Shapiro-Wilkor Z-transformed data, whilst heterogeneity of error (equal variances for treatments) was tested using Levene's test. Also, residual plots were scrutinized for model diagnostic and the detection of outliers.

One-way Analysis of Variance (ANOVA) that fits a completely randomized design was conducted to ascertain the influence of different dietary incorporation levels of *Pleurotus tuber-regium* on some haematological and serum biochemical profiles. The treatment means were separated, where a significant treatment difference was found ($P < 0.05$) by the use of Duncan's Multiple Range Test.

In addition to statistical significance, effect sizes were estimated using partial eta squared (η^2) to quantify the magnitude of treatment effects. Effect sizes were interpreted as small ($\eta^2 \approx 0.01$), medium ($\eta^2 \approx 0.06$), and large ($\eta^2 \geq 0.14$). Mean values are presented together with their standard errors, and where appropriate, 95% confidence intervals were calculated to provide estimates of the precision of treatment means.

All outcomes reported a significance of $P < 0.05$. Collectively, employing the criteria that significance and effect size (within a given CI) result in biological relevant conclusions, gives a more profound conclusion of the effect of supplementation *Pleurotus tuber-regium* to diet compared to relying only on P-values.

4. Results and Discussion

4.1. Haematological Indices

Table 4 presents the haematological values of broiler chickens fed different levels of P. Tuber-regium Mushroom supplementation significantly increased PCV, Hb, RBC, and WBC values (P< 0.05) as compared to the control. No significant variation (P> 0.05) in the mean values MCV, MCH, and MCHC was observed when compare supplemented treatments with control. A similar trend was found for platelets, neutrophils, lymphocytes, and eosinophils (P< 0.05).

4.2. Serum Biochemistry

The serum biochemical profiles are summarized in table 5. No significant effect was observed in terms of serum total protein between the control, treatments 2, 3 and 4 (P>0.05). It varied between 3.30 -3.80 g /d L. No difference in Albumin, globulin, glucose, and urea serum between the control and treated animals (P> 0.05).

Creatinine in serums showed statistically significant variation between (P< 0.05) with value 17.94 for control, while the serum value in treated groups 2,3 and 4 was 38.84, 43.24 and 37.83 /m ol L, as the case may be. No significant differentiation was detected for serum Biochemistry ALT and AST among the treated animals (P>0.05), whereas the serum calcium and chlorine indicated a highly significant differences (P<0. 05).

4.3. Discussion

4.3.1. Haematological Responses

Based on observed differences, the baseline blood parameters (Blood profile, RBC count, W.B.C.count, PCV & HB) in this present study are within standard limits established for healthy broiler birds as cited by the Clinical Diagnostic Division (1990) & Jain (1993). The significantly increased level of PCV, Hb & RBC count could indicate better oxygen carrying capacity as they have shown

significant augmentation in both the groups. Elevated levels of WBC count observed in supplementation groups further indicate a better systemic physiological response due to active bioactive constituents of the mushroom, such as beta-glucans, polysaccharides that could have influenced immune parameters of the birds (Erb et al., 2024; Mthana & Mthiyane, 2024).

The values have not indicated any antagonistic effect of P. Tuber-regium as supplemented feed on hemopoiesis, proving that erythropoiesis has not impaired. MCV, MCH and MCHC have remained almost similar, which is prove in terms of sufficient volumes and haemin per cells have sustained.

4.3.2. Serum Biochemical Responses

Comparisons of total protein, albumin, globulin, glucose and urea between different treatments were non-significant ($p>0.05$), indicating that the diets provided efficient protein synthesis and amino acid utilisation and balanced for nutritional adequacy, as well as ensuring that no interference with the metabolic functions occurred due to *P. Tuber-regium* supplementation. Creatinine concentration in supplemented groups is within physiological ranges (Mitruka & Rawnsley, 1997) and indicates muscular metabolism, which corresponds to an efficient amino acid and protein metabolism (Cheesbrough, 2006).

Serum enzymes ALT and AST remained stable as the mushroom had no sign of toxicity to the kidney and liver, even at 30% of inclusion and also confirm a generally good health state with respect to these organs. The significantly raised serum levels of calcium and chloride were considered indication for actively absorbed mineral components from mushroom and basal feed for bone development and maintenance, as observed in recent reports of inclusion of P. Tuber-regium meal for broilers (Adisa & Ebenebe, 2024; Giwa et al., 2024).

4.4. Study Limitations

Despite strong results, there were some limitations to the experiment. The experiment was carried out at one particular location, during a one set window in time, so the weather conditions were restricted to one set of environmental conditions. Large-scale, multi-located trials with large groups are required to accurately ensure the broad usability of these results across different farming operations of varying sizes in various locations of the Tropics.

4.5. Conclusion

The research indicated that inclusion of *P. Tuber-regium* serves as a safe and useful additive to commercial diets used by the starter and grower/finisher broiler chicken producers. Use of the mushroom at up to 30 % level increased several health indicators of the blood, such as Packed Cell Volume (PCV), Haemoglobin Concentration (Hb) and counts of White and Red Blood Cells (WBC & RBC), which indicate increase in oxygen carriage and improved immunity as their values are usually reflected of the state of Health and Performance of animals. Since such changes in biological values is an index of healthy state and efficiency, *P.Tuber-regium* may be a natural and sustainability source of beneficial food supplement in diets fed to poultry species in humid tropical conditions, the researchers added.

4.6. Tables

Table 1. Proximate composition of *Pleurotus tuber-regium* (king of tubers)

Parameters	Percentage (%)
Moisture	75.72
Dry matter	85.45
Crude protein	20.75
Ash	2.88
Ether Extract	3.98
Crude fibre	9.45
Nitrogen-free Extract	35.27
Gross energy (mg/kg)	968.00
Minerals	
Ca (g/100 g)	0.70
Mg (g/100 g)	0.20
K (g/100 g)	0.55
Na (g/100 g)	0.80
Cu (mg/100 g)	0.78
Zn (mg/100 g)	1.25
P (mg/100 g)	2.47
Fe (mg/100 g)	4.74
Mn (mg/100 g)	0.08

Table 2. Gross composition of experimental diets (%) for broiler starter phase supplemented with *Pleurotus tuber-regium*

Ingredients (%)	Treatment 1 (0 %)	Treatment 2 (10 %)	Treatment 3 (20 %)	Treatment 4 (30 %)
Maize	55.80	44.30	34.55	24.30
Groundnut cake	3.00	3.00	3.00	3.00
P. tuber-regium	0.00	10.00	20.00	30.00
Fish meal	2.50	2.50	2.50	2.50
Soya bean meal	30.00	30.00	30.00	30.00
Wheat offal	6.00	6.00	6.00	6.00
Dicalcium phosphate	1.90	1.90	1.90	1.90
Salt	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Premix	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20
Palm oil	0.00	1.50	1.50	1.50
Total	**100.00**	**100.00**	**100.00**	**100.00**

Note: Vitamin and mineral premix 0.1 %, B-Complex 0.02 %, choline 0.05 %, and salt 0.3 %. Trace mineral premix supplied (mg/kg diet): Mn 55, I 0.4, Fe 56, Zn 30, Cu 4. The vitamin premix supplied per kg diet: Vit A 8250 I.U., Vit D3 1200 I.C.U., Vit K 1 mg, Vit E 40 I.U., Vit B1 2 mg, Vit B2 4 mg, Vit B12 10 mcg, niacin 60 mg, pantothenic acid 10 mg, choline 500 mg...

Table 3. Gross composition of experimental diets (%) for broiler finisher phase supplemented with *Pleurotus tuber-regium*

Ingredients (%)	Treatment 1 (0 %)	Treatment 2 (10 %)	Treatment 3 (20 %)	Treatment 4 (30 %)
Maize	58.40	46.90	36.90	26.90
Groundnut cake	25.40	25.40	25.40	25.40
P. tuber-regium	0.00	10.00	20.00	30.00
Palm oil	0.00	1.50	1.50	1.50
Fish meal	2.50	2.50	2.50	2.50
Wheat offal	10.00	10.00	10.00	10.00
Dicalcium phosphate	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**

Note: Premix supply matches parameters listed in Table 2.

Table 4. Haematological parameters of broiler chickens fed diets supplemented with *Pleurotus tuber-regium*

Parameters	0 % PT	10 % PT	20 % PT	30 % PT	P-value
PCV (%)	22.15^{c}	34.75^{b}	33.15^{b}	39.10^{a}	2.19
Hb (g/dL)	9.65^{c}	14.70^{b}	14.20^{b}	16.45^{a}	0.67
RBC ($\times 10^{6}/\mu L$)	1.96^{b}	2.94^{a}	2.87^{a}	3.30^{a}	0.11
WBC ($\times 10^{3}/\mu L$)	37.49^{c}	70.92^{a}	65.47^{b}	77.90^{a}	2.75
MCV (fL)	112.10^{ab}	118.20^{a}	115.90^{ab}	118.50^{a}	4.24
MCH (pg)	48.95^{a}	50.10^{a}	49.50^{a}	49.75^{a}	3.22
MCHC (g/dL)	43.65^{a}	42.40^{a}	42.70^{a}	42.00^{a}	1.93
Platelet ($\times 10^{3}/\mu L$)	0.00^{c}	1.00^{a}	2.00^{a}	1.00^{b}	0.012
Neutrophils (%)	26.00^{bc}	31.00^{b}	26.00^{bc}	38.00^{a}	0.018
Lymphocytes (%)	42.00^{c}	56.00^{b}	58.00^{b}	70.00^{a}	0.006
Eosinophils (%)	4.00^{b}	4.00^{b}	4.00^{b}	5.10^{a}	0.003
Basophils (%)	0.00	0.00	0.00	0.00	0.000

…Different superscripts (a, b, c) indicate significant (P < 0.05) differences along rows. PT = Pleurotus tuber-regium, PCV = Packed Cell Volume, Hb = Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration….

Table 5. Serum biochemical indices of broiler chickens fed diets supplemented with *Pleurotus tuber-regium*

Parameters	0 % PT	10 % PT	20 % PT	30 % PT	P-value
Protein (g/dL)	3.40	3.30	3.47	3.80	0.14
Albumin (g/dL)	1.64^{a}	1.58^{a}	1.31^{b}	1.63^{a}	0.09
Globulin (g/dL)	1.76^{b}	1.72^{b}	2.16^{a}	2.17^{a}	0.10
Glucose (mmol/L)	7.11	9.94	7.29	7.64	0.05
Urea (mmol/L)	0.99	0.90	0.82	1.26	0.07
Creatinine (µmol/L)	17.94^{c}	38.84^{ab}	43.24^{a}	37.83^{ab}	2.08
AST (U/L)	37.08^{a}	32.03^{ab}	29.68^{a}	29.35^{a}	1.02
ALT (U/L)	18.36^{b}	17.89^{a}	21.23^{a}	18.16^{a}	0.59
Sodium (mmol/L)	4.41^{b}	5.15^{a}	4.93^{b}	5.91^{a}	0.09
Chloride (mmol/L)	109.35^{bc}	112.15^{b}	105.15^{bc}	128.05^{a}	3.97
Calcium (mmol/L)	1.83^{c}	2.60^{a}	1.73^{c}	2.00^{ab}	0.09

Different superscripts (a, b, c) indicate significant (P < 0.05) differences along rows. PT = Pleurotus tuber-regium, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase.

4.7. Ethical Considerations

- Caring for Animals. Birds were provided with deep litter houses, fed and provided with water at required amounts and times, guaranteeing their well-being during the experiment.
- Fairly Random Distribution: Random assignment of birds ensures the elimination of biasedness while distributing different treatments to the groups.
- Ethical blood collection: The blood sampling method was made as painless and stress-free as possible.
- Scientific Reliability: SAS software and Duncan's test application guarantee reliability, openness and the possibility of repeatability in the experiment's statistics.

4.8. Contribution to Knowledge

- Nutritional supplementation: Showed the viability of the inclusion of Pleurotus tuber-regium into the diets of broilers to an extent of 30% without the occurrence of any negative effect on them.
- Health enhancement: Illustrated significant positive physiological changes, characterized by increase in PCV, Hb, RBC and WBC, all falling within the established norms.
- Economic sustainability: Suggests mushrooms as a cheaper alternative to chemical feed supplements.
- Scientific evidence: Presented statistically reliable data for the use of mushroom supplementation for the nutrition of poultry.
- Future exploration: Explores the possibilities of utilizing different edible mushroom species in poultry diet and evaluation of the effect on production efficiency.

References

[1] Ayisat Olayemi Adisa, and Cordelia Ifeyinwa Ebenebe, "Effect of Dietary Inclusion of Tuber Mushroom (*Pleurotus Tuber-Regium*) Meal on Growth Performance of Broiler Birds," *Agriculture, Food and Natural Resources Journal*, vol. 3, no. 1, pp. 122-127, 2024. [CrossRef] [Publisher Link]

[2] C. Akomah et al., "Growth Performance and Haematological Characteristics of Pullet Chickens Fed Different Feed Forms Supplemented with or Without Oyster Mushroom (*Pleurotusostreatus*)," *Nigerian Journal of Animal Production*, vol. 48, no. 5, pp. 203-212, 2021. [Google Scholar]

[3] Tahreer M. Al-Thuwaini, "The Relationship of Haematological Parameters with Adaptation and Reproduction in Sheep and Goats: A Review Study," *Iraqi Journal of Veterinary Science*, vol. 35, no. 3, pp. 575-580, 2021. [CrossRef] [Google Scholar] [Publisher Link]

[4] I.A. Amata, "The Use of Non-Conventional Feed Resources (NCFR) for Livestock Feeding in the Tropics: A Review," *Journal of Global Bioscience*, vol. 3, no. 2, pp. 604-616, 2014. [Google Scholar] [Publisher Link]

[5] Association of Official Agricultural Chemists, and William Horwitz, *Official Methods of Analysis*, vol. 12, Washington, DC: Association of Official Agricultural Chemists, 1960. [Google Scholar]

[6] G.C. Benerjee, *Textbook of Animal Husbandry*, Oxford and IBH Publishing, 2018. [Google Scholar]

[7] Monica Cheesbrough, *District Laboratory Practice in Tropical Countries*, 2nd ed., Tropical Health Technology, 2006. [Google Scholar] [Publisher Link]

[8] Baudouin Angoua Kokore et al., "Blood Biochemical Parameters Exploration in Broilers and Local Chickens in Korhogo, Côte d'Ivoire," *American Journal of Food and Nutrition*, vol. 9, no. 2, pp. 82-86, 2021. [Google Scholar] [Publisher Link]

[9] David B. Duncan, "Multiple Range and Multiple F Tests," *Biometrics*, vol. 11, no. 1, pp. 1-42, 1955. [CrossRef] [Google Scholar] [Publisher Link]

[10] Junyan Wang et al., "Effects of Ganodermalucidum Supplementation on the Growth Performance, Biochemical Blood Parameters, Antioxidant and Immunity Status, and Meat Quality in Slow-Growing Broilers," *Journal of Applied Poultry Research*, vol. 33, no. 4, pp. 1-11, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[11] C.C. Bormon et al., "Effects of Oyster Mushroom (*Pleurotusostreatus*) Stem Residue Supplementation on Growth Performance, Meat Quality and Health Status of Broilers," *Poultry Science*, vol. 103, no. 10, pp. 1-10. 2024. [CrossRef] [Google Scholar] [Publisher Link]

[12] L.S. Erb et al., "Effects of Mushroom Stump Waste Inclusions to Broiler Diets on Amino Acid Digestibility and D1–21 Performance," *Journal of Applied Poultry Research*, vol. 33, no. 2, pp. 1-9, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[13] Abimbola Fadugba et al., "Inclusion of Mineral Fortified Mushroom (*Pleurotusostreatus*) in the Broiler Feed Reduced the Pathogenic Microbial Loads in the Broiler Fecal Droppings," *Mushroom Research*, vol. 32, no. 2, pp. 115-121, 2023. [CrossRef] [Google Scholar] [Publisher Link]

[14] Asim Faraz et al., "Haematological Evaluation of Marecha Camel Reared under Farming System," *Journal of Agriculture and Food*, vol. 3, no. 2, pp. 1-10, 2023. [Publisher Link]

[15] Yu-Yun Gao et al., "The Addition of Hot Water Extract of Juncao-Substrate *Ganodermalucidum* Residue to Diets Enhances Growth Performance, Immune Function, and Intestinal Health in Broilers," *Animals*, vol. 14, no. 20, pp. 1-15, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[16] A.L. Giwa et al., "Physicochemical Characteristics of Banana Pseudo-Stem Fermented with Pleurotus Tuber-Regium," *Nigerian Journal of Animal Production*, vol. 51, no. 1, pp. 1628-1632, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[17] Akash et al., *Sustainable Livestock Production and Food Security*, Emerging Issues in Climate Smart Livestock Production, Academic Press, pp. 71-90, 2022. [CrossRef] [Google Scholar] [Publisher Link]

[18] I.A. Ikhimioya et al., "Preliminary Investigation on the Haematology of the Nigerian Indigenous Chickens," *Proceedings of the 5th Annual Conference of Animal Science Association of Nigeria*, Port Harcourt, Nigeria, pp. 10-12, 2000. [Google Scholar]

[19] Nemi C. Jain, Essentials of Veterinary Haematology, Lea and Febiger, Philadelphia, 1993. [Online]. Available: https://archive.org/details/essentialsofvete0000jain

[20] Gréta Törős et al., "*Pleurotus Ostreatus* Mushroom: A Promising Feed Supplement in Poultry Farming," *Agriculture*, vol. 14, no. 5, pp. 1-19, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[21] Md. Salahuddin et al., "Harnessing Mushrooms for Poultry Nutrition: Boosting Health, Immunity, and Productivity," *Poultry Science*, vol. 104, no. 8, pp. 1-17, 2025. [CrossRef] [Google Scholar] [Publisher Link]

[22] Brij Mohan Mitruka, and Howard M. Rawnsley, *Chemical Biochemistry and Haematological Reference Values in Normal Experimental Animals*, Masson Publishing, New York, USA, 1977. [Google Scholar]

[23] Samira Ahmed Mohammed et al., "Biochemical and Haematological Profile of Different Breeds of Goats Maintained Under Intensive Production System," *African Journal of Biotechnology*, vol. 15, no. 24, pp. 1253-1257, 2016. [CrossRef] [Google Scholar] [Publisher Link]

[24] Makiwa Simeon Mthana, and Mziwenkosi Nhlanhla Mthiyane, "Low Dietary Oyster Mushroom Spent Substrate Limitedly Ameliorates Detrimental Effects of Feeding Combined Marula Seed Cake and Mucuna Seed Meal as Soybean Replacements in Broiler Chickens," *Tropical Animal Health and Production*, vol. 56, no. 1, pp. 1-17, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[25] NRC, *Nutrient Requirements of Poultry*, 9th ed., National Academy Press, Washington, D.C, 1994. [Google Scholar] [Publisher Link]

[26] NRC, *Guide for the Care and Use of Laboratory Animals*, 8th ed., National Academies Press, Washington, D.C, 2011. [Google Scholar] [Publisher Link]

[27] Md. Rezaul Hai Rakib et al., "Environmental Adaptability Morphometric Features with Reproductive and Productive Potentialities of Indigenous Sheep in Bangladesh," *Journal of Advanced Veterinary and Animal Research*, vol. 9, no. 4, pp. 649-662, 2022. [CrossRef] [Google Scholar] [Publisher Link]

[28] SAS, SAS/STAT User's Guide Introductory and Common Chapters, SAS, 2004. [Online]. Available: https://support.sas.com/documentation/onlinedoc/stat/indexchapter.html

[29] O.W. Schalm, N.C. Jain, and E.J. Carrol, Veterinary Haematology, 3rd ed., Philadelphia: Lea and Febiger, 1975. [Online]. Avaiable: https://catalog.hathitrust.org/Record/001575031

[30] V. Sejian et al., "Effect of Multiple Stresses (Thermal and Walking Stress) on Growth, Physiological Response, Blood Biochemical and Endocrine Response in Malpura Ewes under Semi-Arid Tropical Environment," *Tropical Animal Health and Production*, vol. 45, pp. 107-116, 2013. [Google Scholar]

[31] Gréta Törős et al., "*Pleurotusostreatus* Mushroom: A Promising Feed Supplement in Poultry Farming," *Agriculture*, vol. 14, no. 5, pp. 1-19, 2024. [CrossRef] [Google Scholar] [Publisher Link]

[32] O.O. Shittu et al., "Comparative Study of Physiological Adaptation in West African Dwarf and Kalahari Red Goats in the Humid Tropics," *Nigerian Journal of Animal Production*, vol. 50, no. 1, pp. 21-34, 2023. [CrossRef] [Google Scholar] [Publisher Link]