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Effect of Different Levels of Bentonite in Litter on Blood Parameters of Broiler Chicken

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Abstract | This study was conducted in the poultry to evaluate the impact of different levels of bentonite on blood parameters of broiler chicken. For this purpose, a total of 180 one-day-old, unsexed Ross 308 broiler chicks were randomly assigned to five experimental treatments, each with three replicates per treatment and consisting of 12 chicks per replica. Bentonite was incorporated into the litter at varying levels including the control group (no bentonite), the second treatment (150 g/m^2), the third treatment (250 g/m^2), the fourth treatment (350 g/m^2), and the fifth treatment (450 g/m^2). Birds were fed with two commercial diets where a starter diet (days 1–21) containing 23.03% crude protein and 2,988.49 kcal/kg metabolizable energy, was followed by a grower diet (days 22–35) containing 21.55% crude protein and 3,054.29 kcal/kg metabolizable energy. The statistical analysis of physiological and cellular blood parameters revealed a significant increase ($P \leq 0.05$) in white blood cell count, packed cell volume, and haemoglobin levels in the treatments supplemented with bentonite compared to the control group. Although the red blood cell counts also increased in these treatments, the differences were not statistically significant, except in the second treatment, which showed no significant difference from the control group in hemoglobin concentration. Furthermore, no statistical differences were observed in the heterophil percentage, lymphocyte percentage, or the heterophil-to-lymphocyte ratio. Regarding biochemical blood parameters, a significant improvement ($P \leq 0.05$) was noted in liver enzymes (AST and ALT), as well as in total protein, albumin, and globulin levels in the bentonite-supplemented treatments. On the other hand, the control group exhibited lower blood glucose and cholesterol levels compared to the different treatments. Taken together, the data highlight the importance of environmental enrichment to support the better performance of broiler poultry.

Keywords | Bentonite, Haematological and biochemical blood parameters, Broiler, Litter

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INTRODUCTION

Litter quality plays a critical role in the health and performance of broiler chickens in commercial poultry production. Many factors impact the productivity of poultry, from the hatching stage to market readiness (Abdulateef *et al.*, 2024a, b). Some researchers have applied various methods, including nutritional and managerial approaches, to reduce oxidative stress in the body and alleviate physiological stress (Al-Azzami and Mohammed, 2023; Saad *et al.*, 2023, 2024). Typically composed of feces, feed residues, feathers, and spilled drinking water, litter can become a breeding ground for harmful conditions when moisture accumulates, and acidity increases. This leads to elevated ammonia levels, negatively impacting bird health and increasing the risk of pathogen transmission (Karamanlis *et al*., 2008; Garcia *et al*., 2010). Given that modern broiler production relies on genetically improved birds bred for rapid growth and high productivity, maintaining optimal litter quality is essential for ensuring sustainable performance and minimizing environmental stress.

Bentonite, a naturally absorbent clay, has emerged as a promising additive for improving litter conditions. Its moisture-absorbing and chemical-binding properties help reduce ammonia and moisture levels, creating a healthier environment for poultry. Research suggests that modifying litter with bentonite can have a positive effect on physiological and biochemical parameters in poultry (Klasing, 2007; Dawkins and Layton, 2012).

Blood parameters, such as blood cell counts, protein concentrations, and enzyme levels, serve as reliable indicators of poultry health and the internal environmental conditions within housing facilities. This study aims to assess the effects of supplementing different levels of bentonite in the litter used for rearing Ross 308 broilers, with a focus on key haematological and biochemical parameters. Additionally, we seek to determine the optimal bentonite supplementation level that maximizes physiological and biochemical benefits, ultimately improving litter management practices, enhancing productivity, and safeguarding poultry health and welfare.

MATERIALS AND METHODS

This study was conducted at the Poultry Research Farm, Department of Animal Production, College of Agriculture, University of Basrah, from October 9, 2024, to November 13, 2024. A total of 180 one-day-old Ross 308 broiler chicks were obtained from Fadak Hatchery in Basrah Governorate. The chicks were randomly assigned to five experimental groups, each comprising three replicates, with 12 birds per replicate.

The birds were housed in elevated cages, positioned 100 cm above the ground, and each cage was enclosed with plastic barriers measuring 100 × 100 cm. The flooring was covered with a 5–7 cm thick layer of wood shavings, supplemented with varying levels of bentonite, except for the control group, which received no bentonite supplementation. Cylindrical feeders and manual drinkers with a 6-litre capacity were used, and the birds were provided with a commercial diet ad libitum throughout the experimental period.

Measurements and studied parameters

Blood sampling: At 35 days of age, blood samples were collected from the leg vein of broiler chickens, with three samples per treatment group. Approximately 2.5 mL of blood was transferred into EDTA tubes for analysis of cellular parameters, while the remaining 2.5 mL was centrifuged at 3000 rpm for 15 minutes to separate the serum for biochemical analysis.

Hematological parameters

Total White Blood Cell Count (WBC) was determined using Natt and Herrick's solution Al-Daraji *et al*. (2008), and a hemocytometer, following the formula:

$$\text{Total WBC count } (\times 10^3/mm^3) = (\text{Total cell count in 9 squares} + 10\% \text{ of total WBC count}) \times 200$$

- Packed Cell Volume (PCV): Measured using method (Archer, 1965).
- Haemoglobin Concentration (Hb) was estimated by dividing the PCV value by 3, as described previously (Campbell, 1995).

Heterophil (H), Lymphocyte (L), and H/L Ratio was determined in blood smears, prepared and stained with Wright-Giemsa stain, then examined under a 100× magnification microscope. The H/L ratio was calculated using the formula:

$$H/L \text{ ratio} = (\text{Number of heterophils per 100 WBCs}) / (\text{Number of lymphocytes per 100 WBCs})$$ (Al-Daraji *et al*., 2008)

Blood biochemical parameters

Total serum protein was measured using a BIOLABO-SAS kit (France) at a 550 nm wavelength and calculated as:

$$\text{Total protein } (g/100\ ml) = (\text{Absorbance of sample} / \text{Absorbance of standard solution}) \times 6$$

Globulin concentration wad determined using a BIOLABO-SAS kit at 540 nm wavelength, with the following calculation:

$$\text{Globulin } (g/100\ ml) = (\text{Absorbance of sample} / \text{Absorbance of standard solution}) \times 5$$

Albumin Concentration was derived by subtracting the globulin concentration from the total protein concentration. AST and ALT Enzyme Activities were assessed using BIOLABO kits at a 505 nm wavelength, with results expressed in IU/L based on a standard curve.

Blood Glucose Concentration was measured with a BIOLABO SA kit at a 550 nm wavelength, using the formula:

Glucose (mg/100 ml) = (Absorbance of sample/ Absorbance of standard solution) × 100

The collected data were analyzed statistically using SPSS software (version 2016). Differences among treatment means were evaluated using the Least Significant Difference (LSD) test, with statistical significance set at P < 0.05.

RESULTS AND DISCUSSION

The results indicate that bentonite supplementation had no significant effect (P ≤ 0.05) on red blood cell count among the different treatment groups at 35 days of age (Table 1). However, significant differences (P ≤ 0.05) were observed in white blood cell (WBC) counts, with treatment five recording the highest count (31.35 million cells/mm^3), which was significantly higher than that of the control group (24.75 million cells/mm^3). Similarly, treatments four, three, and two also exhibited substantially higher WBC counts than the control, with values of 30.30, 29.95, and 29.65 million cells/mm^3, respectively. Furthermore, a significant increase (P ≤ 0.05) in packed cell volume (PCV) was observed among the treatment groups. Treatment five recorded the highest PCV value (46.50%), whereas the control group had the lowest (34.98%). The PCV levels for treatments four, three, and two were 41.91%, 39.59%, and 35.13%, respectively.

Similarly, significant differences (P ≤ 0.05) were observed in haemoglobin concentration. Treatment five recorded the highest haemoglobin level (14.15 g/ml), while treatment two had the lowest (10.50 g/ml), which was not significantly different from the control group (10.60 g/ml). The haemoglobin concentrations in treatments three and three were 12.70 g/ml and 12.00 g/ml, respectively.

Conversely, data revealed no significant effects of bentonite supplementation on heterophil percentage, lymphocyte percentage, or the H/L ratio, although the control group showed slightly higher numerical values in these parameters (Table 2).

The observed significant variations in cellular blood parameters (white blood cells, PCV, and haemoglobin levels) among the treatment groups can be attributed to improvements in litter quality due to bentonite supplementation. Bentonite is known to help reduce ammonia levels, minimize the presence of harmful gases such as methane, carbon dioxide, and hydrogen sulfide, regulate moisture content and temperature, and maintain a stable acidic pH during the first three weeks. Additionally, it helps reduce bacterial load and improve overall poultry welfare conditions. These improvements likely alleviated stress factors, thereby enhancing physiological health and immune function in broilers. This effect is consistent with findings by Aly *et al.* (2018), who demonstrated that clay minerals significantly reduce ammonia emissions in poultry houses, leading to improved respiratory health and blood parameters. Furthermore, Prayitno *et al.* (1997) have reported that bentonite supplementation in litter enhanced mineral availability and subsequently improved haematological values in broilers.

The increased white blood cell count in broilers supplemented with bentonite may reflect a positive immune response to improved environmental conditions. These findings are consistent with previous studies suggesting that changes in haematological parameters serve as indicators of stress levels, overall health status, and energy metabolism in poultry (Hrabčáková *et al.*, 2014; Karamanlis *et al.*, 2008; Alabi *et al.*, 2015; Qi *et al.*, 2017; Scope *et al.*, 2002; Gebregeziabhear, 2015; Attia at al., 2017). Recent studies by Jahanian and Ashnagar (2018) have shown that reduced ammonia exposure in poultry houses correlates with improved lymphocyte proliferation and enhanced immune response. The immunomodulatory effect of improved litter conditions has been linked to

Table 1: Effect of adding different levels of bentonite to broiler litter on blood cellular parameters (red and white blood cells, packed blood cells, and haemoglobin percentage) at 35 days of age (mean ± standard error).

Treatment/ Indicators	RBC (x10^9/L)	WBC (x10^9/L)	PCV %	Hb %
Control	3.93±0.170	b24.75 ±1.050	34.98 b±2.640	10.60 b±0.800
T2	4.29 ±0.730	ab29.65 ±1.750	35.13 b±3.770	10.50b±1.300
T3	5.26 ± 0.820	Ab29.95 ±1.350	39.59 ab±1.310	12.00ab±0.400
T4	5.17 ±0.930	ab30.30 ±1.200	41.91ab± 2.970	12.70ab±0.900
T5	6.10 ±0.840	a31.35 ±1.850	46.50 a± 0.300	14.15 a±0.150
P value	N.S	*	*	*

* Significant (different letters vertically represent significant differences at the P≤0.05 level), N.S means no significant differences between the various experimental treatments, Treatments: T1: Control treatment, no added wood shavings broiler litter was used, T2: Second treatment, 150g/m2 of bentonite was added to the broiler litter, T3: Third treatment, 250g/m2 of bentonite was added to the broiler litter, T4: Fourth treatment, 350g/m2 of bentonite was added to the broiler litter, T5: Fifth treatment, 450g/m2 of bentonite was added to the broiler litter, RBC: Red blood cells, WBC: White blood cells, PCV: Packed blood cells, Hb: Hemoglobin.

Table 2: Effect of adding different levels of bentonite to broiler litter on blood cellular parameters (heteroblasts, lymphocytes, and the ratio between heteroblasts and lymphocytes) at 35 days of age (mean ± standard error).

Treatment/ Indicators	Hetero %	Lympho %	H/L Ratio
Control	42.00±1.000	58.00±1.000	0.72±0.030
T2	35.50±2.500	64.50±2.500	0.55±0.060
T3	38.00±2.000	62.00±2.000	0.61±.0300
T4	37.50±4.500	62.50±4.500	0.60±.1150
T5	36.00±1.000	64.00±1.000	0.56±0.025
P value	N.S	N.S	N.S

N.S means no significant differences between the different experimental treatments, Treatments T1: Control treatment, sawdust broiler litter was used without addition, T2: Second treatment, 150g/m2 of bentonite was added to the broiler litter, T3: Third treatment, 250g/m2 of bentonite was added to the broiler litter, T4: Fourth treatment, 350g/m2 of bentonite was added to the broiler litter, T5: Fifth treatment, 450g/m2 of bentonite was added to the broiler litter, Hetro: Heteroblasts, Lymphocytes: Lymphocytes, H/L: Ratio between heteroblasts and lymphocytes.

reduced oxidative stress, as demonstrated by Kheravii *et al*. (2017), who found decreased levels of malondialdehyde and increased antioxidant enzyme activities in birds raised on treated litter.

The data presented in Table 3 revealed significant differences (P ≤ 0.05) among the treatment groups in biochemical parameters, including glucose, ALT, AST, total protein, albumin, globulin, and cholesterol levels, at 35 days of age.

Glucose concentration varied notably across treatments, with treatment five exhibiting the highest level (260.68 mg/100 mL), significantly surpassing all other groups. In contrast, treatment three recorded the lowest glucose concentration (204.23 mg/100 mL), although it was not significantly different from treatment four (208.01 mg/100 mL). The control group and treatment two showed intermediate glucose levels of 224.62 mg/100 mL and 235.02 mg/100 mL, respectively, both of which were significantly lower than those in treatment five.

ALT levels also displayed significant variation across the groups. The control group had the highest concentration (3.57 IU/L), while treatment two recorded the lowest (1.22 IU/L). Treatments five, four, and three exhibited moderate ALT levels of 3.24, 3.37, and 1.80 IU/L, respectively.

Similarly, AST levels showed significant differences, with the control group presenting the highest concentration (365.99 IU/L). Treatment two recorded the lowest AST level (232.52 IU/L), whereas treatments five (299.27 IU/L) and four (284.19 IU/L) had significantly higher levels than treatment two, though they were not substantially different from each other. Treatment three (260.39 IU/L) also exhibited higher AST levels than treatment two.

Total protein concentration varied significantly among treatments. Treatment three had the highest total protein level (3.15 g/100 ml), whereas treatment two recorded the lowest (2.72 g/100 ml), which was not significantly different from the control group (2.73 g/100 ml). Treatments five and four showed moderate values of 3.07 g/100 ml and 2.93 g/100 ml, respectively.

Albumin concentration differed significantly across the groups, with treatment three recording the highest level (1.61 g/100 mL). Treatment two had the lowest concentration (1.56 g/100 mL), which was comparable to that of the control, treatment four, and treatment five (1.55, 1.56, and 1.57 g/100 mL, respectively).

Cholesterol levels also exhibited significant variation, with treatment four registering the highest concentration (138.10 mg/dl) and treatment five the lowest (136.72 mg/dl). The control group, treatment two, and treatment three showed intermediate cholesterol levels.

Table 3: Effect of adding different levels of bentonite to broiler litter on blood chemistry and vital parameters (mean ± standard error).

Treatment/ Indicators	Glucose (mg/dl)	ALT (U/L)	AST (U/L)	T.Protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Globulin (g/dl)
Control	214.62 c±1.105	3.57 a±.105	365.99 a±2.495	2.73c±.005	1.55b±.020	101.71 c±3.520	1.18 b±.015
T2	235.02 b±2.260	1.22 e±.030	232.52 d±6.735	2.72c±.035	1.56b±.020	138.10 b±2.465	1.16 b±.015
T3	204.23 d±1.525	3.24 b±.100	260.39 c±.765	3.15a±.060	1.69a±.020	140.83 b±1.210	1.46 a±.040
T4	208.01 d±.665	1.80 d±.095	284.19 b±4.540	2.93b±.005	1.54b±.010	152.88 a±3.240	1.39 a±.005
T5	260.68 a±1.995	2.37 c ±.030	299.27 b±5.310	3.07ab±.095	1.57b±.025	136.72 b±3.935	1.50 a±.070
P value	*	*	*	*	*	*	*

*Significant (different letters vertically represent significant differences at the P≤0.05 level), Treatments T1: Control treatment, broiler sawdust was used without addition, T2: Second treatment, 150g/m2 of bentonite was added to the broiler litter, T3: Third treatment, 250g/m2 of bentonite was added to the broiler litter, T4: Fourth treatment, 350g/m2 of bentonite was added to the broiler litter, T5: Fifth treatment, 450g/m2 of bentonite was added to the broiler litter, ALT Alanine aminotransferase AST Aspartate aminotransferase, T.Protein Total protein, Albumin, Cholesterol, Globulin.

Globulin concentration varied significantly, with treatment three having the highest value (1.50 g/100 mL), while treatment two recorded the lowest (1.16 g/100 mL), which was not significantly different from the control group (1.18 g/100 mL).

These biochemical improvements can likely be attributed to the enhanced litter quality resulting from the addition of bentonite. The addition of bentonite helped reduce ammonia and other harmful gases, improved the overall litter condition, and contributed to better poultry welfare. These factors collectively led to improved health status and physiological performance in broilers. The decline in liver enzymes (ALT and AST) suggests a reduction in ammonia exposure, which may have mitigated potential liver stress. Additionally, higher serum protein levels could indicate enhanced liver function and improved nutritional status. Similarly, Marzouq and Saad (2025) demonstrated that supplementing broiler diets with 3 % vegetal charcoal markedly improved growth rates and feed conversion efficiency, increased breast and thigh meat yields (indicative of superior carcass quality), reduced intestinal counts of *Salmonella* and *E. coli* while enhancing *Lactobacillus* populations, and elevated haematological indices (PCV, Hb, total protein) associated with better health status. These outcomes further substantiate the premise that mineral- or clay-based additives optimize productive and physiological performance by improving the gut environment and mitigating toxic load.

These findings align with previous research that has established a strong correlation between improved litter conditions, reduced ammonia levels, and enhanced physiological and biochemical parameters in broilers (Zarnab *et al*., 2019; Taherparvar *et al*., 2019; Aldu-hafeez *et al*., 2009; Abdel-Fattah *et al*., 2008; Hrabčáková *et al*., 2014). Recent research by Kheravii *et al*. (2017) have provided evidence that exposure to high ammonia levels induces hepatocellular damage, resulting in elevated serum transaminase levels. The significant reduction in liver enzymes observed in bentonite-treated groups supports the hepatoprotective effects of improved litter quality. Additionally, Sharma *et al*. (2016) demonstrated that improved environmental conditions significantly enhanced protein metabolism and reduced stress-induced catabolism in broilers. The elevation in serum proteins, particularly globulin, in our study is consistent with findings by Sokolenko *et al*. (2024), who reported positive correlations between optimal litter conditions and enhanced immunoglobulin production in poultry.

The glucose variations observed across treatments may reflect differences in stress levels and energy metabolism. Gorelik *et al*. (2020) reported that ammonia stress increases glucocorticoid release, leading to enhanced gluconeogenesis and altered glucose homeostasis in broilers. The improved glucose patterns in bentonite-treated groups suggest reduced metabolic stress, which aligns with findings by Ibrahim *et al*. (2018) who demonstrated that litter amendments significantly reduce cortisol levels and improve energy utilization in broilers.

The dose-dependent effects observed across treatments are consistent with research by Coufal *et al*. (2006) and Wang *et al*. (2020), who established optimal application rates for litter amendments in commercial poultry production. Our findings support the importance of appropriate bentonite supplementation levels to achieve maximum benefits while avoiding potential negative effects on mineral absorption, as highlighted by (Safaeikatouli *et al*., 2012).

CONCLUSION

Incorporating bentonite into litter had a notable positive impact on key haematological and biochemical parameters, indicating enhanced physiological health and more efficient feed utilization in broilers. These findings suggest that bentonite supplementation could serve as a valuable strategy for enhancing poultry management practices, ultimately contributing to improved bird welfare, optimized environmental conditions, and increased production efficiency.

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NOVELTY STATEMENT

Study examines the effect of adding bentonite at varying levels (150-450 g/m^2) to poultry litter on blood parameters and liver functions of broiler chickens, where the results showed a notable improvement in blood indicators and physiological condition of the birds, confirming the effectiveness of using bentonite as a strategy to improve the poultry breeding environment in commercial systems.

AUTHOR CONTRIBUTION

HFS conducted methodology, writing original draft preparation. MNR contributed in writing review and editing. All authors have read and agreed to the published version of the manuscript.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflicts of interest

The authors declared no conflict of interest.

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