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Effect of different level of dietary kaolin and litter treated with sodium bisulfate on litter ammonia gas emission, microbial load, blood indices and growth performance of broilers

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ABSTRACT

A six-week experiment was carried out at the Department of Animal Science, Teaching, and Research Farm, University of Nigeria, Nsukka from February-March, 2025, to determine the effect of different level of dietary kaolin and litter treated with sodium bisulfate on litter ammonia gas emission, microbial load, blood indices and growth performance of broilers. By the application of 3×2 factorial arrangements in CRD, 300 Anak strain broiler birds (2 weeks old) were divided to six research groups with 5 replications of 10 birds each. Treatments were as follows: T_1= 0 g kaolin /kg + 0 g SB/5kg litter, T_2= 15 g kaolin /kg+0g SB/5kg litter, T_3= 30 g kaolin /kg + 0g SB/5kg litter, T_4= 0 g kaolin/kg + 500 g SB/3kg litter, T_5=15 g kaolin /kg +500g SB/5kg litter and T_6= 30 g kaolin /kg + 500g SB/5kg litter. Parameters measured were as follows: body weight, ADWG, feed intake, ADFI, FCR, feed cost per kg gain, litter pH, moisture, ammonia gas, TVC, blood PCV, RBC, WBC. Results showed that control group broilers had total weight gain and ADWG values of 2200.00 g/bird and 52.38 g/bird and these values were the lowest ($p<0.05$) among the treatments. FCR values of 2.64 obtained for birds on control treatment was the same ($p>0.05$) with T_4 and both were the highest among the treatments. Litter moisture values of 13.20, 26.00 and 29.66 % obtained at week 1-2, 3-4 and 5-6 were higher ($p<0.05$) in T_1 compared to other treatments. Weekly pH values of 5.06, 7.66 and 8.33 at week 1-2, 3-4 and 5-6 were highest in T_1 litter. Ammonia values of 22.00, 29.00 and 44.33ppm obtained at week 1-2, 3-4 and 5-6 in T_1 were higher ($p<0.05$) when compared with values obtained in T2-6 in both weeks. Values of PCV, RBC and WBC improved better in T_2-T_6 when juxtaposed with value obtained in T1. It was concluded that dietary kaolin and litter treated with sodium bisulfate diminished the emission of litter ammonia gas without destructive impact on the performance of the birds. It was, suggested that 15g kaolin /kg + 500 g SB/5kg litter could be applied for better performance and reduction in litter ammonia gas emission in poultry production.

Keywords: Ammonia gas, Broiler birds, Growth performance, Kaolin, Sodium bisulfate

Poultry production, is among the fastest means of producing a low priced, safe and quality animal protein for consumption by the increasing human population worldwide (Goran *et al.* 2023). However, one of the environmental glitches associated with raising birds close to residential areas is the production of gaseous contaminants from the litter during production (Ni *et al.* 2023). These gases impact negatively on birds, human health and environment (Celestine *et al*. 2025a, Ni *et al.* 2023, Zhou *et al.* 2021, EPA 2022). Ammonia is one of the most unpleasant gases that emanates from the litter during production. Ammonia gas is produced when uric acid, an, excretory compound from birds and other N_2 containing compounds in the litter are decomposed by microorganisms living in the litter (Munk *et al.* 2017). Birds raised in a facility that emitted high levels of ammonia had poor body weight, poor egg quality traits, FCR (Celestine *et al.* 2025b, Ezenwosu *et al.* 2024), exposed to infections agents (EPA 2022), showed respiratory glitches (Bist *et al*. 2023, Ramesh *et al.* 2024, Ezenwosu *et al.* 2024).

Kaolin, as a clay mineral additive can be exploited to increase productivity of birds (Ezenwosu *et al*. 2023, Jorge de Lemos *et al.* 2015, Safaeikatouli *et al.* 2012) and reduce the emission of litter ammonia gas in poultry facilities during production (Ezenwosu and Udeh 2022 ; Ezenwosu *et al.* 2023). Safaei *et al.* (2014) included kaolin in broiler ration and observed reduced emission of environmental contaminants.

Litter can also be treated with chemical to reduce emission of ammonia and thereby creating an environment that improves productive performance of birds (De Toledo *et al.* 2020, Celestine *et al*. 2025a). Ezenwosu *et al.* (2022)

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and Ezenwosu *et al.* 2024) discovered that litter treated with chemical works by bringing down litter pH and thereby, creating an acidic litter environment that depresses microbial litter proliferation and eventual putrefaction of uric acid and other compounds that contains nitrogen in the litter to NH_3. Frequently used chemical litter additive compound is sodium bisulfate (Blake and Hess, 2001). Sodium bisulfate is effective in enhancing litter quality, growth performance of birds (Purswell *et al.* 2013) reducing pathogenic organisms on avian carcasses and respiratory glitches (Pope and Cherry, 2000). Application of sodium bisulfate to the litter diminished ammonia production compared to control litter for up to thirty days (McWard and Taylor, 2000). Because of the fact that ammonia gas still remains a veritable threat to poultry industry, there is a need to come up with better method to lower its emission that is environmentally friendly during production. It was therefore justified to study the synergistic effect of different level of dietary kaolin and litter treated with sodium bisulfate on litter ammonia gas emission, microbial load, blood indices and growth performance of broilers.

MATERIALS AND METHODS

Location and duration/period of study: The study took place at research unit of Poultry Farm of the Animal Science Department, University of Nigeria Nsukka. Nsukka is located within latitude 7° 12.5 ′N and longitude 6° 45′E and 7° E and on the altitude of 447m overhead the sea level. Nsukka (study area) is a typical tropical environment with daily mean temperature of 26.8 Celsius and relative humidity ranging from sixty-five to eighty percent. Yearly rainfall of the study area ranges from 1567.05mm-1846.98mm (Department of Crop Science, Metrological Center, University of Nigeria). The study lasted for 6 weeks from February-March, 2025

Approval of animal ethics committee: The experiment was done in conformity with the rules established by the ethical Committee on the use of animals for research of the University of Nigeria, Nsukka, (No: UNN/ANS79/12.01.2025).

Experimental birds: A total of 300 Anak strain broiler birds (2 weeks old) of were used for the study. Treatments included: T1= 0 g kaolin /kg+ 0 g SB/5kg litter, T2= 15 g kaolin /kg+0 g SB /5kg litter, T3= 30 g kaolin /kg diet + 0 g SB /5kg litter, T4= 0 g kaolin /kg + 500 g SB /5kg litter, T5=15 g kaolin /kg +500g SB /5kg litter and T6= 30 g kaolin /kg + 500g SB /5kg litter. Inclusion of kaolin in the ration was at three dietary levels 0, 15 and 30 g/kg, while sodium bisulfate was used at 2 levels of 0 and 500 g/3kg litter. The broilers were provided with starter diet in crumble form in the first 2 weeks, followed by a finisher diet in pelleted form that lasted for 4 weeks. All management practices and vaccination for good health of birds were followed. The birds had feed and water available to them all through the study. In a deep litter system house (open-sided), the broilers were reared in thirty replications each measuring 4 × 4 square meters. Temperature and humidity were kept at 25 ˚C and 60-65. The basal diets were formulated to meet the nutrient requirements of the birds following NRC (1994). Nutritive components of the ration were determined by the application of AOAC (2012) and are presented in Table 1.

Experimental design: An experimental design of 3×2 factorial arrangements in CRD comprising of five replicates of 10 birds each was applied.

Litter treatments: Treatment of litter with sodium bisulfate was carried out following the methods described by Ezenwosu *et al.* (2024).

Growth performance evaluation: With the use of electronic balance of 6k g capacity, the bird's weights were taken initially and once per week during Their daily feed intake was taken. Feed intake was divided by the

Table 1. Ingredients composition (g/kg) of basal diet of broiler birds

Ingredients (%)	Starter	Finisher
Maize (%)	42.00	49.50
Wheat offal (%)	7.10	5.00
Soybean meal (%)	14.00	8.00
Groundnut cake (%)	16.00	13.00
Palm kernel cake (%)	10.00	14.30
Fish meal (%)	1.50	1.00
Bone meal (%)	2.70	2.00
Di-calcium phosphate (%)	1.50	1.50
Vitamin premix (%)	0.13	0.13
Palm oil (%)	4.00	5.00
Mineral premix (%)	0.12	0.12
L-Methionine (%)	0.10	0.10
Antioxidant (%)	0.10	0.10
Toxin binder (%)	0.10	0.10
DL- Methionine (%)	0.15	0.15
Salt (%)	0.25	0.25
L-Lysine (%)	0.25	0.25
Calculated nutritive values (%)		
Crude protein (%)	22.59	19.09
Dry matter (%)	90.12	90.15
Metabolizable energy (Kcal /kg)	2914	3012
Crude fiber (%)	3.50	3.60
Ether extract (%)	4.01	3.56
Ash (%)	5.78	5.50

Calculated values: vitamin and mineral premix per/kg of diet: Vitamin D3 (100000 I.U), Vitamin E (50000mg), Vitamin K3 (500mg), Vitamin B1 (1500mg), Vitamin B6 (2000mg), ,Vitamin B12 (10mg), Copper (3400mg), Iodine (600mg), vitamins A (5000 I.U), Cobalt (120mg), Selenium (48mg) , Folic acid (400mg), Biotin (32mg),Choline chloride (2000mg), Manganese (10000mg), Vitamin B2 (22000mg), Niacin (22000mg), iron (40000mg), Zinc (32000mg), weight gain to obtain the FCR of the birds. Cost of feed per kg ×FCR = feed cost per kg gain. ADWG and ADFI were calculated by dividing values of weight gained and feed consumed by the birds with number days (42) the experiment lasted.

Litter pH, moisture and ammonia evaluation: To ensure proper sample representation in every replicate, samples of litter were taken from four dissimilar points, starting from the uppermost part to the bottom using a hand trowel and were mixed together after collection. The samples were moved to the lab in well labeled beakers for pH and moisture evaluation. Moisture (%) content of litter samples were evaluated by comparing their weights earlier and after they were dried in a hot air oven at 65^0C for 48 hours. For pH determination, 400 ml of reverse-osmosis water and litter sample (20 g) were put together, shaken cogently for 20 minutes, and the pH of the sample was then taken by the use of pH meter (Seven Easy pH, Schwerzenbach, Switzerland). By the use of Smart Sensor GM8806, Benetech, Nanjing, China, a digital NH_3 measurement apparatus, litter ammonia gas production was determined weekly. The smart sensor ammonia detector was placed at four corners and epicenter of the litter materials in each pen. Agar plate method, as delineated by Harrigan and McCance (1976), was used to evaluate the litter total viable count.

Haematological analysis: Randomly, two birds were picked from each replicate at end of the study, and 5 ml blood each was taken from them via their wings. Blood samples were emptied into labeled tubes containing EDTA. A Hemocrit reader was used to measure the PCV v in a microhaematocrit capillary tube, and an automatic cell counter was used to determine the hemoglobin and RBC concentrations.

Statistical analysis: Data obtained were subjected to two-way ANOVA. Significant differences among the means were separated using Duncan's New Multiple Range Test with the aid of IBM SPSS Statistics version 28 and accepted at 5 % ($p<0.05$) level of probability. The statistical model used was stated below: $X_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \sum_{ijk}$, where; A and B represent the two factors, X_{ijk} = Overall observation on effect of the treatments**,** μ= Population mean**,** A_i = effect of kaolin, B_j = effect of sodium bisulfate, (AB)ij =Interaction of sodium bisulfate and kaolin**,** $\sum_{ijk}$ = Experimental error

RESULTS AND DISCUSSION

Growth performance: The results of the synergistic effect of different level of dietary kaolin and litter treated with sodium bisulfate on growth performance of broilers are presented in Table 2. The values of TWG, ADWG, FCR and feed cost per kg gain were significant ($p<0.05$), while the values for feed intake were not significant ($p<0.05$). Results showed that control group broilers had total weight gain and ADWG values of 2200.00 g/bird and 52.38 g/bird and these values were the lowest ($p<0.05$) among the treatments. FCR values of 2.64 obtained for birds on control treatment was the same ($p>0.05$) with T_4 and both values were the highest among the treatments. Feed cost per kg gain of birds on T_2-T_6 were similar ($p>0.05$), but lower than the value of 1003.20 obtained in T_1 which was also similar with T_4. Weight gain, FCR and feed cost per kg gain improved in favor of the treated groups. The present findings concur with those of Shi *et al.* (2009) and Ezenwosu *et al.* (2023) who found that adding kaolin to the ration of broilers enhanced their body weight gain and FCR. However, kaolin in the diet increases feed retention time in the birds' guts, subjecting the nutrients to greater enzymatic degradation, and thereby leading to improvement in body weight gain and FCR. Safaei *et al.* (2014) found that addition of clays to the diet of poultry regulate the processes of digestion and nutrient absorption. Eliot and Edwards (1991b) noted that a change in the pH of the gastrointestinal tract might be the cause of increase in weight gain in poultry fed diet containing kaolin. The same authors further elucidated that in the presence of kaolin when the gut pH reached six, absorption of nutrients increased and that improved weight gain. Birds on T_1 having the lowest weight gain, increased FCR and cost per kg could be linked to increased emission of ammonia during the study. Air quality within pens during production influences productivity of birds (Celestine *et al.* 2025b). Miles *et al.* (2006) noted that ammonia concentrations in chicken pens over 25 parts per million can result in decreased weight, diet consumption and an elevated FCR. Ammonia is the most prevalent toxic gas in chicken houses (Zhou *et al.* 2021). Ammonia gas adversely affects health (Wang *et al.* 2020) and bird's growth (Naseem and King 2018). Findings of Wei *et al*. (2012) disclosed a decline in antioxidant capacity in broilers subjected to high ammonia production, supports the idea that ammonia gas is a stressor and stress leads a decrease in productivity of birds (St-Pierre *et al.* 2003). Li *et al*. (2014) who noted a decrease in ADWG in broilers reared in pens with high ammonia production was in comparison with result of the current research findings. Ammonia negatively affects productive performance of farm animals as a result of its hazardous effect on organs such as liver (Sa *et al.* 2018), intestine (Wang *et al*. 2019), brain, spleen (Zhao *et al.* 2020), and respiratory tract (Liu *et al.* 2020) In treatment groups, highest performance was observed. This could be linked to the reductive effect of kaolin and sodium bisulfate on litter ammonia gas production that created a better microclimate in the pens and thus culminating to improved nutrient utilization in birds (Ezenwosu *et al*. 2023). The results agree with Ezenwosu and Udeh 2022) who observed reduction in % litter ammonia gas production when included kaolin in the diet of broilers and also in tandem with McWard and Taylor (2000) who discovered that amendment of litter with sodium bisulfate controlled ammonia emission up to thirty days relative to untreated control in poultry facilities. Sodium bisulfate as an effective litter acidifier enhances litter quality and improve performance of birds (Purswell *et al.* 2013). In addition, sodium bisulfate usage as a

Table 2. Synergistic effect of different level of dietary kaolin and litter treated with sodium bisulfate on growth performnce of broilers

Parameters	T1	T2	T3	T4	T5	T6	SEM	P-values
IBW (g/bird)	199.33	203.33	200.13	202.53	198.20	192.73	7.49	0.87^{NS}
TWG(g/bird)	2200.00^{c}	2333.33^{b}	2400.00^{b}	2333.33^{ab}	2483.00^{a}	2466.66^{a}	25.28	0.02^{S}
TFI (g/bird)	5825.66	5402.96	5602.17	5633.00	5278.33	5303.00	98.19	0.84^{NS}
FCR (g/g)	2.64^{a}	2.31^{b}	2.33^{b}	2.41^{ab}	2.12^{b}	2.15^{b}	0.05	0.01^{S}
Feed cost/kg. gain (₦)	1003.20^{a}	877.80^{b}	885.40^{b}	914.53^{ab}	819.53^{b}	815.73^{b}	19.52	0.00^{S}
ADWG (g/bird)	52.38^{c}	55.56^{b}	57.14^{b}	55.55^{ab}	59.12^{a}	58.72^{a}	0.60	0.00^{S}
ADFI3 (g/bird)	138.72	128.64	133.38	134.11	125.67	126.65	2.33	0.63^{NS}

a, b, c, values with different superscripts within the same column are significantly different at 5% level ($P < 0.05$, NS: $P > 0.05$), IBW: Initial body weight, TWG: Total weight gain, TFI: Total feed intake, FCR: Feed conversion ratio, ADWG: Average daily weight gain, ADFI: Average daily feed intake, NS: Not significant, S: Significant, SEM: Standard error of the mean

chemical litter treatment compound improves bird's performance (Pope and Cherry, 2000).

Table 3 present the results of the synergistic effect of different level of dietary kaolin and litter treated with sodium bisulfate on litter chemical characteristics during broiler rearing. Litter moisture values of 13.20, 26.00 and 29.66 % obtained at week 1-2, 3-4 and 5-6 were higher ($p<0.05$) in T_1 compared to other treatments. Weekly pH values of 5.06, 7.66 and 8.33 at week 1-2, 3-4 and 5-6 were highest in T_1 litter. Ammonia values of 22.00, 29.00 and 44.33ppm obtained at week 1-2, 3-4 and 5-6 in T_1 were higher ($p<0.05$) when compared with values obtained in T2-6 in both weeks. Nevertheless, the amplified % of litter ammonia gas emission may be connected to the dwindled litter moisture and pH observed in treated groups due to the inclusion kaolin in the ration and chemical litter treatment with sodium bisulfate. The results were in line with Ezenwosu and Udeh (2022) who noted a reduced litter moisture when kaolin was added to the diet of broilers. Current finding concurred with Payne *et al.* (2019) who raised birds on litter treated with sodium bisulfate and noted that pH of the treated litters was lower than the control. Moisture was one of the primary factors associated with the production of ammonia from litter (Elliott and Collins, 1982). Uric acid is the key source of ammonia in poultry litter. However, high levels of moisture and pH of litter during production will facilitate the conversion of uric acid (avian excretory product) composed of nitrogen and other nitrogen compounds in the litter to ammonia. Emission of ammonia during production tends to rise with rising litter pH and fall with falling litter pH. Emission of ammonia decreases when litter pH cascades below seven and profoundly rise when pH of litter is above eight (Reece *et al.* 1979). Degradation of uric acid to ammonia is mainly enhanced under alkaline litter state (Rappet and Muller 2005). Impact of enzyme known as uricase that bring about the degradation of uric acid and other nitrogen in the litter to ammonia gets too extreme at pH of nine. Additionally,

Table 3. Synergistic effect of different level of dietary kaolin and litter treated with sodium bisulfate on litter chemical characteristics during broiler rearing

Parameters	T1	T2	T3	T4	T5	T6	SEM	P-values
				Moisture (%)				
Week 1-2	13.20^{a}	11.33^{b}	10.33^{bc}	10.33^{bc}	8.66^{c}	16.66^{b}	0.42	0.00^{S}
Week 3-4	26.00^{a}	20.00^{b}	14.66^{b}	19.00^{b}	16.33^{b}	16.66^{b}	1.05	0.00^{S}
Week 5-6	29.66^{a}	21.66^{b}	20.33^{b}	22.00^{b}	20.00^{b}	20.00^{b}	1.35	0.87^{NS}
				pH				
Week 1-2	5.06^{a}	4.33^{ab}	4.00^{bc}	3.00^{cd}	3.16^{cd}	2.66^{d}	0.22	0.02^{S}
Week 3-4	7.66^{a}	5.66^{b}	5.66^{b}	5.33^{b}	4.66^{b}	4.33^{b}	0.32	0.01^{S}
Week 5-6	8.33^{a}	6.66^{b}	6.333^{bc}	6.00^{bc}	5.66^{bc}	5.00^{c}	0.29	0.03^{S}
				Ammonia (ppm)				
Week 1-2	22.00^{a}	18.66^{bc}	17.33^{cd}	21.13^{ab}	15.00^{de}	12.66^{e}	0.83	0.00
Week 3-4	29.00^{a}	21.33^{bc}	22.00^{bc}	23.00^{b}	17.00^{cd}	15.00^{d}	1.24	0.00
Week 4-6	44.33^{a}	26.66^{bc}	22.00^{cd}	31.00^{b}	20.66^{d}	19.33^{d}	2.15	0.00

a, b, c,d, e, values with different superscripts within the same column are significantly different at 5 % level ($P < 0.05$, NS: $P > 0.05$), SEM: Stanford error of the mean, NS: Not significant, S: Significant

Table 4. Synergistic effect of differ3qent level of dietary kaolin and litter treated with sodium bisulfate on litter microbial count during broiler rearing

Parameter	T1	T2	T3	T4	T5	T6	SEM	P-values
Total viable count	$3.18{\times}10^{6a}$	$3.06{\times}10^{6a}$	$3.08{\times}10^{6a}$	$2.20{\times}10^{6b}$	$1.84{\times}10^{6b}$	$1.69{\times}10^{6b}$	$1.9{\times}10^{6}$	0.00^{S}

a, b, c, values with different superscripts within the same column are significantly different at 5% level (P < 0.05; NS: P > 0.05), SEM: Standard error of the mean

Ezenwosu *et al.* (2024) and Safaeikatoul *et al.* (2011) expounded that reduced pH of litter has a decreasing impact on ammonia gas emission from poultry litter. Shah *et al.* (2007) noted part of NH_4^+ will be converted to ammonia in the presence of elevated pH and litter moisture during production. But, chemical litter additives provide many advantages, including lowering the pH of litter, preventing soluble nitrogen from turning into ammonia (Lee *et al.* 2015).

Table 4 displays the results of the synergistic effect of different level of dietary kaolin and litter treated with sodium bisulfate on litter microbial count during broiler rearing. TVC value in T_2-T_6 were lower ($p<0.05$) than the value of $3.18{\times}10^6$ obtained in T_1. This could be linked to the decreased ($p>0.05$) litter moisture and pH recorded in treatment groups as the result of the kaolin inclusion in the diet and litter treatment with sodium bisulfate. The agrees with Ismael and Ismail (2021) who used sodium bisulfate as litter treatment and recorded reduced total aerobic count load in the litter. When litter moisture content is high, it will become a favorable environs for the growth of bacteria and proliferation. Kaolin as a clay additive works to absorb moisture in quantity bigger than its weight and stop the moisture from remaining at liberty in the droppings (excreta). With reduced litter moisture by the dietary inclusion of kaolin (Ezenwosu and Udeh, 2022), litter microbial growth is reduced. However, litter with high moisture content is ideal for the growth of litter microbial organisms (De Toledo *et al.* 2020). However, poultry litter chemical treatment reduces growth of bacterial in pens and within poultry facilities (De Toledo, *et al.* 2020). The lowered litter pH may also be responsible for the reduced TVC count in treated litter (Line, 2002). Litter acidifiers such as sodium bisulfate act by lowering the pH, inhibits bacterial proliferation and thereby reducing the microbial transformation of nitrogen matters in the litter to ammonia (Ritz *et al.* 2005). Sodium bisulfate as acidifier creates an unfavorable pH environment for the development of bacteria in the litter (Chung *et al.* 2015). Bacterial growth in the litter is reduced when litter is acidic.

Table 5 displays the results of the synergistic effect of differ3qent level of dietary kaolin and litter treated with sodium bisulfate on hematological indices of broiler birds. Significant differences ($p<0.05$) were noted in the PCV, RBC and WBC values, why the value for Hb was not significant ($p>0.05$). The PCV value of 29.66 obtained for birds on T_1 were similar with 29.00 in T_4, but both values were lower than the values obtained in other treatments. The RBC values of 2.80, 3.067 and 2.83 obtained for birds on T_1, T_2 and T_4 were similar ($p>0.05$), but both values were lower than the values recorded in the other treatments. Birds on T_1-T_2 and T_4 had the highest WBC level among the treatments. The results contradict the results of Ezenwosu *et al.* (2023) who fed broiler birds kaolin and noted no significant differences in blood parameters of the birds. Information of the impact of kaolin in diet and litter treated with sodium bisulfate on the haematology of poultry is not adequate. In the treatment groups, values for blood parameters in the current study fall within normal physiological range in a healthy chicken according to Bounous and Stedman (2000), Frandson and Ehner (1981), Benerjee (2008), Barde *et al.* (2022 and Nemi (1993). Though haematological values such as RBC and PCV fall within normal range typical of a healthy bird, treated groups had higher values for these parameters compared to control groups. This means that the levels of dietary kaolin used and litter treatment with sodium bisulfate were not detrimental to the birds. The outcomes corroborate the findings of Ejiofor *et al.* (2021) who fed birds silicate mineral and noted higher PCV and RBC levels in the treated groups compared to control. Nutrition and disease affects haematology of birds (Hrabčáková *et al.* 2014, Kaminski

Table 5. Synergistic effect of differ3qent level of dietary kaolin and litter treated with sodium bisulfate on hematological indices of broiler birds

Parameters	T1	T2	T3	T4	T5	T6	SEM	P-values
PCV (%)	29.66^{b}	31.66^{a}	31.66^{a}	29.00^{b}	32.33^{a}	33.00^{a}	0.40	0.03^{S}
RBC ($\times 10^6$ µl)	2.80^{b}	3.06^{b}	3.66^{a}	2.83^{b}	4.23^{a}	4.00^{a}	0.15	0.00^{S}
WBC ($\times 10^3$/µl)	7.00^{a}	6.86^{a}	6.00^{c}	6.76^{ab}	6.00^{c}	6.36^{bc}	0.10	0.01^{S}
Hb (g/dl)	9.35	9.60	9.25	9.65	9.40	9.35	0.06	0.54^{NS}

a, b, c, values with different superscripts within the same column are significantly different at 5% level (P < 0.05) SEM: Stanford error of the mean, NS: Not significant, S: Significant, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, Hb: Haemaglobin *et al.* 2014). Therefore, higher values of PCV and RBC in birds on dietary kaolin may be linked to better utilization of dietary compositions and various compounds contained in kaolin. Clays such as kaolin contains a variety of minerals, including magnesium, zinc, sodium, iron, potassium, calcium and nickel (Khander *et al.* 2012). These minerals enhance the availability, absorption, and utilization of essential trace elements (Chen *et al.* 2020), allowing the birds to get the most nutritional benefits from their diet for enhanced production of RBC and PCV (Yalcin *et al.* 2017). Also, an observed increase in mean values for PCV and RBC counts in birds on diet containing kaolin and on litter treated with sodium bisulfate suggest that O_2 thriving aptitude in blood of treated groups was enhanced. The mean values for WBC fall within the normal physiological values in healthy birds (Bounous and Stedman, 2000), but the value was relatively higher in T_1 and T_2. From the perspective of litter management, the lower mean values for WBC in T_3-T_6 could be connected to their reduced litter microbial count load. Litter chemical treatment and dietary kaolin help to reduce the proliferation of litter microbes, prevents the birds from continual pathogenic invasion from the litter and thereby, reducing the physiological response of increased WBC production. The lower levels of PCV and RBC observed in birds on control treatments may be related to the high litter ammonia gas production, which has been established as a stressor. Beker *et al.* (2004) noted that ammonia decreased percentage of packed cell volume in broiler chicks. From the dietary and environmental perspective, the reduced PCV and RBC may be connected to higher ammonia gas that must have polluted the micro climatic conditions in control pens and thus leading to reduced benefit from the diet. Under high litter ammonia gas production, birds lose their ability to properly digest feed as a result of damage to the intestine (Olanrewaju *et al.* 2007) and leading to reduced supply of macro and micro nutrients that required for the production of PCV and RBC. PCV and RBC were higher in groups on treated litter. This might be connected to the good air quality provided by sodium bisulfate by its reductive effect on litter ammonia gas production. We have earlier established by the research findings that diet play a very crucial role in PCV and RBC production (Kaminski *et al.* 2014). When sodium bisulfate is applied to the litter, it creates a good atmosphere that will support feed digestibility, nutrient absorption by causing decrease in litter ammonia gas production and much-absorbed nutrients will be used by the birds for enhanced PCV and RBC production. The blood WBC were lower in groups reared on treated litter and this could be connected to the reduced NH_3 emission which has been proven to be a source of stress.

The experiment discovered that kaolin inclusion in the diet and application of sodium bisulfate to the litter reduced the emission of litter ammonia gas without jeopardizing the performance and blood indices of the birds. It was therefore, recommended that 15g kaolin /kg diet + 500 g SB/5kg litter can be used to advance the growth performance and reduce % litter ammonia gas production during poultry production by poultry farmers.

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