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Impact of kaolin on growth performance, haematological and some serum biochemical indices of broiler chickens

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Abstract The aim of this study was to investigate the impact of kaolin on the growth performance, haematological indices and some serum biochemical indices of broiler chickens. A total of 261 'Anak strain' mix sexed of day-old chicks of broiler chickens at two weeks of age were allotted to 4 dietary treatments in a completely randomized experimental design with 6 replicates of 9 birds each. Dietary treatments at both the starter phase at 14 days old up to 28 days old age and treatment at the finisher phase at 28 days old up to 56 days old age included:0 g/kg ration, 5 g/kg ration, 10 g/kg ration and 15 g/kg ration. The results of the weekly feed intake, weight gain and feed conversion ratio showed that weight gain, feed intake and feed conversion ratio improved ($P < 0.05$) in favor of the treatment groups. In overall and daily performance of the birds, feed intake and weight gain were higher ($P < 0.05$) in the treatment group than in the control group. However, serum biochemical and haematological indices investigated among the treatments were not significant ($P > 0.05$). It was recommended that up to 5-15% kaolin can be safely included in rations for improved performance in broiler production.

Keywords: Anak strain, broiler chicken, growth performance, haematology, kaolin, serum

1. Introduction

One of the fastest means of producing quantity and quality farm animal protein for human consumption is through broiler production, because of its diminutive fat content, elevated protein and amino acid balance (Sleman et al 2015). Furthermore, the poultry industry is the greatest vivacious sector, with the highest growth reflected in the amplified demand for food worldwide (Neves et al 2014).

However, according to Hassan et al (2018) numerous synthetic feed additives have been utilized not only to enhance the efficiency of feed, but also to improve performance and enhance broiler health. However, the European Union has placed a prohibition on the utilization of these products (antibiotics) in poultry production owing to the amplified glitches related to antibiotic resistance in birds and antibiotic residue in chickens (Carvalho and Santos 2016). The destructive effects of the use of antibiotic growth promoters such as, penicillin, tetracycline and amphenicol have been detected in foods (Diarra and Malouin 2014). The antibiotic residues in meat, when consumed, may cause antibiotic resistance in humans (Landers et al 2012). According to Kummerer (2009) as women consume meat containing tetracycline residue, it interferes with development of teeth in young children. This is also the case with beta-agonists, such as clenbuterol, which may cause food poisoning, palpitations, muscle tremors and tachycardia (Hoffman et al 2001). However, in contemporary animal production, the exclusion of antibiotics in animal ration as a result of its negative impact on the health of both animal and human beings has been the prime focus of scientists as they study to discover various materials that can serve as alternatives to antibiotics.

However, numerous nutritional supplements that have utilized as growth promoters in place of antibiotics are: prebiotics, probiotics, organic acids, amino acids, immunostimulants, enzymes, and phytogenic feed additives (Ahsan et al 2016; Mashayekhi et al 2018; Abouelezz et al 2019).

Furthermore, the use of clays such as kaolin, acting as an inert ingredient in feed, has also been very common in broiler and in laying hen nutrition (Safaeikatouli et al 2011). Clays such as kaolin inclusion in ration reduces the passage rate of digester and improve feed digestibility, thus, resulting in an improved animal performance (Safaeikatouli et al 2012). Kaolin as a clay can improve the integrity of intestines via elimination of toxins generated by harmful microorganisms living in the gastrointestinal tract (Xu et al 2003). Inert ingredients such as kaolin have been used in poultry nutrition to improve growth performance and decreasie the opposing effects of mycotoxins (Safaeikatouli et al. 2011). The mode of action of this process mostly relies on the protection of epithelium by forming a colloid that covers the intestinal mucosa, averting both irritation and lesions, and by binding the bacteria and toxins and thus, eradicating them along with faeces (Vila-Donat et al 2018). Ration supplemented with kaolin may also cause an increase in villus height and in the villus altitude to crypt depth ratio, which in

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turn increases the surface area of the gastrointestinal tract and thus increases nutrient digestibility (Subramaniam et al 2015). Ani et al (2014) discovered that the inclusion of clay up to % 5 clay caused enhancement in growth performance and decreased production cost. Kim et al (2011) when evaluating the addition of kaolin in the ration of laying hens observed a significant improvement in the feed conversion ratio compared to the control group. Eliot and Edwards (1991b) explained that the reason for the rise in the weight gain of broilers fed kaolin supplemented rations was the change in the pH of the gastrointestinal tract. They also reported that in the presence of kaolin when the gut pH reaches 6, absorption of some nutrients, including cations, increases which improves weight gain. The primary and current focus of scientists is to research and discover alternatives to antibiotics. Therefore, the present study was designed to investigate the impact of varying levels of kaolin on growth performance, haematological and some serum biochemical indices in broiler chickens.

2. Materials and Methods

2.1. Study site

The study took place at the Department of Animal Science Teaching and Experimental Farms, University of Nigeria Nigeria. Nsukka lies within longitude 6° 45'E and 7° E and latitude 7° 12.5 'N and an altitude 447 m above sea level. The climate of the study environment is naturally tropical, with relative humidity ranging from 65 to 80% and 26.8 0 ^{O}C mean daily temperature (Okonkwo and Akubuo 2007). The yearly rainfall ranges from 1567.0-1846.98 mm (Metrological Center, Crop Science Department, University of Nigeria, Nsukka Enugu State). The study lasted for 6 weeks.

2.2. Characteristics of kaolin

The tested kaolin, was purchased from Joe Chem, Chemical Store, Nsukka Enugu State, Nigeria. Kaolin is tinny clay, usually white in color, formed by the weathering of aluminous minerals and it is classified as a phyllosilicate, due to its absorption capacity and absence of primary toxicity. The inner layer is composed of an octahedral- alumino- layer joined to the tetrahedral silica layer via apical oxygens. It has a low cation exchange capacity and usually absorbs water and forms a plastic, paste-like substance.

2.3. Experimental birds and management

A total of 216 'Anak strain' day-old broiler chickens at two weeks of age were allotted to 4 dietary treatments in a completely randomized experimental design with 6 replicates of 9 birds. The birds were housed in a deep litter system with fresh wood shavings as litter. Prior to the arrival of the birds from the hatchery, the brooding house was cleaned with detergent and disinfected with strong disinfectant after which wood shavings were spread. The brooding house was heated with charcoal pot a few hours before the arrival of the birds. Drinkers and feeding troughs were acquired, disinfected and strategically positioned. Clean drinking water and feed were procured before the arrival of the birds. Overall flock prophylactic administration and routine vaccination were given. A stress pack was given to the birds in drinking water at 100 g/50 liters to increase appetite and energy supply. Clean water and treatments were provided ad libitum throughout the 6 weeks of the feeding trial. A thermometer was used to monitor the temperature of the study environment.

2.4. Experimental diets

The feeding trial lasted for 6 weeks. Starter and finisher diet compositions are presented in (Tables 1 and 2). The proximate compositions of the experimental diets were analyzed according to the Association of Official Agricultural Chemists (AOAC 2019) methods.

2.5. Data collection

2.5.1. Growth performance parameters

At the beginning of the research, the birds were weighed to obtain their initial body weight. Afterward, their average live-weights were measured on weekly basis by weighing all the birds in each replicate. Live weight gain was used to calculate the average daily weight gain per bird in each replicate. Feed intake was taken from the first day of the study to the end. Feed intake was measured by first weighing the feed before being given to the birds. Then, the difference between the feed provided the preceding day and leftover feed in the feeding trough the next morning was divided by the number of birds in each replicate to obtain the daily feed intake per bird for each replicate. The feed conversion ratio was calculated by dividing the feed intake of birds by the body weight gain of birds. Average daily feed intake was obtained by dividing the daily feed intake of birds by the number of days the feeding trial lasted. Average daily weight gain was obtained by dividing weight gained by birds by the number of days the feeding trial lasted.

Table 1 Ingredients (%) and chemical composition (g/kg DM) of experimental diets for broiler starter.

Ingredients (%)	Diets			
	0g	5 g	10 g	15 g
Maize	42.00	42.00	42.00	42.00
Wheat offal	7.00	7.00	7.00	7.00
Soybean meal	14.00	14.00	14.00	14.00
Groundnut cake	20.00	20.00	20.00	20.00
Palm kernel cake	10.00	10.00	10.00	10.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	4.25	4.25	4.25	4.25
Vitamin premix	025	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Total	100	100	100	100
Kaolin (g/kg ration)	0.00	5.00	10.00	15.00
Calculated compositions (%)				
Crude protein (%)	22.57	22.57	22.57	22.57
Metabolizable Energy (Mcal/KgME)	2800	2800	2800	2800
Crude fibre (%)	4.80	4.80	4.80	4.80
Crude fat (%)	3.90	3.90	3.90	3.90
Chemical composition (%)				
Crude matter	90	89.90	90.10	90.05
Crude fibre	4.80	4.81	4.80	4.81
Crude protein	22.56	22.57	22.59	22.59
Crude fat	3.90	3.89	3.91	3.90
Crude ash	7.80	7.89	8.00	8.05
Carbohydrate	56.87	56.89	56.91	56.90
Nitrogen Free extract	50.94	50.74	50.71	50.70

.0 g/kg ration, 5 g/kg ration, 10 g/kg ration, 15 g/kg ration. *Each 2.kg of vitamin premix contains: vitamin A: 1000000 IU; vitamin D3: 2,200.000 mg; vitamin B1: 1500 mg; vitamin B2: 5000 mg; vitamin K3:2000 mg, vitamin B12: 10 mg; vitamin B6:1500 mg, vitamin E: 10000 mg; Biotin: 20mg; Niacin: 15,000 mg, Folic acid: 500 mg and 5000 mg calpan.

Table 2 Ingredients (%) and chemical composition (g/kg DM) of experimental diets for broiler finisher.

Ingredients (%)	Diets			
	0g	5g	10g	15g
Maize	38.00	38.00	38.00	38.00
Wheat offal	13.00	13.00	13.00	13.00
Soybean meal	8.00	8.00	8.00	8.00
Groundnut cake	14.00	14.00	14.00	14.00
Palm kernel cake	20.00	20.00	20.00	20.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	4.25	4.25	4.25	4.25
Vitamin premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Total	100	100	100	100
Kaolin (g/kg ration)	0	5.00	10.00	15.00
Calculated composition (%)				
Crude protein (%)	20.00	20.00	20.00	20.00
Metabolizable Energy (Mcal/KgME)	3000	3000	3000	3000
Crude fibre (%)	5.85	5.85	5.85	5.85
Crude fat (%)	4.20	4.20	4.20	4.20
Chemical composition (%)				
Crude matter	90.20	91.05	90.11	90.33
Crude fibre	5.85	5.65	5.65	5.81
Crude protein	20.10	20.09	20.08	20.00
Crude fat	4.21	4.11	4.23	4.24
Crude ash	5.85	5.79	5.65	5.89
Carbohydrate	62.21	62.21	62.21	62.21
Nitrogen Free extract	54.19	55.41	54.50	54.39

.0 g/kg ration, 5 g/kg ration,10 g/kg ration, 15 g/kg ration *Each 2.kg of vitamin premix contains: vitamin A: 1000000 IU; vitamin D3: 2,200.000 mg; vitamin B1: 1500 mg; vitamin B2: 5000 mg; vitamin K3:2000 mg, vitamin B12: 10mg; vitamin B6:150 0mg, vitamin E: 10000mg; Biotin: 20mg; Niacin: 15,000 mg, Folic acid: 500 mg and 5000mg calpan.

2.5.2. Serum biochemical indices

The albumin determination: Done according to the method of Grant (1987). Alkaline phosphatase (ALP): Calculation: alkaline phosphatase activity was calculated as follows:

$$\text{Activity of ALP (in U/L)} = \frac{\text{Absorbance of sample} \times 30}{\text{Absorbance of standard} \quad 1}$$

The Aspartate Aminotransferase (AST) was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine. The color intensity was measured against the blank at 546 nm. The activity of AST was read from a standard curve. The determination of Alanine Aminotransferase (ALT) was performed according to the method of Reitman and Frankel (1975).

The Total protein content in blood plasma was determined using Peterson modifications of the microlowry method using a protein assay kit (Sigma diagnostics, p 5656, Sigma, MO USA) principle. The protein concentrations were determined using biuret method. Bilirubin was done according to the method of Jendrassik and Grof (1938) as contained in Randox assay kits.

2.5.3. Haematological indices

At the end of the last week of the feeding trial (6 weeks), 5 birds were randomly selected from each treatment, and blood samples were collected. Blood samples were taken in labelled sterile universal bottles containing ethylene diamine tetra-acetic acid (EDTA) Blood samples in EDTA-containing bottles were used to analyse full blood counts with the help of an auto hematology analyser.

2.6. Statistical design and analysis

Data produced were subjected to analysis of variance (ANOVA) in CRD using statistical package (SPSS 2003) Windows version 8.0. Mean differences were separated using Duncan's New Multiple Range Test (Duncan 1955) as outlined by Obi (2002). The statistical model used to test the effects of treatments on growth performance, haematological and serum biochemical parameters was:

$$X_{ij} = \mu + T_i + E_{ij}$$

Where: X_{ij} = individual observation; μ = population mean; T_i = treatment effect; E_{ij} = experimental error.

3. Results

3.1. Growth performance

The results of the kaolin meal supplementation on weekly feed intake, body weight gain and feed conversion ratio of broiler chickens are presented in Table 3. Feed intake values among the treatments from weeks 1-4 were not significant ($P > 0.05$). The feed intake values of birds on 0 g at week 5 and week 6 were lower ($P < 0.05$) than the values observed for birds on 5 g, 10 g and 5 g. Body weights gain values of birds among the treatments at weeks 1-2 were not significant ($P > 0.05$). Weight gain values of birds on 0 g at weeks 3-6 were lower than the values observed for birds on 5 g, 10 g and 5 g. At weeks 1 and 4, the feed conversion ratio values were not significant ($P > 0.05$). In summary, feed conversion ratio values of treatment groups (5 g, 10 g and 15 g). were significantly $P < 0.05$) lower than that of control group (0 g).

3.2. Overall and daily performance

The general and daily performance of kaolin meal supplementation on the growth performance of broiler chickens is shown in Table 4 and in Figures 1-3. Total feed intake, total weight gain, average daily feed intake and average daily weight gain value of control group were significantly lower ($P < 0.05$) than the treatment groups (5 g, 10 g and 15 g). Feed conversion ratio value of birds on 0 g was higher ($P < 0.05$) than the values observed for birds on 5 g, 10 g and 15 g respectively.

3.3. Haematological indices

The results of the impact kaolin meal supplementation on haematological indices of broiler chickens are presented in Table 5. There were no significant differences ($P < 0.05$) in the values of the haematological indices studied.

3.4. Serum biochemical indices

The results of the impact of kaolin meal supplementation on some serum biochemical indices of broiler chickens are shown on Table 6. The serum biochemical indices investigated were not significant ($P > 0.05$).

Table 3 The impact kaolin meal supplementation on weekly feed intake, body weight gains and feed conversion ratio of broiler chickens (n=54 per treatment).

Parameters	0 g	5 g	10 g	15 g	SEM
Feed intake (g/bird)					
Week 1	445.67	477.33	460.67	479.00	8.384
Week 2	746.00	811.33	766.33	723.33	23.395
Week 3	916.67	930.00	900.00	916.67	17.687
Week 4	1050.00	1066.67	1076.67	1100.00	20.201
Week 5	1244.00^{c}	1340.00^{ab}	1378.67^{a}	1276.33^{bc}	19.05
Week 6	1378.67^{c}	1451.00^{ab}	1490.26^{a}	1410.00^{b}	21.20
Body weight (g/bird)					
Week 1	330.00	346.67	356.67	350.00	8.115
Week 2	310.00	363.33	410.00	430.00	23.479
Week 3	463.33^{c}	510.00^{bc}	600.00^{ab}	693.00^{a}	31.607
Week 4	470.00^{b}	566.67^{ab}	630.00^{a}	640.00^{a}	27.943
Week 5	556.67^{b}	580.00^{b}	686.67^{a}	732.00^{a}	22.754
Week 6	433.33^{c}	570.00^{b}	626.67^{ab}	679.67^{a}	29.555
Feed conversion ratio (g/g)					
Week 1	1.36	1.38	1.27	1.37	0.450
Week 2	2.40^{a}	2.23^{ab}	1.92^{ab}	1.71^{b}	0.102
Week 3	1.97^{a}	1.82^{ab}	1.51^{bc}	1.34^{c}	0.091
Week 4	2.25	1.94	1.71	1.71	0.102
Week 5	2.23^{ab}	2.34^{a}	2.00^{bc}	1.74^{c}	0.076
Week 6	3.08^{a}	2.54^{b}	2.37^{bc}	2.07^{c}	0.125

$^{a,\ldots c}$ Means on the same row with different superscript are significantly different ($P < 0.05$), 0 g/kg ration, 5 g/kg ration, 10 g/kg ration, 15 g/kg ration.

Table 4 The impact of kaolin meal supplementation on the growth performance of broiler chickens (n=54 per treatment).

Parameters	0 g	5 g	10 g	15 g	SEM
Overall performance					
Initial weight	523.33	526.67	523.67	533.33	2.826
Total feed intake (g/bird)	5723.333^{b}	6076.333^{a}	6072.600^{a}	5905.333^{ab}	57.472
Total weight gain (g/bird)	2563.33^{c}	2936.67^{b}	3310.00^{a}	3525.00^{a}	115.41
Feed conversion ratio (g/g)	2.17^{a}	2.06^{ab}	1.83^{bc}	1.67^{c}	0.066
Daily performance					
Average daily feed intake (g/bird)	136.41^{b}	144.67^{a}	144.48^{a}	140.52^{ab}	1.34
Average daily weight gain (g/bird)	60.98^{c}	69.92^{b}	78.80^{a}	83.92^{a}	2.75

$^{a,\ldots c}$ Means on the same row with different superscript are significantly different ($P < 0.05$), .0 g/kg ration, 5 g/kg ration, 10 g/kg ration, 15 g/kg ration

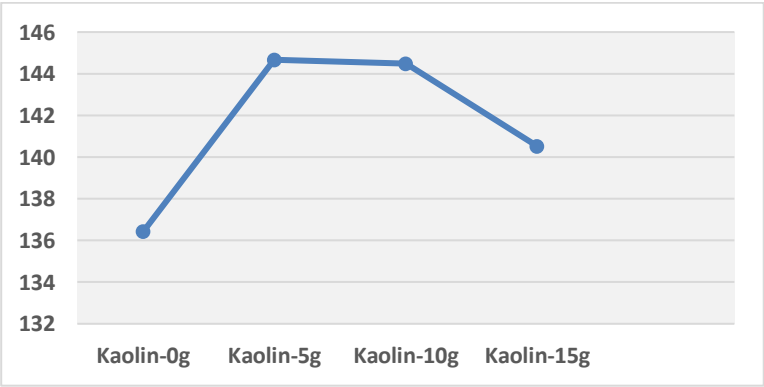

Figure 1 Average daily feed intake of broiler chickens fed varying kaolin supplemented rations.

4. Discussion

4.1. Growth performance

The impact of kaolin meal supplementation on weekly feed intake, body weight gain, feed conversion ratio, overall and daily performance of broiler chickens is shown in Table 3-4 and Figures 1-3. From the results, the weight gain and feed conversion ratio were significantly improved ($P < 0.05$) in favor of treatment groups (5 g, 10 g, and 15 g) that were fed 5%, 10%, and 15% kaolin inclusion respectively.

These results are in agreement with Prvulovic et al (2008) and Shi et al (2009) who observed that the inclusion of clay minerals such as kaolin in broiler rations enhanced body weight gain and feed conversion ratio. Additionally, weight gain in

favor of the treatment groups in this present study is also in agreement with the findings of Ani et al (2014) who observed that inclusion of clay in broiler diets up to five percent led to improvement in growth performance of broiler chickens.

The improvement in weight gain and feed conversion ratio observed in this present study could be due to the presence of kaolin in the ration, which might have increased feed retention time in the gut of the birds, subjecting the nutrients to enzymatic action for quite a long time and thus, increasing digestibility. This assertion is affirmed by Safaei et al (2014) who observed that dietary supplementation of clays such as kaolin can control the processes of digestion and nutrient absorption, promoting a longer retention time of the digester, allowing its greater digestion and absorption to improve the performance of farm animals in terms of weight gain.

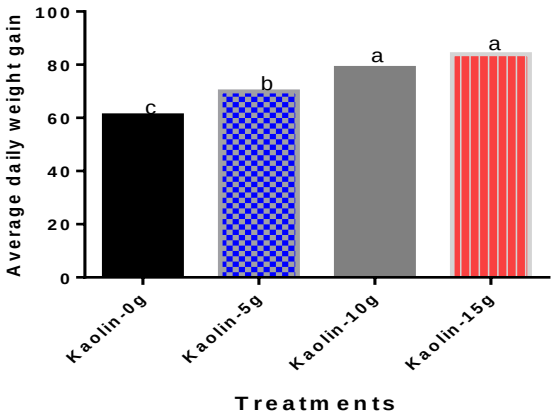

Figure 2 Average daily weight gain of broiler chickens fed varying kaolin supplemented ration.

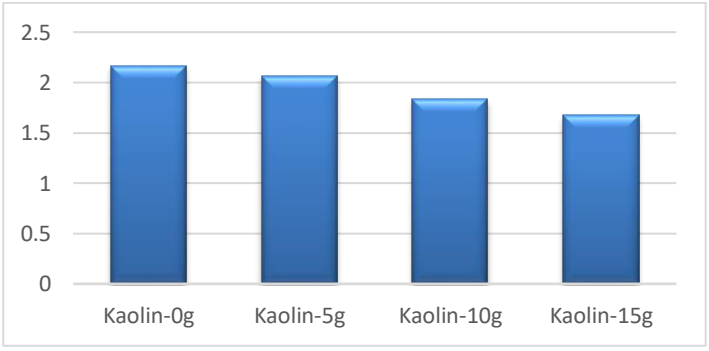

Figure 3 Feed conversion ratio of broiler chickens fed varying kaolin supplemented ration.

Kaolin as a clay can improve intestinal integrity via the excretion of toxins generated by pathogenic microorganisms living in the bird's gastrointestinal tract (Xu et al 2003). Research findings have shown that ration supplementation with kaolin may cause an increase in the height of villus and in the villus altitude to crypt depth ratio, which in turn will increase the surface area of the gastrointestinal tract and thus increase nutrient digestibility (Subramaniam et al 2015). Villi are nutrient absorption structures located in the small intestines, the healthier and increased their surface areas are, the more nutrients will be absorbed, thus leading to improved growth performance.

According to Macari et al (2002) the absorptive capacity of the intestine will be directly proportional to the size of the villi and poultry with higher villi may have a better absorption of nutrients. Hu et al (2013) observed improvements in intestinal integrity measured by the villus height by providing clay (montmorillonite) in the diet of broilers. These authors also opined that maintaining intestinal integrity is essential for the proper functioning of epithelial cells and that the use of clays when feeding poultry is a great alternative to maintain intestinal health for improved animal performance.

The improved feed conversion ratio observed for the birds fed kaolin supplemented ration agrees with Kim et al (2011) who evaluated the effect of kaolin in the ration of laying hens and observed a significant improvement in feed conversion ratio compared to the control group. Moreover, Eliot and Edwards (1991b) also observed that the reason for the rise in the weight gain of broiler chickens fed kaolin was the change in the pH of the gastrointestinal tract. They further explained that in the presence of kaolin when the gut pH reaches 6, absorption of some feed nutrients, including cations, increases and which improves weight gain.

Health of the gastro-intestinal tract is a key factor in feed digestibility and nutrient absorption. A healthy gut will culminate in improved feed digestion, and nutrient absorption, thus leading to an enhanced feed conversion ratio. Kaolin was used to promote the reduction of toxins that cause injuries to the intestinal epithelium, by the absorption and excretion of the pathogen thereby protecting the intestinal mucosa and improving the performance of the poultry (Owen et al 2012). Kaolin as

an inert ingredient has been used in poultry diets to improve growth performance and to alleviate the adverse effects of mycotoxins (Safaeikatouli et al 2011).

Furthermore, kaolin in rations improves weight gain and the feed conversion ratio relies on epithelium protection by forming a colloid that covers the intestinal mucosa, preventing both irritation and lesions and by binding the bacteria and toxins to eliminate them along with faeces (Vila-Donat et al 2018). The improved performance recorded in the present study agreed with the work of Owen et al (2012) who observed that kaolin inclusion in the diet of broiler birds had beneficial effects on growth performance.

Increased feed intake in all treatment groups could be attributed to the presence of kaolin in the rations, which can act as a pellet binder and improve not only pellet quality, but also feed intake. These results are supported by previous research (Southern et al 1994) that included clay called sodium bentonite in broiler rations and recorded increased feed intake and growth performance in broiler chicks.

However, among the treatment groups, birds fed 15% kaolin had the lowest value for feed intake, but the highest weight gain and feed conversion ratio value. This is an indication that higher kaolin inclusion may cause a decrease in feed intake, while at the same time accelerating the rate of feed digestion and nutrient absorption and thus, improving the feed conversion ratio which culminate in improved weight gain and performance. This observation is in line with Safaeikatouli et al (2012) who stated that the inclusion of clays such as kaolin in animal feed could reduce the passage rate of digesters, improving digestibility, which causes birds to consume less feed to meet their nutritional requirements.

4.2. Haematological indices

In the present study**,** kaolin supplementation had no significant effect on haematological indices investigated. These results disagree with Oko et al (2011) who included kaolin in the ration of broiler chickens and observed significant differences in some of the hematological indices investigated.

Table 5 The impact of kaolin meal supplementation on the haematological indices of broiler chickens (n=54 per treatment).

Parameters	0 g	5 g	10 g	15 g	SEM
Packed cell volume (%)	32.33	23.67	29.33	30.33	1.469
Red blood cells (×10^6 /mm^3)	4.36	4.20	4.36	4.53	0.049
Total white blood cells (x10^3/µl)	7.13	6.93	7.60	8.13	0.223
Haemoglobin (g/dl)	12.63	12.56	13.26	11.93	0.394
Neutrophils (%)	58.00	57.33	60.00	60.67	0.674
Lymphocytes (%)	38.67	38.67	37.33	38.00	0.672
Monocytes (%)	2.67	2.33	2.67	2.67	0.229
Eosophils (%)	2.00	2.00	2.67	2.33	0.179
Mean corpuscular haemoglobin concentrate (%)	52.87	51.80	49.48	50.00	2.76
Mean corpuscular haemoglobin (pg)	2.90	3.00	3.03	2.99	0.098
Mean corpuscular volume (um^3)	7377.06	5619.92	6721.03	6687.6000	288.02

[a,...c]Means on the same row with different superscript are significantly different ($P < 0.05$), 0 g/kg ration, 5 g/kg ration, 10 g/kg ration, 15 g/kg ration.

Table 6 The impact of kaolin meal supplementation on some serum biochemical indices of broiler chickens (n=54 per treatment).

Parameters	0 g	5 g	10 g	15 g	SEM
Blood Urea(mg/dl)	12.00	11.00	10.00	10.00	2.787
Alanine aminotransferase (ALT)(u/l)	11.67	12.67	13.00	12.00	2.752
Alkaline phosphate (ALP)(u/l)	50.00	46.33	45.33	44.33	2.162
Total bilirubin (mg/dl)	0.37	0.33	0.67	0.67	0.149
Aspartate aminotransferase (AST)(u/l)	22.00	25.00	20.00	23.35	1.023
Total protein (g/dl)	4.33	4.33	4.00	3.67	0.193

[a,...c] Means on the same row with different superscript are significantly different ($P < 0.05$), .0 g/kg ration, 5 g/kg ration, 10 g/kg ration, 15 g/kg ration.

4.3. Serum biochemical indices

It is well-known that the liver is the epicenter for several digestive, metabolic and productive activities, and as such, is susceptible to varying degrees of chemical and biological damage. Such damage is made obvious by the serum levels of specific enzymes originating from the liver. These enzymes, depending on their levels may cause some disruptions to bodily functions, thereby resulting in poor health and production performance. Increased levels of these enzymes are associated with liver or muscle damage, resulting from the body's response to stress (Lumeij 2008). Additionally, serum parameters have been observed as good indicators of the physiological status of animals and their changes are important in assessing the response of such animal to various physiological situations (Khan and Zafar 2005). However, the values of some liver enzymes among the treatments (0 g, 5 g, 10 g and 15 g) were not significant ($P > 0.05$). This is in agreement with Eraslan et al (2006) and Kermanshahi et al (2009) who reported that clay known as sodium bentonite had no effect on blood serum enzymes when

included in ration. Also, the lack of significant differences in the urea values among the treatments is in agreement with Oko et al (2011).

5. Conclusions

In the present study, the inclusion of kaolin as a mineral clay additive up to 15 g/kg diet was able to improve performance in broiler chickens without deleterious effects. Therefore, it was concluded that kaolin supplementation up to 15 g/kg diet can be used by farmers to enhance performance of broilers.

Ethical Considerations

The experiment was carried out according to the provisions of the Ethical Committee on the use of animals for biomedical research of the University of Nigeria, Nsukka, Enugu, Nigeria.

Conflict of Interest

The authors declare no competing interest.

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References

AOA.C (2019) Association of Official Analytical chemist. Method of Analysis, 21st edition, Washington DC, USA.

Abouelezz K, Abou-Hadied M, Yuan J, Elokil A, Wang G, Wang S, Wang J and Bian G (2019) Nutritional impacts of dietary oregano and Enviva essential oils on the performance, gut microbiota and blood biochemicals of growing ducks. Animal 13:2216-2222.

Ahsan U, Cengiz O, Raza I, Kuter E, Chacher MFA, Iqbal Z, Umar S and Cakir S (2016) Sodium butyrate in chicken nutrition: The dynamics of performance, gut microbiota, gut morphology, and immunity. World's Poult. Sci. J. 72:265-275.

Carvalho IT and Santos L (2016) Antibiotics in the aquatic environments: a review of the European scenario. Environ. Int. 94:736-757.

Diarra MS and Malouin F (2014) Antibiotics in Canadian poultry production and anticipated alternatives. Front. Microbiol. 5:282-290.

Duncan DE (1955) New Multiple Range Test. Biometrics 11:1-42.

Elliot MA, Edwards HM (1991b) Some effects of dietary aluminum and silicon on broiler chickens. Poultry Sci. 70:1390-1402.

Eraslan G, Essiz D, Akdogan M, Karaoz E, Oncu M and Ozyildiz Z (2006) Efficacy of dietary sodium bentonite against subchronic exposure to dietary aflatoxin in broilers. Bull. Vet. Inst. Pulawy 50:107–112.

Grant GH, Silverman LM, Christenson RH (1987) Amino acids and proteins. In: Tietz NW (Ed.). Fundamentals of clinical chemistry, 3rd Edn. WB Saunders Co., London. pp. 291-345.

Hassan HMA, Samy A, Amani A, Youssef W and Mohamed MA (2018) Using different feed additives as alternatives to antibiotics growth promoter to improve growth performance and Carcass traits of broilers. Int. J. Poult. Sci. 17:255-261.

Harrigan W F and McCance M E (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press Incorporated, London.

Hoffman RJ, Robert JR, Hoffman S, Freyberg CL, Poppenga RH and Lewis SN (2001) Clenbuterol ingestion causing prolonged tachycardia, hypokalemia, and hypophosphatemia with confirmation by quantitative levels. J. Toxicol. Clin. Toxicol. 39:339-344.

Hu CH, Qian ZC, Song J, Luan ZS, Zuo AY (2013) Effects of zinc oxide-montmorillonite hybrid on growth performance, intestinal structure, and function of broiler chicken. Poult. Sci. 92:143-150.

Jendrassik L, Grof P (1938) Vereinfachte photometrische methoden zur bestimmung des bilirubins. Biochem. Z. 297:82-89.

Kermanshah H, Hazegh AR. and Afzali N (2009) Effect of sodium bentonite in broiler chickens fed diets contaminated with aflatoxin B1. J. Anim. Vet. Adv. 8:1631–1636.

Khan TA and Zafar F (2005) Haematological study in response to varying doses of estrogen in broiler chicken. International Journal of Poultry Science 4:748-751.

Kummerer K (2009) Antibiotics in the aquatic environmentea review part I. Chemosphere 75:417-434.

Kim JS, Jo JK, Yoon SY, Yun K, Kwon IK, Chae BJ (2011) Effects of kaolin (natural ligneous clay) supplementation on performance and egg quality in laying hens. Jour of Ani Sci and Tech 53:133138.

Lumeij JT (2008) Avian clinical biochemistry. Pages 839–872 in Clinical Biochemistry of Domestic Animals. 6th ed. J. J. Kaneko, J. W. Harvey, and M. L. Bruss, ed. Academic Press, Burlington, MA. 38.

Macari M, Furlan R, Gonzáles E (2002) Ingestão de alimentos: Mecanismos regulatórios. Fisiologia aviária aplicada a frangos de corte. 2nd ed. Jaboticabal (SP): Funep/UNESP.

Mashayekhi H, Mazhari M and Esmaeilipour O (2018) Eucalyptus leaves powder, antibiotic and probiotic addition to broiler diets: Effect on growth performance, immune response, blood components and carcass traits. Animal 12:2049-2055.

Neves DP, Banhazi TM, Naas IA (2014) Feeding Behaviour of Broiler Chickens: a Review on the Biomechanical Characteristics. Brazilian Journal of Poultry Science 16:1-16.

Obi IU (2002) Statistical methods of detecting differences between treatments means and research methodology issues in laboratory and field experiments. 2nd Ed.Express Pub.Ltd. Enug u. pp. 13-21.

Oko OOK, Iso IE, Udoh SP, Mbre JI (2011) Effects of Dietary Kaolin Supplementation on the Growth Performance and Serum Chemistry of Broilers; Nigerian J. Anim. Sci. 13:86-92 86.

Okonkwo WI .and Akubuo CO (2007) Trombe wall system for poultry brooding. Int. J. Poultry Sci. 6:125-130.

Owen OJ, Nodu MB, Dike UA, Ideozu HM (2012) The effects of dietary kaolin (clay) as feed additives on the growth performance of broiler chickens. G J of Agric Sci.

Prvulovic D, Kogic D, Lasic GG and Kosarcic S (2008) The effects of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. Turkish J. Vet. Anim. Sci. 32:1–7.

Reitman S and Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 8:56-63.

Safaei M, Jafariahangari Y, Mutalib MSA and Rezaei R (2014). Asian journal of animal and veterinary advances 9:64-71.

Safaeikatouli M, Jafariahangari Y, Baharlouei A and Shahi G (2011) The Efficacy of Dietary Inclusion of Sodium Bentonite on Litter Characteristics and Some Blood Hormones in Broiler Chickens. Journal of Biological Sciences, 11:216-220.

Safaeikatouli M, Boldaji F, Dastar, Hassani S (2012) The effect of dietary silicate minerals supplementation on apparent ileal digestibility of energy and protein in broiler.

Shi Y, Xu Z, Sun Y, Wang C, Feng J (2009) Effects of different types of montmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. Turkish J. Vet. Anim. Sci. 33:15–20.

Sleman SM, BeskiRobert A, SwickPaul AI (2015) Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr. 1:45-53.

Southern LL, Ward TL, Binder TD, Hebert LG (1994) Effect of sodium bentonite or hydrated sodium calcium aluminosilicate on growth performance and tibia mineral concentrations in broiler chicks fed nutrient deficient diets. Poult. Sci. 73:848- 854.

SPSS (2003) Statistical Package for social Sciences. Window Version 8.0.

Subramaniam M, Kim IH (2015) Clays as dietary supplements for swine: A review. J. Anim. Sci. Biotech. 6:2–9.

Xu ZR, Hu CH, Xia MS, Zhan XA. and Wang MQ (2003) Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science 82:1030-1036.