EFFECT OF VARYING DIETARY LEVELS OF KAOLIN ON LITTER MICROBIAL LOAD COUNTS AND SOME SERUM BIOCHEMICAL INDICES IN BROILER CHICKENS

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ABSTRACT
Because of the ban on antibiotics utilization in the poultry by the European Union as the results of the amplified glitches related with their residues in chicken, current focus of scientists is to research and discover various safe alternatives to antibiotics. The aim of this study was to investigate the effect of varying dietary levels of kaolin on litter and some serum biochemical indices in broiler chickens A total of 261 'Anak strain' of mix sexed of day-old chicks of broiler chickens in which 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 starter phase at 14 days old up to 28 days old age and treatment at finisher phase at 28 days old up to 56 days old age includes: kaolin-0g=0g kaolin/kg diet, kaiolin-5g=5g kaolin/kg diet, kaolin-10g =10g kaolin/kg/diet and kaolin-15g=15g kaolin/kg diet. Results of litter microbial load counts were significantly ($p<0.05$) lower treatment groups (kaiolin-5g, kaolin-10g and kaolin-15g when compared to control group (kaolin –0g). Generally, litter microbial load counts decreased as the level of kaolin in the feed increased. However, serum biochemical 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 ration for enhancing litter quality in broiler production.
KEY WORDS: Anak strain, Broiler chicken, Growth performance, Haematology, Kaolin, Serum,

INTRODUCTION

Numerous synthetic feed additives have been utilized not only to enhance efficiency of feed, but also to improve performance and enhance broiler's health Hassan *et al.* (2018). Unfortunately, European Union has placed a prohibition on the utilization of these products (antibiotics) in animal production such as poultry as a results of the amplified glitches related with their residues in chicken (Carvalho and Santos, 2016). However, numerous nutritional supplements that have been utilized as growth promoters in place of antibiotics are: prebiotics, probiotics, organic acids, amino acids, immune-stimulants, enzymes, and phytogenic feed additives (Mashayekhi *et al.* 2018; Abouelezz *et al.* 2019). Other feed additives include, sodium butyrate and rosemary powder (Alagawany *et al.* 2019)

Furthermore, the use of clays like kaolin, acting as an inert ingredient in feed, has also been very common in broiler and laying hen's nutrition (Safaeikatouli *et al.* 2011). Kaolin as a clay can improve integrity of intestines via elimination of toxins generated by harmful microorganisms living in the gastrointestinal tract (Xu *et al.* 2003). According to Eliot and Edwards (1991b) the reason for the rise in the weight gain of broilers fed kaolin supplemented ration 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 and this improves weight gain. Since the primary and current focus of scientists is to research and discover alternatives to antibiotics. Therefore, the present study was designed to investigate the effect of varying dietary levels of kaolin on litter microbial counts and some serum biochemical indices in broiler chickens.

MATERIALS AND METHODS

Location and duration of the study

The study took place at the Department of Animal Science Teaching and Experimental Farms, University of Nigeria, Nsukka. The study lasted for 6weeks

Experimental birds and management

A total of 216 'Anak strain' of mix sexed of day-old chicks of broiler chickens in which at two weeks of age were allotted to 4 dietary treatments in a completely randomized experimental design with 6 replicates of 9 birds each. Treatment were as follows: (**Kaolin-0g** =0g kaolin/kg diet, **Kaolin-5g** =5g kaolin/kg diet, **Kaolin-10g** = 10g kaolin/kg diet and **Kaolin-15g** =15g kaolin/kg diet respectively). The tested kaolin was bought from Joechem Ventures Nsukka. The birds were housed in a deep litter

system with fresh wood shavings as litter. Clean drinking water and feed were made ready before the arrival of the birds. Overall flock prophylactic administration and routine vaccination were given. Clean water and treatments were provided *ad libitum* throughout the 6 weeks of the feeding trial.

Experimental diets:

Commercial Top starter and finisher feeds were used in the conduct of the study

Data Collection

Serum biochemical indices and litter microbial counts

At the end of the study, two birds each from each replicate were randomly selected for serum biochemical study. Two mL of blood was collected from the wing vein using a sterile syringe and needles. Blood collected was emptied into a labelled treated vacutainer tube without anticoagulant. The following biochemical indices determined were listed below:

Albumin determination: Done according to the method of Grant (1987).

Alkaline phosphatase (ALP): Calculation: alkaline phosphatase activity was calculated as follows:

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

Aspartate Aminotransferase (AST): This was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine. The color intensity was measured against the blank at 546nm. The activity of AST was read up from a standard curve. Alanine Aminotransferase (ALT) was determined according to the method of Reitman and Frankel (1975). Total protein content in blood plasma was determined using Peterson's modifications of the micro-lowry method using a protein assay kit (Sigma diagnostics, p 5656, Sigma, MO, USA).

The total viable count was determined by pour plate method using Nutrient Agar as described by Harrigan and McCance (1976). The mold count was done according to the method of Harrigan and McCance (1976). The coli-form count was determined by pour plate method using Agar as described by Harrigan and McCance (1976).

Statistical design and analysis: Data produced were subjected to the 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).

RESULTS AND DISCUSSIONS

Table 1: Results of litter microbial load of broiler chickens fed varying levels of kaolin as feed additive

Parameters (cfu/g)	**Kaolin-0g**	**Kaolin-5g**	**Kaolin-10g**	**Kaolin-15g**	**SEM**
Total viable counts	3.6×10^{10a}	2.0×10^{9b}	1.5×10^{9b}	2.0×10^{9b}	3234422653.11
Mold counts	5.0×10^{2a}	3.0×10^{2ab}	1.8×10^{2ab}	1.1×10^{2b}	637.99
Coli-form counts	8.6×10^{3a}	2.6×10^{3b}	2.8×10^{3b}	1.8×10^{3b}	8667.54

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

Table 2: The impact kaolin meal supplementation on some serum biochemical indices of broiler chickens (n=54 per treatment)

Parameters	Kaolin-0g	Kaolin-5g	Kaolin-10g	Kaolin-15g	SEM
Blood Urea(mg/dl)	12.00	11.00	10.00	10.00	2.78
Alanine aminotransferase (ALT)(u/l)	11.67	12.67	13.00	12.00	2.75
Alkaline phosphate (ALP)(u/l)	50.00	46.33	45.33	44.33	2.16
Total bilirubin (mg/dl)	0.37	0.33	0.67	0.67	0.14
Aspartate aminotransferase (AST)(u/l)	22.00	24.01	20.00	23.35	3.02
Total protein (g/dl)	4.33	4.33	4.00	3.67	1.29

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

Results of the litter total viable counts, coli form and mold values of broiler chickens fed varying dietary levels of kaolin as feed additive is presented in Table 1. From the result, values of total viable count, Coli forms and Mold counts recorded in kaolin-0g (control) were significantly (P<0.05) higher in treatment groups (Kaolin-5g, Kaolin-10g Kaolin-15g). This can be linked to the moisture reduction effect of kaolin in the litter. High litter moisture and pH (alkalinity) encourages the proliferation and activities of litter microbes. From the study done by Ezenwosu *et al.* (2022) control group recorded the highest (P<0.05) moisture and pH values when compared to kaolin supplemented groups. This affirms the observed increase (P<0.05) in total viable counts, coli form and mold load values in control treatment. High moisture content of litter encourages proliferation of litter microbes than when it is low (Elliott and Collins, 1982). Reduction in litter pH creates an acidic environment that is detrimental to the litter microbial growth and putrefaction of litter. This also affirms the major reason why microbial load values observed in treatment groups were significantly (P<0.05) the lowest. Parizadian *et al.* (2013) also observed that the broiler's litter when moist is a propitious environment for the growth of bacteria and fungi; and also, the main cause of ammonia production and emission from the litter.

The results of the effect of varying levels of kaolin on some serum biochemical indices of broiler chickens are shown on Table 2. Serum biochemical indices investigated were not significant (p>0.05). However, in this present study., values of some liver enzymes among the treatments 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 the blood serum enzymes when included in ration. Also, the lack of significant differences in the urea values among the treatment is in agreement with Oko *et al.* (2011).

CONCLUSION

Kaolin improved litter quality by reducing the litter microbial load counts. Therefore, kaolin supplementation is recommended for use by broiler producers up to 15g/kg feed.

REFERENCES

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(10), 2216-2222.

Alagawany M., Elnesr S.S. and Farag M.R. (2019). Use of liquorice (Glycyrrhiza glabra) in poultry nutrition: Global impacts on performance, carcass and meat quality. World's Poult. Sci. J. 75, 293-303.

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

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

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

Ezenwosu. C. and Udeh F. U. (2022). Using kaolin as a feeding protocol to control ammonia gas production from the litter in broiler chicken production. Livestock Research for Rural De,velopment 34 (9) 2022

Elliott, H., Collins, N. (1982). Factors affecting ammonia release in broiler houses. Trans ASAE 25:413–418.

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, G.H., Silverman, L.M., Christenson, R.H. (1987). Amino acids and proteins. In: Tietz NW (Ed.). Fundamentals of clinical chemistry, 3rd Edn . WB Saunders Co., London. pp. 291-345.

Hassan, H.M.A., Samy, A., Amani, A., Youssef, W. and Mohamed, M.A. (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(6), 255-261.

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

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

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.

Obi, I.U. (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.Pp13-21.

Oko, O. O. K., Iso, I. E., Udoh, S. P. and Mbre, J. I. (2011). Effects of Dietary Kaolin Supplementation on the Growth Performance and Serum Chemistry of Broilers; Nigerian J. Anim. Sci. 2011, 13:86-92 86.

Parizadian, K B., Shams, S. M., Hassani, S. and Mostafalo, Y. (2013). Effects of physical size of clinoptilolite on growth performance, serum biochemical parameters and litter quality of broiler chickens in the growing phase. Poult. Sci. J. 1 (2): 93-104

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.28(1):56-63.

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.*

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

Xu Z, R., Hu, C.H., Xia, M. S., Zhan, X.A. and Wang, M.Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science, 82: 1030-1036. https://doi.org/10.1093/ps/82.6.1030.