Haematology of Birds and Microbial Analysis of Recycled Poultry Litter Treated with Graded Levels of Aluminium Sulphate (Alum)

ABSTRACT

The study was carried out at the poultry unit of the Department of Animal Science teaching and research farm, Ahmadu Bello University, Zaria to determine the Chemical and Microbial Analysis of Poultry Litter (wood shavings) Treated with Graded Levels of Aluminium sulphate (Alum). The alum used was obtained from the Sabon-garimarket in Zaria, Kaduna State. Aluminium sulphate (alum) was applied to the wood shavings by mixing it with alum thoroughly using hands covered with hand gloves. The rates of alum application was as follows: T1 control (normal wood shavings with no alum), T2 (5% alum by kg weight of wood shavings), T3 (10% alum by kg weight of wood shavings) and T4 (15% alum by kg weight of wood shavings). Five sets of litter samples were obtained fortnightly from each pen from different locations i.e. the four corners and centre from which the microbial load were measured. At the termination of the experiment (day 56), two birds from each pen having representative weights for the group (6 birds per Treatment) were selected and 1.5ml of blood was taken via the wing vein. Haematological parameters were analyzed by an auto haemo-analyser (BC2800 vet auto haemo analyser) at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. This result shows significant ($P<0.05$) difference among all the parameters analysed except MCHC. The result shows a significant decrease in the total bacteria, *E. coli*and *Salmonella spp*. in the alum treated litter groups (5%, 10% and 15% alum treated litter) compared to the control group (0% alum treated litter), the mould and yeast load was significantly increased in alum treated litter groups (5%, 10% and 15% alum treated litter) compared to the control group (0% alum treated litter).The study conclude that treating recycled poultry litter with alum can reduce microbial load of the litter. Birds reared on recycled poultry litter have significantly higher haemoglobin and PCV compared to the control.

Key words: Haematology, microbial load, recycled poultry litter, Alum

INTRODUCTION

Chicken litter is a mixture of faeces, wasted feeds, bedding materials, and feathers (Kim *et al*., 2012). Over 14 million tons of chicken litter is produced every year in the US, most of which is usually recycled and spread on arable land as a low cost organic fertilizer (Enticknap *et al.*, 2006). Poultry manure contains significant amounts of nitrogen because of the presence of high levels of protein and amino acids. Owing to its high nutrient content, poultry litter has been considered to be one of the most valuable animal manure as organic fertilizer (Sharpley *et al*., 2009). Chicken litter is also the source of human pathogens, such as *Salmonella*, *Campylobacter jejuni*, and *Listeria monocytogenes*, which can potentially contaminate fresh produce or the environment and are frequently associated with foodborne outbreaks (Chinivasagam *et al.*, 2010; Wilkinson *et al.*, 2011).Although the application of poultry litter for commercial farming has rarely been associated with food borne outbreaks, enhanced consumer awareness of food safety issues has increased the scrutiny of agricultural practices(Zhao and Jiang, 2014).Microbial concentrations in chicken litter can reach up to 10^{10} CFU/g, and Gram-positive bacteria, such as *Actinomycetes*, *Clostridia*/*Eubacteria*, and *Bacilli*/*Lactobacilli*, account for nearly 90% of the microbial diversity (Bolan *et al.*, 2010).

UNDER PEER REVIEW

Pathogens in chicken litter represent the major group of bacteria of special interest to litter processors. A variety of pathogens can be found in chicken litter or chicken litter-based organic fertilizers, such as *Actinobacillus*, *Bordetalla*, *Campylobacter*, *Clostridium*, *Corynebacterium*, *Escherichia coli*, *Globicatella*, *Listeria*, *Mycobacterium*, *Salmonella*, *Staphylococcus*, and *Streptococcus* (Bolan *et al*., 2010). Alum treatment has been widely used to reduce pathogens before land application of chicken litter (Rothrock *et al.*, 2008), and it can also be applied as an effective way to reduce ammonia volatilization and water-soluble phosphorus runoff from poultry litter in chicken houses (Gandhapudi *et al.*, 2006). Pathogens in poultry wastes, such as *Salmonella* and *Campylobacter*, have been shown to be reduced significantly or eliminated by alum treatment (Line, 2002). Rothrock *et al*. (2008) used denatured gradient gel electrophoresis (DGGE) and quantitative real-time polymerase chain reaction (PCR) to characterize pathogenic microbial communities in alum-treated poultry litter.

MATERIALS AND METHODS

Experimental site and Location

The study was carried out at the poultry unit of the Department of Animal Science teaching and research farm, Ahmadu Bello University, Zaria. The pen is located in northern guinea savannah zone of Nigeria, latitude 11^0 09' 76'' N and longitude 7^0 38' 20'' E at an altitude of 610 mm above sea level. The climate is relatively dry with a mean annual rainfall of 700-1400mm, occurring between the months of April and September (Ovimaps, 2015).

Experimental Diets and Material

Broiler starter and finisher diets were formulated to meet the nutrient requirement of broilers (NRC, 1994) and used in feeding the experimental birds throughout the period of the study in both experiment one and two. The experimental diets are shown in Table 1. The alum used was obtained from the Sabon-garimarket in Zaria, Kaduna State.

Experimental Animals and their Management

Two hundred and forty (240) day old Marshall Strain broiler chicks of mixed sexes were used for the study. The birds were brooded together using kerosene stoves and electric bulbs in two pens for the first one week due to extremely cold weather conditions. The birds were fed a common diet during this period and were subsequently weighed and randomly assigned to four treatment groups. The treatments were replicated three times with 20 birds per pen. They were housed under a deep litter system with 15kg wood shavings per pen in a completely randomised design. Aluminium sulphate (alum) was applied to the wood shavings by mixing it with alum thoroughly using hands covered with hand gloves. The rates of alum application was as follows: T1 control (normal wood shavings with no alum), T2 (5% alum by kg weight of wood shavings), T3 (10% alum by kg weight of wood shavings) and T4 (15% alum by kg weight of wood shavings). Feed and water were supplied *ad libitum* throughout the 56 days study period and routine vaccination schedule was administered.

Table 1: Ingredients Composition and Calculated Analysis of the experimental Diets

Ingredients	Composition (%)	
	Starter (0 – 4 weeks)	Finisher (5 – 8 weeks)
Maize	51.90	54.50
Groundnut cake	16.00	22.20
Soya bean cake	25.00	15.00
Palm oil	2.00	3.40

UNDER PEER REVIEW

Lime stone	1.00	0.90
Bone meal	3.00	2.80
Common Salt	0.30	0.30
Premix*	0.25	0.30
Lysine	0.25	0.30
Methionine	0.30	0.25
Total	**100.00**	**100.00**
Calculated analysis		
Crude protein (%)	23.20	21.80
Metabolisable energy (kcal/kg)	2929	3037
Ether extract (%)	6.57	7.74
Crude fibre (%)	4.18	3.78
Calcium (%)	1.23	1.13
Available Phosphorus (%)	0.52	0.49
Lysine (%)	1.13	1.19
Methionine (%)	0.96	0.86
Feed cost (₦/kg)	91.80	88.00

***Composition of premix supplies the following per kg of feed: Vit. A = 12000IU, Vit. E = 15000IU, Vit. D_3 = 2500IU, Vit. C = 30,000mg, Folic acid = 100mg, Nicotine acid = 5000mg, Panthotenic acid = 15000mg, Fe = 1750mg, I = 40,000mg, Zn = 50,000mg, Mn = 100mg, CU = 1500mg, Cu = 200mg, Si = 100mg, Biotin = 600mg, Metabolisable energy calculated according to formulae of Peuzenga (1985). M.E = (37 x %CP) + (81 x %EE) + (35.5 x %NFE).*

Data collection and Analyses

Blood sample collection and haematological analysis

At the termination of the experiment (day 56), two birds from each pen having representative weights for the group (6 birds per Treatment) were selected and 1.5ml of blood was taken via the wing vein. Haematological parameters were analyzed by an auto haemo-analyser (BC2800 vet auto haemo analyser) at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The parameters determined were red blood cell (RBC) count, white blood cell (WBC) count, total protein (TP), packed cell volume (PCV), haemoglobin (Hb), differentials, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Litter sample collection

Five sets of litter samples were obtained fortnightly from each pen from different locations i.e. the four corners and centre from which the microbial load, pH, total nitrogen (N), soluble reactive phosphorus, VFA and NH_4^+ concentration were measured. Litter samples were taken by removing the first 10mm of the exposed surface from each location set. The samples from each pen were mixed and homogenized to make one sample and was refrigerated before being taken to the laboratory for analyses.

Microbial analysis of litter

Each homogenous sample mixture from each pen was pooled in one sterile flask as representing sample from one pen and were analysed for microbes in the Department of Microbiology, Ahmadu Bello University Zaria. The flasks were shaken and then serial dilutions were made on each. Each dilution were streaked on plate count containing Nutrient Agar (NA) for total bacteria count, eosine methylene blue Agar (EMB) for *E. coli*, bismuth sulfide agar (BSA) for *Salmonella spp.* and potato dextrose agar (PDA) for mould and yeast

UNDER PEER REVIEW

and incubated at room temperature. Suspected colonies were inoculated in triple sugar iron agar for confirmation. Standard plate counting techniques were used for total bacteria, *E. coli, Salmonella spp.* and mold and yeast counts as described by Yardimci and Kenar(2008). Sample for *Eimeria spp.* was taken to the parasitology laboratory at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for *Eimeria*parasite count using the modified McMaster egg/oocyst count technique (Zanjac and Conboy, 2012).

Statistical analyses

All the data collected from the experiment were subjected to analysis of variance (ANOVA) using the general linear model of statistical analysis system (SAS, 2001) software package and the mean separation was done using Duncan multiple range test.

RESULTS

Haematological Parameters of Broiler Chickens Raised on recycled Litter treated with graded levels of Alum

The effect of recycled litter treated with graded levels of on the haematological parameters of broiler chickens was presented in Table 2. This result shows significant ($P<0.05$) difference among all theparameters analysed except MCHC. The packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), lymphocyte, mean cell volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly ($P<0.05$) higher in all the alum treated litter groups (5%, 10% and 15% alum treated litter) compared to the untreated control group (0% alum treated litter). The total protein (TP), white blood cell (WBC), heterophil, monocytes and eosinophil were significantly ($P<0.05$) higher in the control group (0% alum treated litter) compared to the alum treated litter groups (5%, 10% and 15% alum treated litter). There is no significant difference in the mean corpuscular haemoglobin concentration (MCHC) across the treatments.

Table 2: Effect of recycled litter treated with graded levels of Alum on Haematological Parameters of Broiler Chicken.

	Treatments				
	Alum Inclusion (%)				
Parameter	0	5	10	15	SEM
Packed Cell Volume (%)	20.00^{b}	28.33^{a}	28.00^{a}	28.33^{a}	0.192
Haemoglobin (g/dl)	5.67^{b}	8.17^{a}	8.10^{a}	8.27^{a}	0.121
Total Protein (g/dl)	6.20^{a}	4.03^{c}	3.97^{c}	4.87^{b}	0.056
Red Blood Cell (10^{12}/l)	3.83^{c}	4.63^{a}	4.60^{a}	4.53^{b}	0.016
White Blood Cell (10^{9}/l)	18.66^{a}	8.93^{c}	8.90^{c}	9.97^{b}	0.115
MCV (fl)	52.18^{c}	61.14^{b}	60.87^{b}	62.49^{a}	0.342
MCH (pg)	14.77^{b}	17.63^{a}	17.61^{a}	18.23^{a}	0.308
MCHC (g/dl)	28.33	28.83	28.93	29.18	0.707
Deferential					
Heterophil (%)	22.66^{a}	17.33^{bc}	17.66^{b}	16.00^{c}	0.430
Lymphocyte (%)	52.66^{b}	75.00^{a}	75.33^{a}	75.66^{a}	0.844
Monocyte (%)	11.00^{a}	4.333^{b}	4.000^{b}	4.333^{b}	0.419
Eosinophil (%)	8.33^{a}	3.33^{bc}	3.00^{c}	4.00^{b}	0.254
Band (%)	5.33^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.166

UNDER PEER REVIEW

[abc] = Means on the same row with different superscripts are significantly (P<0.05) different. MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, SEM = Standard error of mean.

Microbial Load of recycled litter treated with graded levels of Alum

The result of the fortnightly effect of alum treated litter on the microbial load of the Litter is presented on Figure 1-4. The result shows a significant decrease in the total bacteria, *E. coli* and *Salmonella spp*. in the alum treated litter groups (5%, 10% and 15% alum treated litter) compared to the control group (0% alum treated litter), the mould and yeast load was significantly increased in alum treated litter groups (5%, 10% and 15% alum treated litter) compared to the control group (0% alum treated litter). The *Eimeria spp* parasite load in the litter remained at below detection level throughout the period of the research.

The total bacteria load of the litter at week 0 (after treating with alum) were $2.27x10^6$cfu/g, $3.79x10^5$cfu/g, $3.56x10^5$cfu/g and $3.25x10^5$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, the total bacteria load for 0%, 5%, 10% and 15% for week 2 were $1.08x10^7$cfu/g, $2.64x10^5$cfu/g, $2.37x10^5$cfu/g and $2.18x10^5$cfu/g and $9.90x10^7$cfu/g, $6.11x10^5$cfu/g, $5.91x10^5$cfu/g and $5.13x10^5$cfu/g for week 4 respectively. At week 6, the total bacteria of the litter were $1.23x10^9$cfu/g, $4.24x10^6$cfu/g, $4.14x10^6$cfu/g and $3.98x10^6$cfu/g for 0%, 5%, 10% and 15% alum treated litter respectively and $4.20x10^{10}$cfu/g,$9.69x10^7$cfu/g, $9.59x10^7$cfu/g and $8.97x10^7$cfu/g for 0%, 5%, 10% and 15% alum treated litter respectively at week 8.

The *E. coli* load of the litter at week 0 (after treating with alum) were $3.26x10^5$cfu/g, $4.39x10^4$cfu/g, $4.23x10^4$cfu/g and $3.86x10^4$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 2, the *E. coli* load of the litter were $4.47x10^6$cfu/g, $3.45x10^5$cfu/g, $3.37x10^5$cfu/g and $8.44x10^4$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 4, the *E. coli* load of the litter were $7.31x10^7$cfu/g, $6.72x10^5$cfu/g, $6.49x10^5$cfu/g and $1.03x10^5$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 6, the *E. coli* load of the litter were $3.29x10^8$cfu/g, $1.47x10^6$cfu/g, $1.33x10^6$cfu/g and $1.15x10^6$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 8, the *E. coli* load of the litter were $8.93x10^8$cfu/g, $2.22x10^6$cfu/g, $2.15x10^6$cfu/g and $2.11x10^6$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively. The *Salmonella spp.* load of the litter at week 0 (after treating with alum) were $2.33x10^5$cfu/g, $1.18x10^5$cfu/g, $1.31x10^5$cfu/g and $1.29x10^5$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 2, the *Salmonella spp*. load of the litter were $1.57x10^6$cfu/g, $4.63x10^5$cfu/g, $4.64x10^5$cfu/g and $4.44x10^5$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 4, the *Salmonella spp*. load of the litter were $9.91x10^6$cfu/g, $1.58x10^6$cfu/g, $1.21x10^6$cfu/g and $1.18x10^6$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 6, the *Salmonella spp*. load of the litter were $5.33x10^8$cfu/g, $8.79x10^6$cfu/g, $8.45x10^6$cfu/g and $8.10x10^6$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 8, the *Salmonella spp*. load of the litter were $9.23x10^8$cfu/g, $1.93x10^7$cfu/g, $1.36x10^7$cfu/g and $1.01x10^7$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively.

The mould and yeast load of the litter at week 0 (after treating with alum) were $1.20x10^4$cfu/g, $9.06x10^4$cfu/g, $9.26x10^4$cfu/g and $9.30x10^4$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 2, the mould and yeast load of the litter were $8.30x10^4$cfu/g, $4.50x10^5$cfu/g, $5.26x10^5$cfu/g and $6.39x10^5$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 4, the mould and yeast load of the litter were $5.13x10^5$cfu/g, $7.86x10^6$cfu/g, $8.80x10^6$cfu/g and $9.46x10^6$cfu/g for 0%, 5%,10% and 15% alum treated litter respectively, at week 6, the mould and yeast load of the litter were $8.30x10^5$cfu/g, $1.80x10^7$cfu/g, $4.10x10^7$cfu/g and $5.10x10^7$cfu/g for 0%, 5%,10% and 15% alum treated litter

UNDER PEER REVIEW

respectively, at week 8, the mould and yeast load of the litter were $6.40x10^{6}$cfu/g, $1.60x10^{8}$cfu/g, $2.03x10^{8}$cfu/g and $3.50x10^{8}$cfu/gfor 0%, 5%,10% and 15% alum treated litter respectively.

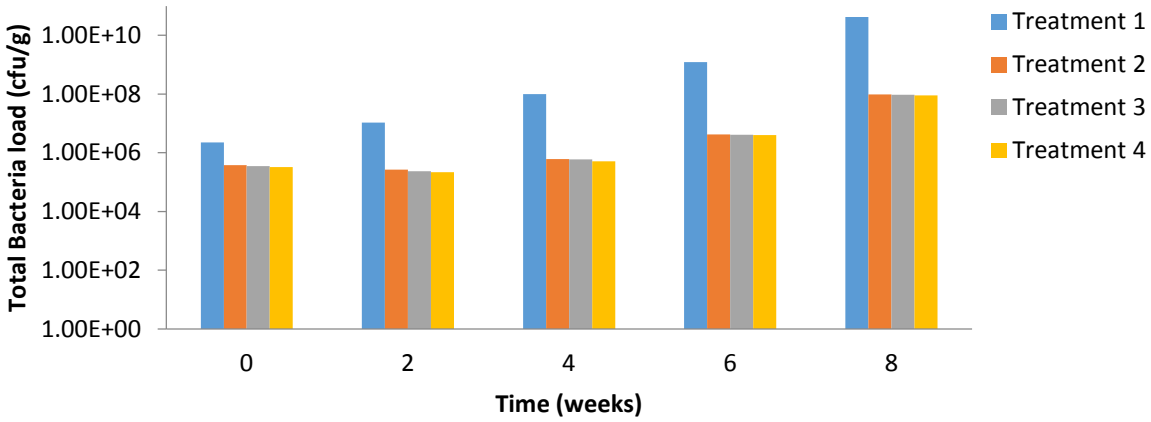

Figure 1: Total Bacteria Load of recycled litter treated with graded levels of Alum

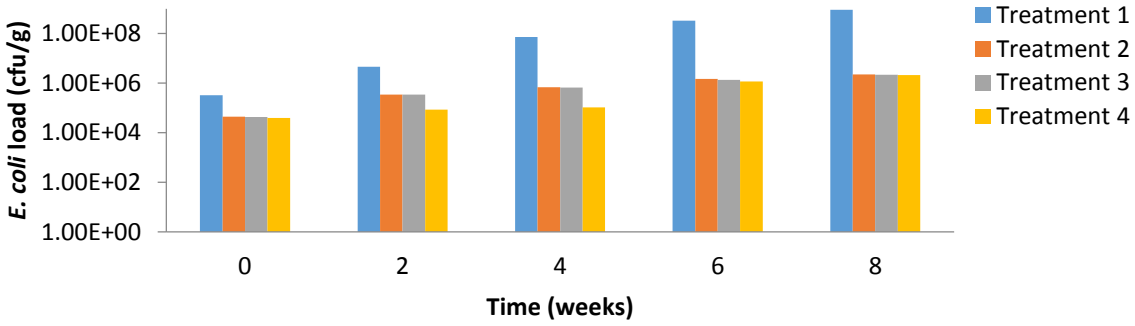

Figure 2: *E coli* Load of recycled litter treated with graded levels of Alum

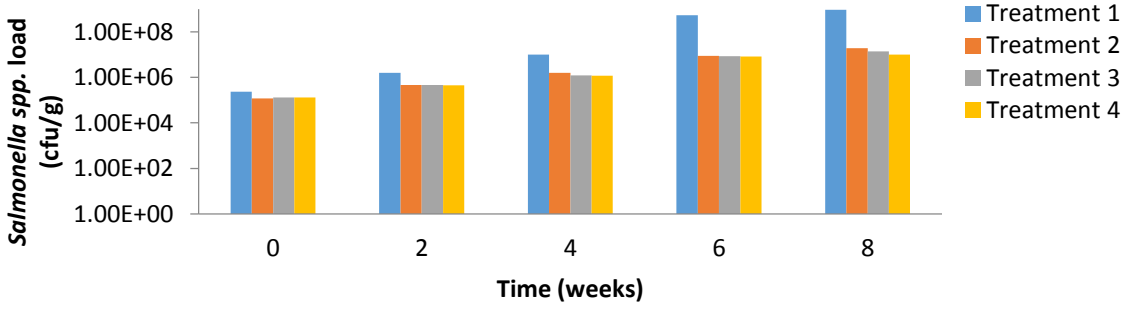

Figure 3: *Salmonella spp.* Load of recycled litter treated with graded levels of Alum

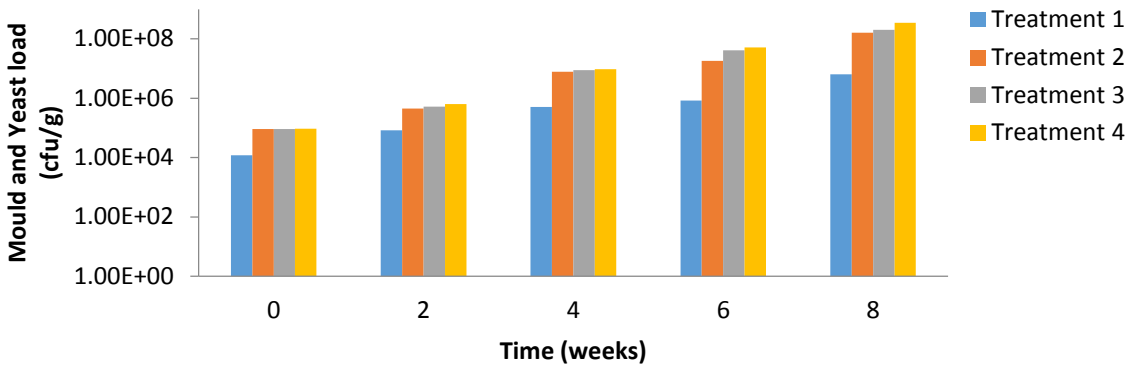

Figure 4: Mould and Yeast Load of recycled litter treated with graded levels of Alum

DISCUSSION

Haematological Parameters of Broiler Chickens Raised on recycled Litter treated with graded levels of Alum

Though the PCV, haemoglobin (Hb), red blood cell, lymphocyte, MCV and MCH in the Alum treated litter groups (5%, 10% and 15% alum treated litter) were significantly higher, they were within the normal range reported by Jain (1993) and Chinrasri and Aengwanich (2007) who reported normal range for PCV, haemoglobin, red blood cell, lymphocyte, MCV and MCH to be 29.75-31.87%,8.22-8.88g/dl, 4.7-4.78x10^{12}/l, 71-75.45%, 62.54-68.81fl and 17.38-18.70pg respectively. The significantly higher values obtained in the control treatment (0% alum treated litter) in total protein, white blood cell, heterophil, monocyte, eosinophil and band were above the normal range reported by Jain (1993) and Chinrasri and Aengwanich (2007) who reported normal range for white blood cell, heterophil, monocyte, eosinophil and band to be 2.57-2.72x10^{9}/l, 15.83-18.3%, 3.00-4.38%, 3.6-4.2% and 1-2% respectively. The disease condition indicated as higher WBC and differentials blood count observed in the control group (0% alum treated litter) may be due to high microbial load in the litter as shown in Figures 1-4. This result agrees with the report of Forbes and Robert (2012) who reported better health status of broiler chicken when raised in alum treated litter.

Microbial Load of recycled litter treated with graded levels of Alum

The significant decrease in total bacteria, *E. coli* and *Salmonella spp*. load of the litter observed at the end of the experiment (week 8) indicates that total bacteria load was reduced by three fold magnitude, *E. coli* load by two fold magnitude and *Salmonella spp.* load by one fold magnitude when poultry litter is treated with alum compared to the untreated litter as seen in the work of Cook *et al.* (2008) who reported two fold reduction in the total bacteria of the litter with alum treatment at 16 weeks. This is similar to the work by Scantling *et al*. (1995), Line (2002) who all reported significant reduction in microbial load in poultry litter treated with alum. This drastic reduction in the total bacteria load, *E. coli* and *Salmonella spp.* can be associated with the low pH in alum treated litter groups (5%, 10% and 15% alum treated litter) as reported by Gandhapudi *et al*. (2006) and Cook *et al*. (2008). The mould and yeast load was seen to be higher in the alum treated litter groups (5%, 10% and 15% alum treated litter) compared to the control (0% alum treated litter) with 6.40 x 10^{6}cfu/g, 1.60 x 10^{8}cfu/g,

UNDER PEER REVIEW

2.03 x 10^8cfu/g and 3.5 x 10^8cfu/g in 0%, 5%, 10% and 15% alum treated litter respectively at the end of the experiment (week 8). This is similar to the report by Cook *et. al.*(2008), who reported 3.5 x 10^7cfu/g and 5.5 x 10^4cfu/g in alum treated and untreated litter respectively, indicating a threefold magnitude higher fungal load in alum treated litter compared to the control untreated litter. This suggests that the addition of alum to poultry litter potentially shifts the microbial loads from bacterially dominated to fungi dominated (Rothrock *et al.*, 2008). The ramifications of this shift in dominance are still unknown, and future work will be aimed at characterizing these fungi and elucidating their role in the acidified litter environment (Rothrock *et al.*, 2008).

CONCLUSION

The study conclude that treating recycled poultry litter with alum can reduce microbial load of the litter, hence improve health status of the birds. And tends to shift the microbial load of the litter from bacteria dominant to fungal dominant, hence reducing the risk of most bacterial diseases in broiler chicken as fungi don't convert ammonium into ammonia. Birds reared on recycled poultry litter have significantly higher haemoglobin and PCV compared to the control.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFRENCES

Bolan, N. S., Szogi, A. A., Chuasavathi, T., Seshadri, B., Rothrock, M. J. Jr. and Panneerselvam, P. (2010). Uses and management of poultry litter. *World's PoultryScienceJournal,66*: 673–698.

Chinivasagam, H. N., Redding, M., Runge, G. and Blackall, P. J. (2010). Presence and incidence of foodborne pathogens in Australian chicken litter. *BrititshPoultry Science journal,*51: 311–318.

Chinrasri, O. and Aengwanic, W. (2007). Blood Cell Characteristics, Hematological Value and average Daily Gained Weight of Thai Indigenous, Thai Indigenous Crossbred and Broiler Chickens. *Pakistan Journal of Biological Sciences*, 10 (2): 302-309.

Cook, K. L., Rothrock, M. J., Warren, J. G., Sistani, K. R. and Moore P. A. (2008). Effect of Alum Treatment on the Concentration of Total and Ureolytic Microorganisms in Poultry Litter. *Journal of Environmental Quality*,37 (6): 2360-2367.

Enticknap, J. J., Nonogaki, H., Place, A. R. and Hill, R. T. (2006). Microbial diversity associated with odour modification for production of fertilizers from chicken litter. *AppliedEnvironmentalMicrobiology*,72: 4105–4114.

Forbes, W. and Robert, B. (2012).Treating Broiler Litter with Alum. Agricultural Extension Service, University of Tennesse. P&SS Info # 318. Pp1-6.

Gandhapudi, S. K., Coyne, M. S., D'Angelo, E. M. and Matocha, C. (2006). Potential Nitrification in Alum treated soil slurries amended with poultry manure. *Journal of Bioresources Technology*, 97:664-670.

UNDER PEER REVIEW

Jain, N. C. (1993). Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia, USA. Pp 133-168.

Kim, J., Diao, J., Shepherd, M. W. Jr., Singh, R., Heringa, S. D., Gong, C. and Jiang, X. (2012). Validating thermal inactivation of *Salmonella* spp. in fresh and aged chicken litter. *AppliedEnvironmentalMicrobiology*, 78: 1302–1307.

Line, J. E. (2002). *Campylobacter* and *Salmonella* populations associated with chicken raised on acidified litter. *PoultryScience*, 81: 1473–1477.

NRC. (1994). *Nutrient requirement of poultry ninth revised edition*. Washington D.C: National Academy Press.

Ovimaps, (2015). Ovi location map; Ovi earth imagery date; December22nd, 2015.

Rothrock, M. J. Jr., Cook, K. L., Warren, J. G. and Sistani K. (2008). The Effect of Alum Addition on Microbial Communities in Poultry Litter.*Journal of Poultry Science*, 87:1493–1503.

Scantling, M., Waldroup, A., Mary, J. and Moore, P. (1995). Microbiological effects of treating poultry litter with aluminiumsulphate. *PoultryScience*, 74*:* 216.

Sharpley, A., Slaton, N., Tabler, T. Jnr., VanDevender, K., Daniel, M., Jones F. and Daniel T. (2009). Nutrient Ana;lysis of Poultry Litter. *Agricultural and Natural Resources*, FSA9529-PD-6-09N.

Statistical Analysis System, (2001). Copyright © by SAS Institute Inc. Cary, NC, USA.

Wilkinson, K. G., Tee, E., Tomkins, R. B., Hepworth, G. and Premier, R. (2011). Effect of heating and aging of poultry litter on the persistence of enteric bacteria. *PoultryScience*, 90: 10–18.

Yardimci, M. and Kenar, B. (2008). Effect of stocking density on litter microbial load in broiler chickens. *Journal of Archiva Zootechnica,* 11:3, 75-81.

Zanjac, A. Z. and Conboy, G. A., (2012). Veterinary Clinical Parasitology 8th Edition, 8-11.

Zhao, C. and Jiang, X. (2014).Microbiological Safety of Chicken Litter or Chicken Litter-Based Organic Fertilizers: A Review. *Agriculture*, 4: 1-29.

UNDER PEER REVIEW