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Research Article

Effect of Acidified Drinking Water on Gut Bacteria Community and Blood Profile of Broiler Chickens

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Abstract

One hundred and fifty (150) day old Abor-Acre chicks were used to determine the effect of organic acids (OAs) fed through drinking water on haematology, serum biochemistry and bacteria community of the gut of broiler chickens. The OAs were acetic (AA), butyric (BA), citric (CA) and formic (FA) acids representing the treatments. Each of the OAs was added to the drinking water at 0.25%. The control had no OA. Each treatment was replicated three times with 10 birds per replicate, arranged in completely randomized design (CRD). Feed and water were offered *ad libitum* for 7 weeks the experiment lasted. Results showed that white blood cells were significantly ($P < 0.05$) increased by BA, CA and FA. The OAs did not significantly ($P > 0.05$) alter other haematological and serum biochemical indices investigated. All the OAs showed antibacterial effect on *Salmonella, E. coli* and *Staphylococcus* in the crop, gizzard, duodenum, ileum and caecum. In conclusion, the OAs could be used as antibacterial agents in drinking water to reduce bacteria bodies in the gut of broiler chickens without deleterious impact on hematological and serum biochemical status of the birds.

Keywords: *Bacteria Community; Drinking Water; Gut; Haematology; Organic Acids; Serum Biochemistry*

Introduction

Diet plays an important role for the well being of broiler chickens considering its significant impact in the development and maintenance of the gut. Hence the gut health should be maintained or enhanced through dietary means for the welfare and productivity of animals. Intensively managed poultry is exposed to infection of microbial entities especially microflora [1]. According to Choct [2] formulating diet for its effects on gut health is fast becoming a reality in monogastric animal industry. Also, Ndelekwute., *et al*. [3] opined that no matter how good the feed is if the health of the gut is poor it cannot be well utilized by the animals.

The gut health among other factors is influenced by endogenous secretions, the presence and number of microflora and nutrient supply to the gut. While the gut harbours over 640 species of bacteria [2] certain components of the feed materials such as non-starch polysaccharides and fibre (cellulose) could cause high digesta viscosity in the gut which favours bacteria growth and multiplication, leading to poor nutrient utilization [4]. Thus, it is essential to reduce this quantum of bacteria population and inactivate them for efficient management of feed by the gut. According to Ndelekwute., *et al*. [3] the gut is like the central processing unit (CPU) of a computer which processes the feed that is then distributed to different organs of the body for necessary metabolic processes. On the other hand, the blood is the medium in which absorbed nutrients are transported in the body and its constituents could be affected by the components of the diet and drinking water.

To maintain both gut health and efficient gut, nutritionists add pharmaceutical antibiotics to the diet of broiler chickens to modulate it to maintain the health of the gut. But due to reported antibiotic resistance in both farm animals and man [5] attention has been shifted to

using other products. The products includes: probiotics, prebiotic, yeast culture, essential oils, spices and organic acids [6-8]. The conventional way of feeding organic acids has been through the feed [9]. It has been suggested that organic acids should also be given through the drinking water [10]. Addition of organic acids to the drinking water for birds could not only sanitize the water but probably modulate the gut for better performance [10,11].

Objective of the Study

This research was intended to determine the effect of feeding drinking water acidified with organic acids on blood parameters and bacteria population of gut of broiler chickens.

Materials and Methods

Site of the experiment

The experiment was conducted at the Teaching and Research Farm of Department of Nutrition and Forage Science of the Michael Okpara University of Agriculture Umudike; Abia State, Nigeria. Umudike is situated on latitude 5° 28^{1}N and longitude 7° 32^{1}E and lies at an altitude of 122m above sea level, with average rainfall of 2000 mm. The average relative humidity during the experiment was 72% and average ambient temperature was 28°C.

Experimental design

One hundred and fifty (150) day old chicks of Abor-Acre strain were used. There were divided into five treatment groups. The experiment was arranged in completely randomized design (CRD).

The treatments were replicated three times each having 10 birds per replicate. The treatment groups (T1 - T5) received no organic acid, acetic acid, butyric acid, citric acid and formic acid respectively. T1 was the control. Acetic, butyric and formic acids were offered to the birds at 2.5 ml/litre (0.25%) and citric acid 2.5 g/litre (0.25%) of drinking water. The citric acid was measured in gram because it was in crystalline form.

Management of experimental birds

Heat was supplied to the birds at day old till the third week using kerosene stove as source of heat. They were given Hb1 vaccine intraocular at day old and lasota through drinking water at 20th day against Newcastle disease.

They were also vaccinated against infectious bursal (Gumboro) disease. The birds were fed formulated starter and finisher diets *ad libitum* (Table 1). The diets were compounded to contain nutrients according to nutrients requirement for broiler chickens in the hot tropical ecosystem which Nigeria geographically falls within (Table 2). The experiment lasted for seven weeks. The acidified water was provided throughout the experiment *ad libitum*. The birds were managed in a deep litter house which was open sided type having wood shavings as bedding materials. The sides of the building were covered with wire mesh.

Ingredients	Starter	Finisher
Maize	55.00	55.00
Soya bean meal	28.00	26.00
Palm kernel cake	10.30	13.30
Fish meal	3.00	2.00
Bone meal	3.00	3.00
Salt (Nacl)	0.25	0.25
Lysine	0.10	0.10
Methionine	0.10	0.10
Premix*	0.25	0.25
Total	100.00	100.00

Table 1: *Ingredients composition of experimental diets*

****Starter Premix supplied per kg diet****: Vitamin A 15,000 I.U, vitamin D_3 13000 iu, thiamine 2 mg, Riboflavin 6 mg, pyridoxine 4mg, Niacin 40mg, cobalamin 0.05g, Biotin 0.08mg, choline chloride 0.05g, Manganese 0.096g, Zinc 0.06g, Iron 0.024g, Copper 0.006g, Iodine 0.014g, Selenium 0.24 mg, Cobalt 0.024 mg and Antioxidant 0.125g. CON: Control; AA: Acetic Acid; BA: Butyric Acid; CA: Citric Acid; FA: Formic Acid.* ****Finisher Premix supplied per kg diet:*** *Vitamin 10, 0001.u., vitamin D_3 12,0001.u. Vitamin E 201.U., Vitamin K 2.5 mg, thiamine 2.0 mg, Riboflavin 3.0 mg, pyridoxine 4.0 mg, Niacin 20 mg, cobalamin 0.05 mg, pantothenic acid 5.0 mg, Folic acid 0.5 mg, Biotin 0.08 mg, choline chloride 0.2 mg, Manganese 0.006g, Zinc 0.03g, Copper 0.006g, Iodine 0.0014g, Selenium 0.24g, cobalt 0.25g and antioxidant 0.125g.*

Nutrients*	Starter diet	Finisher diet
Crude protein	22.10	20.05
Energy (KcalME/kg)	2878	2887
Ether extract	3.92	6.16
Crude fibre	5.01	6.00
Ash	7.04	6.80
Calcium	1.2	1.11
Phosphorus	1.01	0.88
Lysine	1.12	1.05
Methionine	0.45	0.35

Table 2: *Nutrients composition of formulated experimental diets.*
**Calculated.*

Microbiological analysis of gut digesta

At the end of the feeding experiment, digesta was collected from different segments of the digestive system (crop, gizzard, duodenum, ileum, caecum and large intestine). Three birds per treatment representing one per replicate were slaughtered. The abdomen of each bird was cut open and the gut pulled out. Collection of digesta was in sequential order starting from the crop to the large intestine. The digesta within the region of the duodenal fold was taken to be the digesta in the duodenum. The distance between the Merkel's diverticulum and ileo-caecal joint was taken to be the ileal digesta [12]. Each digesta was collected into a Mackartney bottle and quickly stored in a refrigerator at 4°C. Thereafter each sample was subjected to serial dilution technique according to Fawole and Oso [13]. The samples were then incubated at 37°C in a J.P. Selecta incubator (0501991, made in Spain). Bacteria colonies in each culture were characterized to determine the type of bacteria growing in the medium after the cultures were purified [14].

Blood collection and analysis

Blood was collected from the birds and both haematological and blood serum biochemical analyses were carried out. Birds were bled between 8:00 and 9:00hrs. Blood collection was through the jugular vein by the use of hypodermic syringe. Blood for haematological analysis was collected into Mackartney bottles containing dipotassium salt of ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant. Blood samples for biochemical analysis were also collected into Mackartney bottles containing no EDTA and were allowed to clot. Both haematological and blood chemistry parameters were analysed according to the methods of Ochi and Kochatkar [15].

Data transformation and statistical analysis

Bacterial count expressed in colony forming units (cfu) were transformed using Log_{10} according to Alshawabkeh and Tabbaa [12]. All Data collected were subjected to one way analysis of variance (ANOVA). Significant means were separated using Duncan New Multiple Range Text (DNMRT) according to Steel and Torrie [16].

Results and Discussion

Haematological and blood chemistry indices

Table 3 shows the effect of acid treated drinking water on haematological parameters of broiler chickens. Significant difference was observed only in white blood cells of the birds.

Parameters	T1 (CON) (0%)	T2 (AA) (0.25%)	T3 (BA) (0.25%)	T4 (CA) (0.25%)	T5 (FA) (0.25%)	SEM
White Blood Cells ($x10^3/mm^3$)	$28.00^b \pm 2.68$	$28.70^b \pm 2.68$	$29.70^a \pm 2.71$	$29.00^a \pm 2.71$	$29.0^a \pm 2.71$	1.08
Red Blood Cells ($x10^6/mm^3$)	3.90 ± 0.04	3.77 ± 0.04	3.72 ± 0.04	3.95 ± 0.05	3.83 ± 0.04	0.56
Haemoglobin (g/100ml)	12.53 ± 1.93	13.07 ± 1.95	12.97 ± 1.93	12.66 ± 1.94	12.74 ± 0.03	0.78
Packed cell volume (%)	32.35 ± 3.65	31.95 ± 3.63	31.35 ± 3.63	31.38 ± 3.63	31.96 ± 3.70	1.76
MCH (%)	32.35 ± 3.75	34.74 ± 3.89	34.97 ± 3.90	32.14 ± 3.67	33.23 ± 3.92	1.12
MCV (mg / 100ml)	84.66 ± 5.45	84.90 ± 5.48	84.35 ± 5.41	79.60 ± 5.05	83.56 ± 5.38	2.03
MCHC (%)	38.76 ± 3.32	40.91 ± 3.76	42.59 ± 3.80	40.35 ± 3.67	39.85 ± 3.62	1.00

Table 3: *Effect of organic acid treated drinking water on haematological indices of broiler chickens*
abc: Means along the same row with different superscripts are significantly (P < 0.05) different. MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume; CON: Control; AA: Acetic Acid; BA: Butyric Acid; CA: Citric Acid; FA: Formic Acid.

White blood cells of butyric, citric and formic acid groups were significantly higher ($P < 0.05$) than those in acetic acid and the control. It was observed that control group with significant level of pathogenic bacteria in the gut (Table 5 and 6) had lower number of white cells than the three organic acids mentioned (butyric, citric and formic).

White blood cells respond to presence of foreign bodies, wounds and infection in the body [15]. The lower level of white blood cells in acetic acid group could be an indication that the level of acetic acid was not enough to cause deleterious effect to have caused the white blood cells to multiply. Invariably the higher level of white blood cells observed in butyric, citric and formic acids could be linked to probable ulceration of the intestine resulting to response by the white blood cells to increase. Hernandez., *et al*. [17] reported negative effect of organic acids on the histomorphology of broilers in form of luminal erosion. According to Oviedo [18] organic acids caused sub-clinical intestinal lacerations which could have consequently led to response by the white blood cells to increase in number. This is an indication that certain organic acids could trigger response by white blood cells. The increase in white blood cells could not be associated with the presence of pathogenic organisms such as *Salmonella spp* as the number of *Salmonella spp* for instance was higher in control compared to the numbers in organic acid groups. Though the level of white blood cells in butyric, citric and formic acids were higher compared to control and acetic acid groups the levels fell within the normal range for healthy chickens [19]. Also, the level of the micro-organisms in all the treatments could not have caused clinical problem for the white blood cells to increase. For instance, Choct [2] reported that it required 1 x 10^6 cells of *Salmonella spp* to cause clinical problem, which is higher than the numbers observed in this study. White blood cells are known to multiply in defence of the body against pathogenic organisms (such as *Salmonella* and *E. coli*) invasion which was opposite of this. Furthermore, Chaveerach., *et al.* [20] reported increase in the number of degenerated epithelial cells in broilers fed acid treated water.

The effect of the organic acids on blood serum chemistry shown in table 4 indicates that there were no significant differences ($P > 0.05$) in the blood serum biochemical parameters. The non-significant result in all the parameters of serum chemistry signifies that the organic acids at that level was not deleterious and would not have interfered negatively with the physiological processes of the birds. This was clearly shown in the results of the liver enzymes (alkaline-phosphatase, alanine-aminotransferase and aspartate-aminotransferase) evaluated which were statistically similar. This also indicates that the organic acids could not have imparted deleteriously on the liver. While these enzymes play significant roles in nutrient metabolism, high level of the enzymes in the blood is indication of liver injury or infection [21].

Parameters (mg/100 ml)	T1 (CON) (0%)	T2 (AA) (0.25%)	T3 (BA) (0.25%)	T4 (CA) (0.25%)	T5 (FA) (0.25%)	SEM
Total protein	4.97 ± 0.09	4.54 ± 0.08	4.87 ± 0.09	4.60 ± 0.07	4.87 ± 0.09	0.56
Albumin	3.28 ± 0.86	3.32 ± 0.06	3.30 ± 0.96	3.40 ± 0.75	3.36 ± 0.96	0.41
Globulin	1.69 ± 0.05	1.56 ± 0.04	1.57 ± 0.03	1.65 ± 0.05	1.59 ± 0.04	0.24
Urea	2.02 ± 0.06	2.37 ± 0.07	2.43 ± 0.07	2.17 ± 0.05	2.09 ± 0.05	0.08
Cholesterol	142.00 ± 10.55	143.00 ± 10.55	142.67 ± 10.55	145.67 ± 10.59	147.67 ± 10.64	4.09
Creatinine	0.44 ± 0.003	0.46 ± 0.003	0.50 ± 0.004	0.46 ± 0.0003	0.45 ± 0.03	0.03
Glucose	163.33 ± 12.45	166.33 ± 12.40	164.00 ± 12.45	165.67 ± 12.45	163.34 ± 12.40	5.09
ALP	34.00 ± 3.08	36.57 ± 3.10	38.07 ± 3.40	37.92 ± 3.40	38.78 ± 3.41	1.56
ALT (iu/l	19.00 ± 2.34	20.00 ± 2.34	21.67 ± 2.35	21.67 ± 2.35	22.00 ± 2.36	3.23
AST (iu/l	107.00 ± 9.44	115.67 ± 10.0	114.33 ± 10.0	110.33 ± 9.98	117.00 ± 10.05	20.34

Table 4: *Effect of organic acid treated drinking water on blood Serum chemistry indices of broilers. CON: Control; AA: Acetic Acid; BA: Butyric Acid; CA: Citric Acid; FA: Formic Acid; ALP: Alkaline-Phosphates; ALT: Alanine-Aminotransferase; AST: Aspartate-Aminotransferase.*

Bacterial load of gastrointestinal tract (GIT)

Effect of organic acids fed through drinking water on bacterial load in different segments of the gastrointestinal tract or the gut is shown in table 5 and 6. At the foregut (Table 5), total count, *Salmonella, E. coli* and *Staphylococcus* counts were significantly ($P < 0.05$) higher in control than in organic acid groups in all the segments of the foregut. In the hindgut (Table 6), the number of bacteria was significantly ($P < 0.05$) highest in control in all the segments except in the large intestine. In the large intestine the number of *E. coli* in control group was significantly higher than in butyric acid but the same with the other organic acids.

Segments	T1 (CON) (0%)	T2 (AA) (0.25%)	T3 (BA) (0.25%)	T4 (CA) (0.25%)	T5 (FA) (0.25%)	SEM
Crop						
Total count ($x10^4$)	19.02^a ± 2.10	9.85^b ± 1.07	7.02^c ± 1.02	3.82^d ± 0.03	4.58^c ± 0.04	5.09
Salmonella ($x10^4$)	9.32^a ± 1.12	4.02^b ± 0.05	3.42^c ± 0.02	1.82^e ± 0.01	2.25^d ± 0.01	2.41
E. coli ($x10^4$)	2.55^a ± 0.02	2.42^b ± 0.15	1.82^c ± 0.01	1.02^e ± 0.01	1.22^d ± 0.01	0.04
Staphylococcus ($x10^3$)	24.73^a ± 2.52	11.99^b ± 1.98	8.52^c ± 1.89	5.12^d ± 0.87	5.89^e ± 0.88	4.52
Gizzard						
Total count ($x10^3$)	11.12^a ± 1.66	1.28^c ± 0.02	1.48^b ± 0.03	1.28^c ± 0.02	1.48^b ± 0.03	3.09
Salmonella ($x10^3$)	5.35^a ± 0.06	0.65^d ± 0.008	0.75^c ± 0.008	0.65^d ± 0.008	1.25^b ± 0.01	1.01
E. coli ($x10^3$)	2.50^a ± 0.13	0.30^c ± 0.03	0.47^b ± 0.04	0.30^c ± 0.25	0.27^c ± 0.02	0.11
Staphylococcus ($x10^3$)	1.98^a ± 0.04	0.15^c ± 0.002	0.17^c ± 0.002	0.15^c ± 0.002	0.18^b ± 0.002	0.02
Duodenum						
Total count ($x10^3$)	13.88^a ± 2.05	1.18^d ± 0.001	2.05^b ± 0.03	1.58^c ± 0.03	1.52^c ± 0.03	2.11
Salmonella ($x10^3$)	1.85^a ± 0.03	0.35^d ± 0.01	0.95^b ± 0.02	0.75^c ± 0.02	0.71^c ± 0.02	0.02
E. coli ($x10^3$)	1.05^a ± 0.02	0.32^d ± 0.006	0.55^b ± 0.03	0.45^c ± 0.03	0.42^c ± 0.03	0.04
Staphylococcus ($x10^3$)	2.21^a ± 0.01	0.19^c ± 0.05	0.30^b ± 0.02	0.25^{bc} ± 0.02	0.23^c ± 0.02	0.02

Table 5*: Effect of organic acid treated drinking water on bacterial load at the foregut of broiler chickens abcd: Means along the same row with different superscripts are significantly ($p < 0.05$) different. CON: Control; AA: Acetic Acid; BA: Butyric Acid; CA: Citric Acid; FA: Formic Acid.*

Segments	T1 (CON)	T2 (AA)	T3 (BA)	T4 (CA)	T5 (FA)	SEM
Ileum						
Total count (x10^4)	$1.82^a \pm 0.03$	$1.18^b \pm 0.03$	$0.22^c \pm 0.001$	$0.31^c \pm 0.002$	$0.25^c \pm 0.002$	0.03
Salmonella (x10^3)	$2.72^a \pm 0.03$	$0.55^d \pm 0.004$	$1.02^c \pm 0.01$	$1.38^b \pm 0.01$	$1.18^c \pm 0.01$	0.02
E. coli (x10^3)	$1.52^a \pm 0.02$	$0.32^d \pm 0.007$	$0.62^c \pm 0.007$	$0.75^b \pm 0.008$	$0.62^c \pm 0.007$	0.01
Staphylococcus (x10^3)	$3.05^a \pm 0.08$	$1.96^b \pm 0.04$	$0.36^c \pm 0.004$	$0.53^c \pm 0.006$	$0.42^c \pm 0.005$	0.03
Caecum						
Total count (x10^4)	$6.45^a \pm 1.12$	$5.72^b \pm 1.04$	$5.42^c \pm 1.03$	$5.35^c \pm 1.03$	$5.55^c \pm 1.03$	0.78
Salmonella (x10^4)	$3.38^a \pm 0.87$	$1.92^b \pm 0.09$	$1.28^c \pm 0.63$	$1.18^c \pm 0.43$	$1.13^c \pm 0.07$	0.09
E. coli (x10^4)	$3.52^a \pm 0.03$	$3.12^b \pm 0.03$	$2.95^c \pm 0.03$	$2.92^c \pm 0.03$	$3.12^b \pm 0.03$	0.12
Staphylococcus (x10^3)	$6.65^a \pm 1.43$	$3.75^b \pm 0.93$	$2.64^c \pm 0.71$	$2.26^d \pm 0.68$	$1.16^e \pm 0.52$	0.12
Large intestine						
Total count (x10^4)	$2.55^a \pm 0.08$	$1.05^c \pm 0.06$	$1.15^b \pm 0.07$	$0.95^d \pm 0.09$	$1.05^c \pm 0.09$	0.03
Salmonella (x10^3)	$11.17^a \pm 2.33$	$7.87^b \pm 1.07$	$7.67^b \pm 1.07$	$7.50^b \pm 1.07$	$7.00^b \pm 1.05$	1.08
E. coli (x10^3)	$2.45^a \pm 0.08$	$2.15^{ab} \pm 0.08$	$2.02^b \pm 0.07$	$2.18^{ab} \pm 0.07$	$2.15^{ab} \pm 0.07$	0.07
Staphylococcus (x10^3)	$4.74^a \pm 0.98$	$1.69^c \pm 0.06$	$2.21^b \pm 0.08$	$1.83^{bc} \pm 0.06$	$2.03^{bc} \pm 0.08$	0.08

Table 6*: Effect of organic acid treated drinking water on bacterial load at the hindgut of broiler chickens.*
abcd: Means along the same row with different superscripts are significantly ($p < 0.05$) different.
CON: Control; AA: Acetic Acid; BA: Butyric Acid; CA: Citric Acid; FA: Formic Acid.

Overall, the organic acids showed different capacities to reduce bacteria populations in different gut environment. In the crop for instance, formic acid had greater capacity than butyric acid which in turn was better than acetic acid. In the gizzard, acetic and citric acids showed more capacity than others. In the duodenum it was acetic acid followed by both citric and formic acids while it was acetic, butyric and formic acids in the ileum. Butyric, citric and formic acids were better in caecum while in the large intestine there were no clear-cut capability of the acids to reduce bacteria population.

The result of bacteria population indicated that the organic acids in question were antibacterial in nature according to Paul., *et al* [22]. The capability of the organic acids to reduce bacteria population differently at various segments of the gut could be exploited. For instance, acetic acid could be fed if the target is to reduce bacteria population in the duodenum. Furthermore, it calls for feeding of more than one of such organic acids to take care of different segments of the gut. Similar bacteria load observed in the large intestine between acetic acid, citric acid, formic acid and the control could be that the concentration of the acids did reduce in the course of travelling down the gut according to Leeson., *et al* [6]. The ability of butyric acid to reduce the number of bacteria in the large intestine could be as a result of probable fermentation which could have occurred. Paap [23] noted that butyric acid was produced in the hindgut by fermentation. Organic acids have been recommended as water sanitizer and gut acidifier to reduce bacteria load [10,12,18,20,24].

Conclusion

The results of this work clearly showed that the test organic acids were antibacterial, had no deleterious effects on the haematological and blood chemistry parameters. Therefore, they could be incorporated into drinking water for broiler chickens.

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Bibliography

1. Pearson AD., *et al*. "Colonization of broiler chickens by waterborne *Campylobacter jejuni*". *Applied Environmental Microbiology* 59 (1993): 987-996.
2. Choct M. "Managing gut health through nutrition". *British Poultry Science* 50.1 (2009): 9-15.
3. Ndelekwute EK., *et al*. "Challenges Associated with Utilization of Palm Kernel Cake (PKC) in Poultry Nutrition and Feed Additive Applications for their Mitigation". *EC Veterinary Science* 4.5 (2019): 301-307.
4. Lee KW., *et al*. "Growth performance, intestinal viscosity, fat digestibility and plasma cholesterol in broiler chickens fed a rye-containing diet without or with essential oil components". *International Journal of Poultry Science* 3.9 (2004): 613-618.
5. Dibner J. "Organic acids: Can they replace antibiotic growth promoters". *Feed International* 25.12 (2004): 14-16.
6. Leeson S., *et al*. "Effect of butyric acid on the performance and carcass yield of broiler chickens". *Poultry Science* 84 (2005): 1418-1422.
7. Gao J., *et al*. "Effects of yeast culture in broiler diets on performance and immunomodulatory functions". *Poultry Science* 87 (2008): 1377-1384.
8. Ndelekwute EK., *et al*. "Organic acids as feed additives in pig and poultry diets. A Review". *Nigeria Agricultural Journal* 43 (2012): 27-38.
9. Ndelekwute EK. "Effect of finisher diets treated with organic acids on carcass and internal organs of broiler chickens". *Nigeria Journal of Animal Production* 40.1 (2013): 224-231.
10. Marco Q. "Water quality and broiler performance". Broiler Supervisor's Short Course. Bulletin, New Carolina, USA (2008): 11-13.
11. Ndelekwute EK., *et al*. "Effect of organic acid treated drinking water on growth of broiler chickens". *Nigeria Journal of Animal Production* 41.2 (2014): 116-123.
12. Alshawabkeh K. and Tabbaa, J. "Using dietary propionic acid to limit *Salmonella gallinarum* colonization in broiler chicks". *Asian-Australian Journal of Animal Science* 15.2 (2002): 243-246.
13. Fawole MO and Oso BA. "Laboratory Manual of Microbiology". Spectrum Books Ltd., Nigeria (1988): 60-65.
14. Buchanan RE and Gibbons NE. "Manual of Determinative Microbiology". Borthware: Williams and Williams Co. USA (1975): 225-230.
15. Ochi J and Kochatkar A. "Medical Laboratory Science: Theory and Practical". Tata Mac - Graw Hill Pub. Co. India (2008): 115-120.
16. Steel RGD and Torrie JH. "Principles and Procedures of Statistics. A Biometrical Approach". 2nd edition. McGraw-Hill, New York, USA. (1980): 20-90.
17. Hernandez F., *et al*. "Influence of two plant extracts on broilers performance, digestibility and digestive organ size". *Poultry Science* 83 (2006): 169-174.

18. Oviedo EO. "Important factors in water quality to improve broiler performance". Cooperative Extension Service Bulletin, North Carolina, USA, (2006): 15-19.

19. Banerjee GC. "A Text Book of Animal Husbandry". Oxford and IBH Publishing Company (2008): 446-450.

20. Chaveerach P., *et al*. "Effect of organic acids in drinking water for young broilers on campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes". *Poultry Science* 83 (2004): 330-334.

21. Morris H. Ten causes of elevated liver enzymes (2016).

22. Paul SK., *et al*. "Effect of combination of organic acid salts as antibiotic replacer on the performance and gut health of broiler chickens". *Livestock Research for Rural Development* 19.11 (2007): 52-61.

23. Paap P. "Butyrate and its multifarious effects".

24. Waldroup A., *et al*. "Performance characteristics and microbiological aspects of broilers fed diets supplemented with organic acids". *Journal of Food Protection* 58.5 (1995): 482-489.

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