An implication of apple cider vinegar administration on the hematology, serum biochemistry, and liver histology of broiler chickens

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ABSTRACT

Background and Objectives: Organic acids (OA) have been discovered to possess antimicrobial properties, thus resulting in growth improvement in animals. Apple cider vinegar (ACV) contains a blend of OAs in addition to its vitamins and minerals constituent. The aim of this study was to evaluate the effect of intermittent usage of ACV as an alternative growth promoter on the hematology, serum biochemistry, and liver histology of broiler chickens.

Methodology: In a 6-week study, 300 Arbor Acres broiler chicks were randomly distributed into 6 treatments of 50 birds, which were replicated 5 times to contain 10 birds per replicate; this was arranged in a 2 × 3 factorial layouts using a completely randomized design. Birds in 4 treatments were offered graded doses of ACV in their water (5 or 10 mL/L) for either 2 or 3 consecutive days/week, while the birds in the 2 control treatments were administered antibiotics (enrofloxacin).

Main Results: All hematological parameters measured were not significant except the heterophil, which increased with increased dosages of ACV. From the interactive effect of dosage and frequency of administration of ACV, the highest and lowest values of heterophil were obtained from birds offered 10 mL/L ACV thrice weekly and 5 mL/L ACV thrice weekly, respectively. Among the serum parameters, aspartate aminotransferase significantly reduced with a higher administration frequency of ACV, while alanine aminotransferase (ALT) was higher in birds given ACV twice (30.70 ± 3.83 U/L) and thrice weekly (31.15 ± 3.83 U/L) compared to birds on antibiotics (15.10 ± 3.85 U/L). Also, the interactive effect of dosage and frequency of administration of ACV showed that birds offered 10 mL/L ACV twice weekly (37.40 ± 5.41 U/L), and one of the groups offered antibiotics (14.60 ± 5.41 U/L) had the highest and lowest ALT values, respectively. Creatinine level was not significant across all treatments. Results of the liver histology revealed that birds offered 10 mL/L ACV had slight necrosis of the hepatocytes and liver parenchyma.

Conclusions: This study discovered that ACV, an organic growth promoter, should be used at a lower dose and in discontinuous frequency for broiler chickens to prevent possible negative impacts on their liver.

Keywords: Antibiotics, organic acid, blood profile, growth promoter

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INTRODUCTION

Apple cider vinegar (ACV) is one of the rich sources of organic acids. The highest concentrations of organic acid and phenolic agent in ACV are acetic acid and chlorogenic acid, respectively (Natera *et al.,* 2003; Budak *et al.,* 2011). Acetic acid is a short-chain fatty acid (SCFA) notable for its bactericidal activity (Allahdo *et al.,* 2018). These SCFAs also act as energy sources for the intestinal epithelial cells. The epithelial cell plays an important role in food digestion, nutrient absorption, and maintenance of healthy relationships between gut microbiota and the host (Ferket *et al.,* 2005). Other beneficial constituents of ACV include amino acids, enzymes, minerals, and vitamins (Kalaba *et al.,* 2019). Organic acids have been used in poultry production as an alternative to antibiotics and seem to elicit a positive response in growth performance (Kopecký *et al.,* 2012). A number of studies on poultry have suggested that organic acids affect the concentration of bacteria in the caeca and small intestine (Fouladi *et al.,* 2018); have bactericidal action against *Salmonella* in the crop (Thompson and Hinton, 1997); impact the intestinal morphology (Allahdo *et al*., 2018); and as well influence blood parameters (Nosrati *et al.,* 2017). There still exists a dearth of information in the literature on the influence of organic acid on the liver of chickens.

Blood acts as a pathological reflector of the status of an animal exposed to toxicants and other conditions (Olafedehan *et al.,* 2010), and its constituents change in relation to the physical health status of the animal (Togun *et al.,* 2007). The liver is involved in several metabolic processes. Thus, any factor that changes its physiology may induce liver damage, which may consequently affect the entire body function (Rocha *et al.,* 2013). Biochemical tests such as serum enzyme tests of alanine aminotransferase (ALT) may be used to detect liver damage and diseases (Raval *et al.,* 2019). An increase in the activities of these enzymes in the plasma or serum of animals has been suggested to result from damage to various tissues (Sanchez *et al.,* 2002). However, due to reports of low specificity of ALT as a biomarker for liver injury (Okubo *et al.,* 2013), histopathology of the liver can be done to better interpret ALT values (Sikandar, 2018).

In alternative medicine, the establishment of appropriate dosage remains one of the areas of concern. Although, broiler chickens have been observed to be very tolerant of a wide range of acidic water pH (Watkins *et al.,* 2004). However, it is expected that there would be a maximum level that can be tolerated by the birds. In an attempt to promote the positive welfare of broiler chickens that are offered organic acids or products containing it, this study was carried out to investigate the possible implication of varying concentrations of apple cider vinegar (ACV) on hematology parameters as well as liver and kidney function test (both serum enzyme analysis and liver histology).

MATERIALS AND METHODS

Birds, Housing, and Rearing Condition

This research was conducted at the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The climate is tropical humid with a mean annual rainfall of 1,037 mm, mean temperature of 31 ± 3 °C, and relative humidity of 81%. All procedures used in the experiment strictly adhered to the research ethics guidelines of the Federal University of Agriculture, Abeokuta (FUNAAB, 2016), with animal use protocol number (APH 06-05/21). A total of 300-day-old broiler chicks (Arbor Acres strain) were randomly distributed on a weight equalization basis into 6 treatments of a deep litter system. Brooding was done for 2 weeks. Vaccination against infectious bursal disease and Newcastle disease was done on days 7, 14, 21, and 28. Commercial starter and finisher diets containing 2,800 kcal/kg ME with 21% crude protein, and 2,900 kcal/kg ME with 18% crude protein, respectively, were offered *ad libitum* to the birds. The commercial diet (TOPFEEDS® broiler starter and finisher mash) is a product of Premier Feed Mills Company Limited (a subsidiary of Flour Mills of Nigeria Plc.), located in Ibadan, Oyo State, Nigeria. The nutrient composition of both diets is shown in Table 1.

Table 1 Calculated nutrient composition of the commercial diet as declared by the manufacturer[1]

Ingredient	Starter (%)	Finisher (%)
Crude protein	21.00	18.00
Fats and oil	6.00	6.00
Crude fiber	5.00	6.00
Calcium	1.00	1.00
Available phosphorus	0.45	0.40
Lysine	1.00	0.86
Methionine	0.50	0.30
Salt (NaCl)	0.30	0.30

Note: [1]The commercial diet (TOPFEEDS® broiler starter and finisher mash) is a product of Premier Feed Mills Company Limited (a subsidiary of Flour Mills of Nigeria Plc.), located in Ibadan, Oyo State, Nigeria.

Experimental Design

The experiment was a 2 × 3 factorial arrangement (2 dosages and 3 frequencies of administration). Birds were randomly assigned to each of the six treatments in a completely randomized design. Treatment consists of two levels (5 and 10 mL/L) dosage of ACV and three levels (zero, twice, and thrice weekly) frequency of administration of ACV. The experimental layout is as described:

T1: 5 mL/L ACV zero times a week (control treatment for 5 mL/L ACV group)

T2: 5 mL/L of ACV per liter of drinking water twice weekly

T3: 5 mL/L of ACV per liter of drinking water thrice weekly

T4: 10 mL/L ACV zero times a week (control treatment for 10 mL/L ACV group)

T5: 10 mL/L of ACV per liter of drinking water twice weekly

T6: 10 mL/L of ACV per liter of drinking water thrice weekly

The antibiotic was given to the control groups. Each treatment was replicated 5 times with 10 birds per replicate. Store-bought commercially produced organic, unfiltered ACV and a broad-spectrum antibiotic containing 20% enrofloxacin and bromhexine hydrochloride solution were used in this study. The administration of the ACV was from day 2 of brooding till the 6th week of the experiment.

ACV pH Test

The pH of raw and diluted ACV was determined before the experiment using a pH-108A pocket-sized pH meter. The raw ACV had a pH of 3.4, while dilution at 5 mL/L of drinking water and 10 mL/L of drinking water resulted in a pH of 4.60 and 4.85, respectively.

Blood Analysis

At 6 weeks of age, blood samples were collected from 12 birds per treatment into ethylene diamine tetra acetic acid and plain sample bottles for hematological and serum biochemical analysis, respectively. All hematological parameters were analyzed according to the methods described by Dacie and Lewis (1991). Creatinine level was analyzed using the colorimetric method (NCCLS, 1990), while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined spectrophotometrically according to the method of Reitman and Frankel (1957).

Histopathological Examination of the Liver

In the 6th week of the experiment, two (2) birds per treatment were selected, sacrificed, and subjected to necropsy to collect liver samples for histology examination. The liver sections were placed in buffered formalin at 10% for further processing, which was done using the method described by Avwioro (2010).

Statistical Analysis

Data obtained were analyzed as a 2 × 3 factorial analysis of variance using the general linear model (GLM) procedure of MINITAB (2018) statistical software, and all significant means were separated using Tukey Krammer's Test of the same statistical package at $P < 0.05$ significant level.

RESULTS AND DISCUSSION

The main effects of dosage and frequency of administration of ACV on hematological parameters of broiler chickens at 6 weeks of age are presented in Table 2. Only the heterophil was significantly ($P < 0.05$) affected by the dosage of ACV. Birds on 10 mL/L had a higher heterophil value (42.47 ± 1.70%), while birds on 5 mL/L ACV had a lower value (37.10 ± 1.71%). All hematological parameters were not significantly ($P > 0.05$) affected by the frequency of administration of ACV. Likewise, Table 3 shows the interactive effects of dosage and frequency of administration of ACV on hematological parameters of broiler chickens at 6 weeks of age. Only the heterophil value was also significantly ($P < 0.05$) affected. The heterophil ranged from 34.10 ± 2.95% to 47.20 ± 2.95%, with the highest and lowest values obtained from birds offered 10 mL/L ACV thrice weekly and 5 mL/L ACV thrice weekly, respectively. The non-significant effect of ACV and antibiotics administration on all hematological parameters measured except heterophil showed that ACV had a similar impact as antibiotics on the blood and immune status of broiler chickens. According to Bounous and Stedman (2000), the normal ranges of chicken hematological parameters are 2.5–3.5 × 10^{12} L for red blood cells (RBC), 22–35% for packed cell volume, 7–13 g/dL for hemoglobin, and 12–30 × 10^{9} L for white blood cells (WBC). All values obtained for these parameters in this study were within these reference ranges. Similar to this finding was that of Khosravi *et al.* (2010), who also observed that broiler chickens' erythrocyte (RBC), leukocyte (WBC), eosinophil, and lymphocyte were not influenced by the use of propionic acid in the diet at 2 g/kg of feed. However, the heterophil percentage was also reported to be insignificant, which is in contrast to the result of this study. According to Nagatomi (2006), the alteration of heterophil is commonly attributed to an inflammatory response in gallinaceous birds. Heterophils are highly phagocytic due to the beta-defensins found in their granules and are capable of a broad spectrum of antimicrobial activity (Harmon, 1998). It was generally observed that the heterophil percentage of all birds examined during the study was above the normal reference range of 25–30% reported by Reece and Swenson (2004). This could be an indication that all the birds were challenged by an infectious agent during the period. The increased heterophil percentage with increasing ACV dosages observed in this study corroborated the findings of Nosrati *et al.* (2017), who observed that heterophil counts increased with dietary butyric acid supplementation. This could also confirm the immune-stimulating activity of ACV. In a study to evaluate the impact of a chicken's diet supplemented with a microencapsulated feed additive containing organic acids, thymol, and vanillin on immune function outside the gut by Swaggerty *et al.* (2020), heterophils isolated from chicks on the microencapsulated feed additives had higher levels of degranulation and oxidative burst responses than those isolated from chicks on the control diet and it was concluded that these feed additives might peak key immune cells, making them more functionally efficient. Furthermore, Ulsagheer Mohanad *et al*. (2019) and Lilly *et al*. (2011) reported that incorporating ACV into the water of broiler chickens led to improved immunity due to the observed action against some diseases such as Newcastle disease, infectious bursal disease, and *Salmonella*.

Table 2 Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the haematological parameters of broiler chickens at 6 weeks of age

Parameter	Dosage of ACV		P-value	Frequency of administration			P-value
	5 mL/L	10 mL/L		Zero/week	Twice/week	Thrice/week	
PCV (%)	25.80 ± 0.78	27.57 ± 0.77	0.113	26.19 ± 0.93	27.91 ± 0.97	25.95 ± 0.95	0.299
Haemoglobin (g/dL)	8.57 ± 0.26	9.21 ± 0.26	0.084	8.78 ± 0.31	9.28 ± 0.32	8.62 ± 0.32	0.326
RBC ($\times 10^{12}$/L)	2.78 ± 0.13	2.95 ± 0.13	0.334	2.68 ± 0.15	3.06 ± 0.16	2.85 ± 0.15	0.232
WBC ($\times 10^{9}$/L)	14.61 ± 2.83	17.64 ± 2.82	0.451	14.66 ± 3.38	18.68 ± 3.55	15.05 ± 3.46	0.672
Lymphocyte (%)	54.90 ± 1.88	51.33 ± 1.87	0.185	54.98 ± 2.24	51.17 ± 2.36	53.20 ± 2.30	0.509
Heterophil (%)	37.10 ± 1.71^{b}	42.47 ± 1.70^{a}	0.030	37.67 ± 2.04	41.03 ± 2.14	40.65 ± 2.09	0.458
Monocyte (%)	3.28 ± 0.39	2.33 ± 0.38	0.090	2.69 ± 0.46	2.58 ± 0.49	3.15 ± 0.47	0.672
Eosinophil (%)	3.77 ± 0.34	3.23 ± 0.33	0.267	3.27 ± 0.40	3.43 ± 0.42	3.80 ± 0.41	0.644
Basophil (%)	0.07 ± 0.05	0.00 ± 0.05	0.323	0.00 ± 0.06	0.00 ± 0.06	0.10 ± 0.06	0.375

Note: ^{a,b} Means in a row with different superscripts by factor are significantly ($P < 0.05$) different. PCV = packed cell volume, RBC = red blood cell, WBC = white blood cell.

Table 3 Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the haematological parameters of broiler chickens at 6 weeks of age

Parameters	5 mL/L ACV			10 mL/L ACV			P-value
	Zero/week	Twice/week	Thrice/week	Zero/week	Twice/week	Thrice/week	
PCV (%)	26.18 ± 1.28	27.22 ± 1.41	24.00 ± 1.34	26.20 ± 1.34	28.60 ± 1.34	27.90 ± 1.34	0.341
Haemoglobin (g/dL)	8.69 ± 0.43	9.06 ± 0.47	7.97 ± 0.45	8.87 ± 0.45	9.50 ± 0.45	9.27 ± 0.45	0.424
RBC ($\times 10^{12}$/L)	2.69 ± 0.21	3.01 ± 0.23	2.63 ± 0.22	2.68 ± 0.22	3.11 ± 0.22	3.06 ± 0.22	0.578
WBC ($\times 10^{9}$/L)	14.61 ± 4.66	13.34 ± 5.15	15.88 ± 4.89	14.70 ± 4.89	24.01 ± 4.89	14.22 ± 4.89	0.412
Lymphocyte (%)	55.45 ± 3.10	51.44 ± 3.42	57.80 ± 3.25	54.50 ± 3.25	50.90 ± 3.25	48.60 ± 3.25	0.331
Heterophil (%)	37.64 ± 2.81^{ab}	39.56 ± 3.11^{ab}	34.10 ± 2.95^{b}	37.70 ± 2.95^{ab}	42.50 ± 2.95^{ab}	47.20 ± 2.95^{a}	0.044
Monocyte (%)	3.27 ± 0.64	2.67 ± 0.71	3.90 ± 0.67	2.10 ± 0.67	2.50 ± 0.67	2.40 ± 0.67	0.598
Eosinophil (%)	3.64 ± 0.56	3.67 ± 0.61	4.00 ± 0.58	2.90 ± 0.58	3.20 ± 0.58	3.60 ± 0.58	0.953
Basophil (%)	0.00 ± 0.08	0.00 ± 0.09	0.20 ± 0.08	0.00 ± 0.08	0.00 ± 0.08	0.00 ± 0.08	0.375

Note: ^{a,b} Means in a row with different superscripts by factor are significantly ($P < 0.05$) different. PCV = packed cell volume, RBC = red blood cell, WBC = white blood cell.

Table 4 shows the main effects of dosage and frequency of administration of ACV on serum biochemical parameters of broiler chickens at 6 weeks of age. All the parameters measured were not significantly (P > 0.05) affected by the dosage of ACV. However, ALT and AST were significantly (P < 0.05) influenced by the frequency of administration of ACV. For ALT, the highest values were recorded in birds on both twice and thrice-per-week administration frequencies, while for AST, birds on ACV twice/week and thrice/week had the highest (47.15 ± 4.01 U/L) and lowest (27.75 ± 4.01 U/L) values, respectively. With regard to the interactive effects of dosage and frequency of administration of ACV on serum biochemical parameters of broiler chickens at 6 weeks of age (Table 5), all serum biochemical parameters measured except the ALT were not significantly (P > 0.05) affected by the administration of the test ingredient. The highest ALT value was obtained from birds offered 10 mL/L ACV twice weekly (37.40 ± 5.41 U/L), while the lowest value was obtained from one of the groups offered antibiotics (14.60 ± 5.41 U/L). The ALT and AST are among the relevant biomarkers of liver functions (Kasarala and Tillmann, 2016). These enzymes are also present in other organs such as the kidney, heart, and muscles, however, the ALT has been said to be found predominantly in the liver and as a result, it is more specific for the liver (Raval *et al.,* 2019). The AST activity levels observed in this study were within the normal range recommended for chickens, as the peak value according to Altmann (1979) and Meluzzi *et al.* (1992) was 230 U/L and 220 U/L respectively. However, an increased ALT level was observed in broiler chickens offered ACV, particularly those on the 10 mL/L dose. This may be an indication of hepatocellular damage caused by a higher demand for metabolism by the liver. Contrary to our findings, Nourmohammadi *et al.* (2010) reported that ALT and AST enzyme activities in broiler chickens were insignificant when fed a diet acidified with citric acid. Likewise, a reduction in ALT levels was documented for broiler chickens in response to dietary supplementation of garlic powder and phenylacetic acid (Ismail *et al.,* 2021). Although a non-invasive hepatic test, researchers have sometimes reported low specificity of ALT as a biomarker for liver injury (Okubo *et al.,* 2013) hence, the call for liver tissue examination (Sikandar, 2018).

Table 6 and Figure 1 showcase the summary of the findings from the examination of liver tissue samples from chickens in the 6th week of the study. A to F represents treatments 1 to 6 accordingly. Based on the result of the histopathological assessment of liver changes, birds offered 10 mL/L ACV twice and thrice weekly showed evidence of slight necrosis of hepatocytes and liver parenchyma. All other treatment groups showed normal morphology of the hepatocyte. According to Zhou *et al.* (2016), the parenchymal hepatocyte occupies around 70–85% of the entire liver volume. This hepatocyte plays a pivotal responsibility in detoxification, metabolism, and protein synthesis in the body system of animals. Hence, damage to the hepatocyte would impair the proper functioning of the liver. These histology results have, thus, partially justified the significant elevation observed in serum ALT level of birds on both administration frequencies of ACV, and it could be inferred that the higher ALT level observed at both ACV administration frequencies was from birds on the 10 mL/L dosage. Histology examination of the small intestine of broilers administered ACV has been reported in previous studies (Allahdo *et al*., 2018; Jahantigh *et al*., 2021) however, no previous investigation has been done on broiler chickens' liver histology post-ACV administration. Hence, findings from this study can serve as a base for future research on ACV use in broiler production.

Table 4 Main effects of dosage and frequency of administration of apple cider vinegar (ACV) on the serum biochemical parameters of broiler chickens at 6 weeks of age

Parameter	Dosage of ACV		P-value	Frequency of administration			P-value
	5 mL/L	10 mL/L		Zero/week	Twice/week	Thrice/week	
ALT (U/L)	25.50 ± 3.13	25.80 ± 3.13	0.946	15.10 ± 3.83^{b}	30.70 ± 3.83^{a}	31.15 ± 3.83^{a}	0.006
AST (U/L)	36.17 ± 3.28	36.43 ± 3.28	0.954	34.00 ± 4.01^{ab}	47.15 ± 4.01^{a}	27.75 ± 4.01^{b}	0.004
CREA (mg/dL)	0.78 ± 0.10	0.85 ± 0.10	0.616	0.96 ± 0.13	0.66 ± 0.13	0.83 ± 0.13	0.238

Note: ^{a,b} Means in a row with different superscripts by factor are significantly ($P < 0.05$) different. ALT = alanine aminotransferase, AST = aspartate aminotransferase, CREA = creatinine.

Table 5 Interactive effects of dosage and frequency of administration of apple cider vinegar (ACV) on the serum biochemical parameters of broiler chickens at 6 weeks of age

Parameters	5 mL/L ACV			10 mL/L ACV			P-value
	Zero/week	Twice/week	Thrice/week	Zero/week	Twice/week	Thrice/week	
ALT (U/L)	15.60 ± 5.41^{ab}	24.00 ± 5.41^{ab}	36.90 ± 5.41^{ab}	14.60 ± 5.41^{b}	37.40 ± 5.41^{a}	25.40 ± 5.41^{ab}	0.043
AST (U/L)	34.50 ± 5.68	45.10 ± 5.68	28.90 ± 5.68	33.50 ± 5.68	49.20 ± 5.68	26.60 ± 5.68	0.838
CREA (mg/dL)	1.01 ± 0.18	0.53 ± 0.18	0.79 ± 0.18	0.91 ± 0.18	0.78 ± 0.18	0.86 ± 0.18	0.619

Note: ^{a,b} Means in a row with different superscripts by factor are significantly ($P < 0.05$) different. ALT = alanine aminotransferase, AST = aspartate aminotransferase, CREA = creatinine.

Table 6 Histopathological changes in the liver of broiler chickens at 6 weeks of age

Liver section	A	B	C	D	E	F
Portal tract	Mild periportal infiltration of inflammatory cells as well as congested portal vein	Normal	Moderate to severe portal triaditis. Moderate periportal infiltration of inflammatory cells	Mild congestion of the portal vein	Normal	Normal
Liver parenchyma	Focal area of inflammatory cell aggregates observed	Focal area of moderate inflammatory cell aggregates observed	Focal area of moderate inflammatory cell aggregates observed	Focal area of moderate inflammatory cell aggregates observed	Area of necrotic liver cells observed	Focal area of moderate inflammatory cell aggregates observed
Sinusoids	Mild infiltration of inflammatory cells	Appeared normal	Moderate infiltration of inflammatory cells	Moderate infiltration of inflammatory cells	Moderate infiltration of inflammatory cells	Moderate infiltration of inflammatory cells
Hepatocyte	Normal morphology	Normal morphology	Normal morphology	Normal morphology		Necrosis of some hepatocytes

Note: A = control group 1, B = 5 mL/L ACV twice weekly, C = 5 mL/L ACV thrice weekly, D = control group 2, E = 10 mL/L ACV twice weekly, F = 10 mL/L ACV thrice weekly.

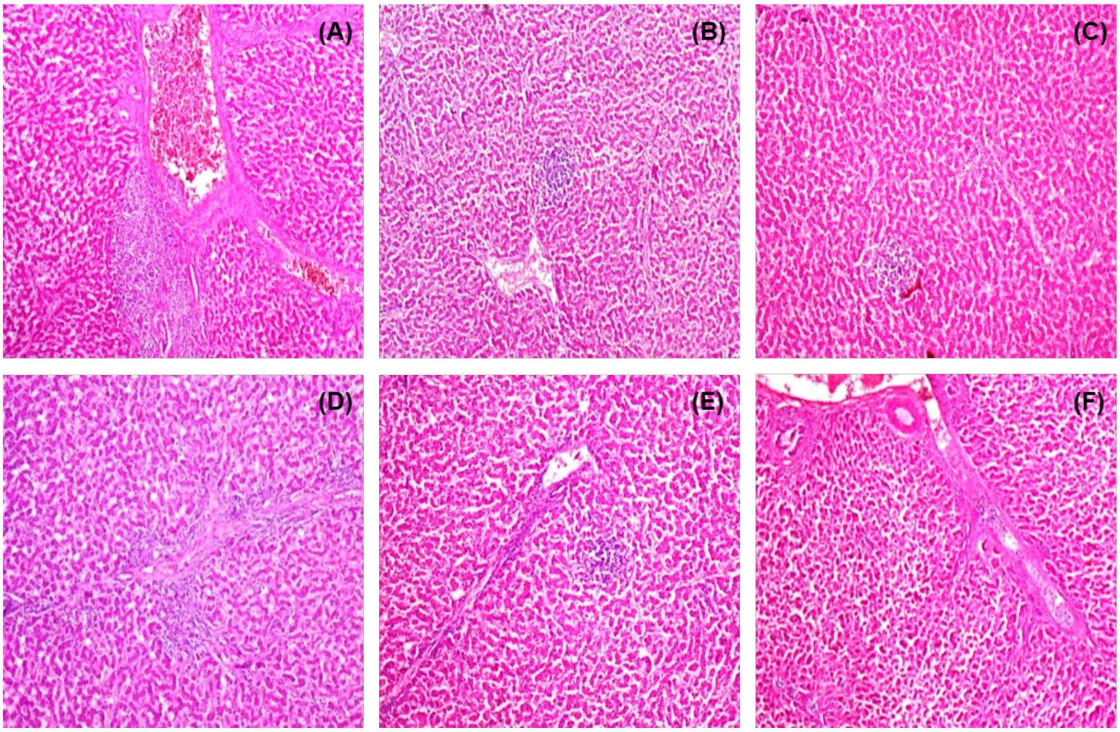

Figure 1 Photomicrograph showing liver section of broiler chickens stained with hematoxylin and eosin (H and E) in control groups and the birds administered apple cider vinegar: (A) control group 1, (B) 5 mL/L ACV twice weekly, (C) 5 mL/L ACV thrice weekly, (D) control group 2, (E) 10 mL/L ACV twice weekly, (F) 10 mL/L ACV thrice weekly (magnification × 100).

CONCLUSION

In a synthetic antibiotic-free broiler production system, farmers may consider the application of apple cider vinegar as an alternative growth promoter however, a lower dosage and administration in a discontinuous mode is encouraged to ensure that the concentration the chickens are offered do not hamper the proper functioning of their liver.

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