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*Original article*

# Anticoccidial effect of apple cider vinegar on broiler chicken: an organic treatment to measure anti-oxidant effect

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## Abstract

The objective of this study was to investigate the anticoccidial effect of apple cider vinegar added to drinking water with the anticoccidial effect of amprolium to feed broiler chicken. The study has adopted an observational approach to evaluate the anticoccidial effect of apple cider vinegar on broiler chicken. The antioxidative changes were measured adding natural apple cider vinegar to drinking water. Four hundred and fifty broiler chickens were purchased from the local market and distributed into three groups ($T_{+vc}$: positive control, $T_{-vc}$: negative control $T_v$: apple cider vinegar) with 150 chickens in each group. The three groups were further replicated into 3 blocks each containing 50 chickens. The groups were fed balanced diet, amprolium was added to the feed of positive control group, and apple cider vinegar was added to the water of $T_v$ group. Measurements of the different variables were started from week 3, at the end of each week 3 birds were chosen randomly, blood samples were collected via the wing vein, and fecal oocysts were counted from intestinal contents of each individual bird using the McMaster technique. Broiler in the control groups $T_{+ve}$ and $T_{-ve}$ showed clinical signs of coccidiosis (blood in feces) and the number of coccidial oocytes in feces increased with time. In the vinegar group, no clinical signs of coccidiosis were observed. Concentrations of total antioxidants and catalase enzyme activity significantly increased ($p \leq 0.05$); while malondialdehyde concentration significantly decreased ($p \leq 0.05$).

**Keywords:** apple cider vinegar, broilers, coccidial oocytes, feed, diet, disease

## Introduction

Coccidiosis has been attributed to the loss of about 1 to 3 billion dollars annually, on the global record within the poultry industry (Muthamilselvan et al. 2016, Cardenas et al. 2017). Avian coccidiosis is characterized as an infectious protozoan disease, caused by gut parasites of the genus *Eimeria* (Williams 2005, Muthamilselvan et al. 2016). These parasites are transmitted into the body via oral route and multiply within mucosal epithelia in different parts of the gastrointestinal tract. As a result, they may lead to the gut damage along with other conditions like inflammation, haemorrhage, diarrhoea, morbidity, and mortality in poultry. This disease annually causes a global loss of over 2.4 billion US dollars in the poultry industry, accompanied with

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setbacks of poor growth performance, replacement of chicks, and medication (Quiroz-castaneda et al. 2015). Different methods are currently being used to constrain avian coccidiosis, which may include anticoccidial chemicals, vaccines, and natural products. Anticoccidial chemicals, coccidiocides, coccidiostats, and ionophores have been used as a conventional strategy to control the avian coccidiosis in modern poultry production (Amare et al. 2012, Ritzi et al. 2014). Moreover, prophylactic in-feed medication for broilers and broiler breeders are worldwide used as a treatment measure against coccidiosis (Kitandu and Juranová 2006). This strategy is cost-effective and successful; yet the presence of drug resistance and public demands for residue-free meat still encourage the development for alternative control strategies (Amare et al. 2012).

The prophylactic use of anticoccidial chemicals in European countries as feed additives has been regulated strictly since 2006. Natural products are emerging as an attractive way to control and manage the coccidiosis. Currently, there are at least four plant products, which are available commercially in the market that can be used as anticoccidial feed additives in chickens and/or other animals (Muthamilselvan et al. 2016). The resistance of avian coccidia to drugs has been increasing dramatically. The limitation in the treatment and the rising public concern about drug remains in chicken meat have stimulated the search for new methods to control coccidiosis (Innes and Vermeulen 2006, Tan 2005, Lillehoj et al. 2007). It has been evaluated that the production of flavoured vinegar was initiated 5000 years back. The Babylonians have produced and traded vinegars in the 6$^{th}$ century, along with different flavours, which mainly include honey, malt, and fruit, medical practitioners have mainly indicated that vinegar is helpful for treating various disorders, which include high fever, stomach ache, croup, poison ivy, and oedema (Budak et al. 2014). Therefore, the anticoccidial effect of apple cider vinegar, added to broiler water, was measured in this study, along with the variations in the blood antioxidant capacity induced by adding apple cider vinegar to the broiler feed.

## Materials and Methods

### Study Setting

The research was conducted at the University of Jordan. The birds were recruited from those which were grown in an open house, on a floor system. An observational study was conducted to investigate the anticoccidial effects of apple cider vinegar on broiler chicken, and measure the subsequent anti-oxidative changes.

### Birds

A total of 600 chicks of Lohmann broiler strain were brought from local market. The birds used in this experiment were free of coccidial infection. The temperature of the skin was evaluated to investigate the intensity of strain among chickens. The congruence among temperature and skin patterns was perceived through physiological observations. From 600 chickens, a total of 450 chickens were observed with low temperature and were considered to have strain. These chickens were distributed into 3 groups (150 birds in each group), which were further divided into 3 replicates (50 birds in each). All animal handling procedures were conducted in accordance with the guidelines, set forth by the Jordanian Society for Animal Production.

### Parasitological techniques of the infectious dose

Wet smears of the mucosa were prepared from intestinal and caecal scrapings for microscopic examination of Eimeria spp. oocysts. Eimeria spp. were identified according to the site of infection and oocyst morphology including size, shape, and color after sporulation according to Alnatour (2002). After collection of the infectious dose a sample of the infectious dose was investigated, 5 Eimeria spp. were identified including E. acervulina (10%), E. brunette (13 %), E. maxima, E. necatrix (12%)(12%), and E. tenella (57%).

### Induced coccidiosis

The infectious coccidial oocysts (*Eimeria* spp.) were isolated from intestines of naturally infected chickens obtained from the local farms. The oocysts were separated using sieving and sedimentation techniques (Soulsby 1982). Oocytes were allowed to sporulate at room temperature in 2.5% potassium dichromate solution. The sporulated oocysts were cleared and counted per 1.0 ml of the solution using the McMaster technique (Soulsby 1982). The oocysts were administered orally to 14 day-old birds and the dose of infectious coccidian oocytes was 3x10$^5$/bird, given via oral administration (Arabkhazaeli et al. 2011). In Mc Master Method, the faeces were mixed with sodium chloride solution, which were then strained using sieve with an aperture of 1mm. The resultant solute was transferred to the McMaster slide and left for 5 minutes. Coccidiosis was induced on 14$^{th}$ day orally (3x10$^5$ sporulated oocysts/bird).

### Apple cider vinegar

Apple Cider Vinegar (ACV) was used by organic broiler producers. It was made by crushing apples and

Table 1. The composition of feed (g/kg) of the basal diets.

| | Starter (0-21 d) | Grower (22-35 d) | Finisher (36-49 d) |
|---|---|---|---|
| Ingredients | | ---------- | |
| Corn | 58.5 | 36.3 | 67.05 |
| Soybean meal (48% CP) | 35.65 | 31 | 26 |
| Palm oil | 1.84 | 1.79 | 3 |
| Limestone (ground) | 1 | 0.96 | 1.68 |
| Dicalcium phosphate | 0.2 | 0.2 | 1.02 |
| NaCl | 0.11 | 0.12 | 0.42 |
| DL-methionine (98%) | 0.10 | 0.1 | 0.2 |
| L-Lysine-HCL (98.5%) | 0.10 | 0.1 | 0.13 |
| Coccidiostat | 0.10 | 0.1 | -- |
| Vitamin premix | 0.1 | 0.1 | 0.1 |
| Mineral premix | 0.1 | 0.1 | 0.1 |
| Choline chloride | 0.1 | 0.1 | 0.1 |
| Antioxidant | 0.1 | 0.1 | 0.1 |
| Antifungal | 0.1 | 0.1 | 0.1 |
| Calculated nutrient composition | | | |
| ME, kcal/kg feed | 3 | 3.075 | 3.15 |
| Protein | 22 | 20 | 18 |
| TSSA (g/kg) | 0.009 | 0.0086 | 0.0081 |
| Methionine (g/kg) | 0.0054 | 0.0051 | 0.005 |
| Lysine (g/kg) | 0.0131 | 0.012 | 0.0107 |
| Therionine (g/kg) | 0.0084 | 0.0076 | 0.0068 |
| Tryptophan (g/kg) | 0.0029 | 0.0027 | 0.0023 |
| Ca (g/kg) | 0.0103 | 0.0098 | 0.0095 |
| P, nonphytate (g/kg) | 0.0045 | 0.0042 | 0.004 |
| Na (g/kg) | 0.0018 | 0.0018 | 0.0018 |

$T_v$ group: no coccidiostats drug was added (apple cider vinegar was added); $T_{-vec}$ group: no coccidiostats drug was added.

squeezing out the liquid. Bacteria and yeast were added to the liquid to start the alcoholic fermentation process. During alcoholic fermentation process, the sugars were turned into alcohol. In a second fermentation process, the alcohol was converted into vinegar by acetic acid forming bacteria (acetobacter). Acetic acid and malic acid gave vinegar its sour taste. Unpasteurized or organic apple cider vinegar has a cobweb-like appearance and can make the vinegar look slightly congealed (5% concentrated). Other constituents of vinegar included polyphenolic compounds, vitamins, minerals, mineral salt, amino acids, and organic acids (Omar et al. 2015). The apple cider vinegar was chemically analysed and its pH, density, and content of organic acids were evaluated before administrating it to the chickens. The pH of apple cider vinegar was evaluated to be 2.5, density was 1.02 grams/ml; whereas the content of organic acids were within normal limits.

## Experimental Design and Treatments

Three treatments ($T_{-vec}$, $T_{+vec,}$ $T_v$) with three replicates were allocated to the birds. The apple cider was added to the drinking water of the chickens in the vinegar group $T_v$ as follows:

- 1-2 weeks: 10 ml/litter of water,
- 3-4 weeks: 20 ml/litter of water,
- 5-7 weeks: 30 ml/litter of water.

Amprolium was added to the feed of $T_{+vec}$ group (at concentration of 1 ppm in anticoccidial drug) (El-Banna et al. 2005). Neither coccidiosis treatment nor amprolium was added to the feed for $T_{-vec}$ group. Table 1 presents the composition of feed (g/kg) of the basal diets.

The treatments ($T_{-vec}$, $T_{+vec}$, $T_v$) were arranged in a Randomized Complete Block Design (RCBD) with three replicates. A total of 9 blocks were made; however, the number of blocks represented the number of replications. The treatments were assigned within the

blocks randomly. A single treatment has been applied per block. The chickens have been grouped into blocks according to the suspected variations that isolate them. The conditions are homogenous within each block, but large differences may exist between different blocks. The faeces of chickens were evaluated to assess the number of coccidial oocytes that appear in their faeces.

### Management of birds

Broiler chicken were purchased from local market and distributed into 3 groups with 3 replicates each containing 50 chicks. All the groups were fed balanced diet, amprolium was added to the feed of the positive control group $T_{+ve}$, and apple cider vinegar was added to the water of $T_v$ group, nothing was added to the feed of the negative control group $T_{-ve}$. The infectious dose of coccidian oocytes was $3x10^5$/bird given via oral administration (Arabkhazaeli et al. 2011).

### Collecting blood samples

Blood samples were collected at the end of each week, via wing veins, using sterile gauge 19 needles and syringes. About 3.5 ml of blood used to be collected in tubes, containing ethylene diamineate acetic acid (EDTA).

### Measurements

Three birds from each replicate were randomly chosen to measure coccidial count in faeces. Blood samples from the three birds were taken to measure:

1. Total antioxidant capacity (Allarad et al. 1998): The Cell Biolabs' Creative proteomics TAC Assay Kit was used to measure the total antioxidant capacity of sample. The kit detects total antioxidant capacity through colorimetric method. This is suitable to be used with serum, cell lysates, plasma, urine, food extracts, and tissue homogenates. It also works with wide range of antioxidants.

2. The catalase enzyme activity was determined by Catalase Activity Assay Kit from Creative proteomics. It is a simple yet sensitive method to measure catalase activity of variety of biological samples. The catalase present in the sample reacts with hydrogen peroxide. However, the catalase activity present in the sample is inversely proportional to the obtained signal.

3. The malondialdehyde (an indicator of lipid peroxidation) was measured using Lipid Peroxidation (MDA) Assay Kit from Creative proteomics, USA. The kit is considered as a sensitive tool for detecting malondialdehyde. Malondialdehyde in the sample reacts with thiobarbituric acid (TBA) to produce MDA-TBA adduct, which can be easily quantified colorimetrically or fluorometrically.

Three birds per replicate were slaughtered to make investigative slides and examine the histopathological changes in the affected part of the chicken intestines. Defecation was also observed for individual chickens. A whole fecal bolus was collected and dissected after observing defecation. The samples were analyzed soon after the collection.

### Oocysts Output

The fecal samples were collected and stored at 4°C to determine the Oocyst per gram (OPG) count and perform Fecal oocyst reduction test (FORT). This method is carried on through the McMaster counting chamber technique that uses saturated NaCl as the flotation medium.

### Histopathological examination

Classical lesions were taken for the histopathological preparation. The cecum part was histopathologicaly analysis. Haematoxylin and eosin (H&E) staining was used to demonstrate the developmental stages in the cecum. Tissues sampled were fixed in 10% neutral buffered formalin, sectioned at 5-6 $\mu$m thicknesses and stained with haematoxylin eosin stain. (Kadhim et al. 2014).

### Statistical analysis

The collected data were properly coded and entered into an excel spreadsheet, which was later entered into SPSS version 17; SPSS Inc. Chicago. The data were analysed by repeated measure of analysis (RMA). Repeated measure of ANOVA has been selected as the parameter for testing. The treatments and noted variables were apparently dependent on each other. RMA is an extension of dependent t-test as it is considered ideal for the assessment. The mean values were further assessed to identify any false occurrence among the data. The differences among group means were considered significant at $p<0.05$.

## Results

In the present study, doses of natural apple cider vinegar administered in drinking water have shown anticoccidial effects against Eimeria species. The effects have been noted in terms of decreasing number of coccidial oocytes in faeces, increasing antioxidative status, and lowering concentration of malondialdehyde in the blood of broil. Coccidial oocyte count in broiler chicken is presented in Table 2. Investigations of broilers' blood indicated that no clinical signs of coccidiosis appeared in chicken infected with coccidial oocytes.

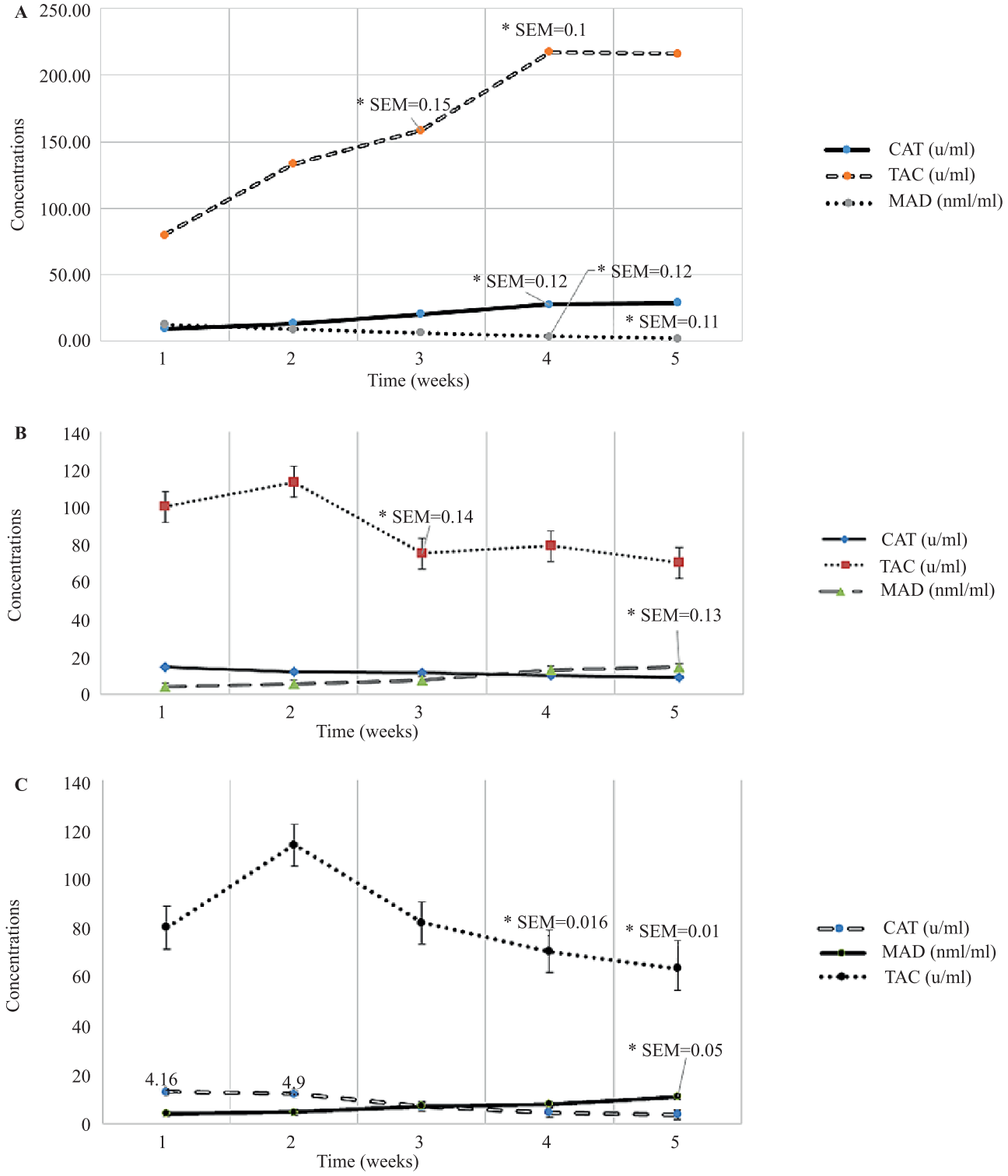


Fig. 1. (A) Total antioxidant capacity (TAC), Catalase enzyme activity (CAT) and Malondialdehyde (MAD) in blood of apple cider vinegar group; (B) Negative control group; (C) Positive control group.

Figure 1a presents total antioxidant capacity (TAC), catalase enzyme activity (CAT) and malondialdehyde (MAD) in blood of the apple cider vinegar group. Figures 1b and 1c present results in negative and positive control groups respectively. Histopathological changes in intestine of 5 and 6 weeks broiler control group are presented in Fig. 2. Sections A, C, E, and G show submucosal parasitic stages; section B shows parasitic stages in the glandular epithelium (arrows); section D shows thickening in the submucosal tissue and destruction of intestinal cells; F section presents inflammatory cells influxes into the submucosa; and sec-

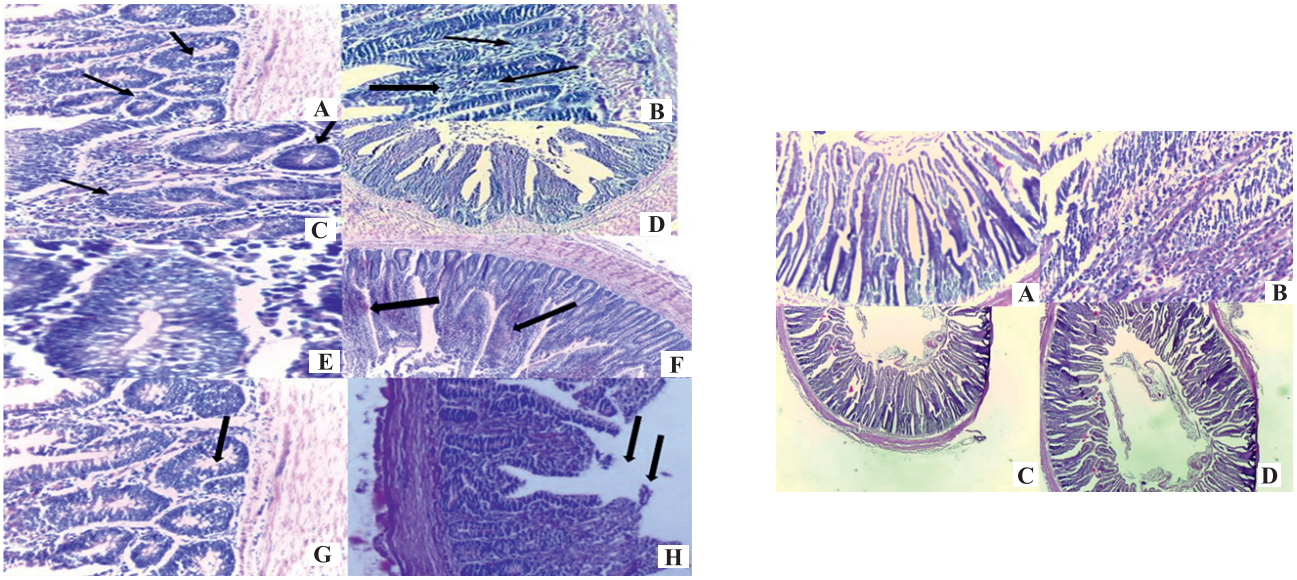


Fig. 2. (A) Histopathological changes in the intestine of 5 and 6 weeks old broiler control group, A, C, G X200, B, D, F and H X100; (B) Histopathological changes in the intestine of 5 and 6 weeks old broilers treated with apple cider vinegar, X100.

Table 2. Coccidial oocyte count in broiler chicken ($T_v$:vinegar group).

| Vinegar group $T_v$ (EPG) | | | | | |
|---|---|---|---|---|---|
| Week | Week | Std. Error | P value[a] | 95% Confidence Interval for Difference[a] | |
| | | | | Lower Bound | Upper Bound |
| 1 | 2 | 60.09 | P≤0.05 | -13879.98 | -12186.69 |
| | 3 | 116.67 | P≤0.05 | 3272.94 | 6560.39 |
| | 4 | 44.10 | P≤0.05 | 4895.40 | 6137.94 |
| | 5 | 104.08 | P≤0.05 | 4383.57 | 7316.44 |
| 2 | 3 | 150.00 | P≤0.05 | 15836.64 | 20063.36 |
| | 4 | 50.00 | P≤0.05 | 17845.55 | 19254.45 |
| | 5 | 158.99 | P≤0.05 | 16643.32 | 21123.35 |
| 3 | 4 | 100.00 | P>0.05 | -808.91 | 2008.91 |
| | 5 | 166.67 | P>0.05 | -1414.84 | 3281.51 |
| 4 | 5 | 145.30 | P>0.05 | -1713.76 | 2380.42 |

Based on estimated marginal means,
* The mean difference is significant at the 0.05 level, [a] Adjustment for multiple comparisons: Bonferroni.
Dependent variable: coccidial oocyte count egg per gram (EPG).

tion H shows sloughing in the epithelial cells (Fig. 2-I). At the same time, sections A, C, and D show normal intestinal cells; section C shows normal submucosal tissue; and B section shows no parasitic stages in the glandular epithelium. Crypt hyperplasia and increased leukocyte infiltration, which is normally found in the intestine of broiler infected with coccidiosis, were not observed in the group treated with apple cider vinegar from day 1 of the experiment (Fig. 2-II). Some of the chickens did not produce enough wet fecal material for applying the OPG counting technique. The chickens were capable of ingesting oocyts from an infected cage, despite of range in oocyts output when the chickens were spray inoculated.

An increase in the number of coccidial oocytes was observed in faeces of the control group. On the other hand, a significant decrease (p≤0.05) in the number of coccidial oocytes in faeces of broiler treated with apple cider vinegar has been found. Moreover, Table 2 shows a significant increase (p≤0.05) in the catalase enzyme activity in blood of broilers treated with apple cider vinegar; whereas the catalase enzyme activity displays a decrease in the control group (Fig. 2). Tables 3 and 4 show coccidial oocyte count in broiler chickens in the control group and within the group that was administered with apple cider vinegar. Figure 2 also shows a significant increase (p≤0.05) in the total antioxidant capacity in blood of broilers treated with apple cider

Table 3. Coccidial oocyte count in broiler chicken ($T_{+v}$epositive control group).

| Control group $T_{+ve}$ (EPG) | | | |
|---|---|---|---|
| | | 95% Confidence Interval for Difference[a] | |
| Std. Error | P value.[a] | Lower Bound | Upper Bound |
| 3041.38 | P≤0.05 | -78350.16 | 7350.16 |
| 1922.09 | P≤0.05 | -66413.80 | -12252.86 |
| 1589.90 | P≤0.05 | -70233.49 | -25433.18 |
| 927.96 | P≤0.05 | -75740.75 | -49592.58 |
| 1691.48 | P>0.05 | -27664.70 | 19998.04 |
| 3929.94 | P>0.05 | -67702.47 | 43035.81 |
| 2166.67 | P>0.05 | -57692.94 | 3359.60 |
| 3329.16 | P>0.05 | -55404.75 | 38404.75 |
| 1452.97 | P≤0.05 | -43804.24 | -2862.42 |
| 1922.09 | P>0.05 | -41913.80 | 12247.14 |

Based on estimated marginal means,
* The mean difference is significant at the 0.05 level. [a] Adjustment for multiple comparisons: Bonferroni.
Dependent variable: coccidial oocyte count egg per gram (EPG).

Table 4. Coccidial oocyte count in broiler chicken (T-ve:negative control group).

| T-ve control group (EPG) | | | |
|---|---|---|---|
| | | 95% Confidence Interval for Difference[a] | |
| Std. Error | P value.[a] | Lower Bound | Upper Bound |
| 3041 | P≤0.05 | -78351 | 7350 |
| 1922 | P≤0.05 | -66414 | -12253 |
| 1590 | P≤0.05 | -70234 | -25434 |
| 928 | P≤0.05 | -75741 | -49593 |
| 1691 | P>0.05 | -27665 | 19998 |
| 3930 | P>0.05 | -67703 | 43035 |
| 2167 | P>0.05 | -57693 | 3359 |
| 3329 | P>0.05 | -55405 | 38404 |
| 1453 | P≤0.05 | -43805 | -2863 |
| 1922 | P>0.05 | -41914 | 12247 |

Based on estimated marginal means,
* The mean difference is significant at the 0.05 level. [a] Adjustment for multiple comparisons: Bonferroni.
Dependent variable: coccidial oocyte count egg per gram (EPG).

vinegar; whereas the total antioxidant capacity in blood of broiler control group shows a decrease (Fig. 1). Figure 2 shows a significant decrease (p≤0.05) in the malondialdehyde concentration in blood of the broiler control group whereas the malondialdehyde concentration in blood of the apple cider vinegar group depicts a decrease.

## Discussion

The present findings are supported by the reported by Abbas et al. (2011) and Nidullah et al. (2010). These researchers have stated that the lower dose of hydrochloric acid (HCl) has the potential to be used as an alternative to the chemotherapeutic drugs for *Eimeria tenella* control. Apple cider vinegar also contains other group of acidsincluding citric, formic, lactic, malic, and succinic acids (Budak et al. 2014). The main histopathological lesions were observed in the control group, which were similar to those observed by McDougald and Reid (1997) Associated with the infection induced by *E. tenella* were very severe. The general effects included changes in the morphology of the villi. The pathological changes were mainly due to the second generation schizonts (Soulsby 1982). Parasites in various stages of development were located throug-

hout the mucosa. Epithelial cell necrosis was more severe, when there were massive accumulations of schizonts with merozoites, marked proliferation of epithelial cells of crypts, and foci of intense mononuclear infiltrates in the submucosal membrane. Moreover, multifocal and discrete interstitial edema at the submucosal membranes was associated with various intralesional forms of the parasite within epithelial cells. (Shirley et al. 2005, Patra et al. 2009, Adamu et al. 2013).

The main histopathological lesions were observed in the $T_v$ group and they were mild (Fig 2). It has been characterized principally by the inflammatory cell influxes that the submucosa along with the thickening of mucosal and submucosal layer showed slight congestion of blood vessel necrosis and thickened epithelial mucosa (Kadhim 2014). It is advised to use apple cider vinegar, which is commercially available for the prevention and treatment of coccidiosis (Quiroz-castañeda and Dantán-gonzález 2015). Nidaullah (2010) utilized aqueous extracts from different medicinal plants, which have been reported to cause a reduction in oocysts count ($p<0.05$) with increased concentration of herbal plants in the respective recipe. Similar approach has been applied by Tipu et al. (2002) who fed Neem fruit to broilers and observed reduction in coccidial oocysts count per gram of feces.

Anticoccidial drug development has increased due to urgent need to control this disease. Several strategies have been used to treat or prevent the occurrence of coccidiosis. Moreover, new alternatives to these drugs were emerging, where some of these alternatives were being obtained from the plants. An advantage of using natural extracts like apple cider vinegar, is the approach of lowering the risk of developing resistance towards drugs. Also, the residues of such natural products in meat are friendly to human consumers and may have no adverse effects on their health. This research has proven that medicinal herbal alternatives like natural apple cider vinegar, can be used to prevent and treat infectious diseases in broiler chicken like coccidiosis.

## Limitations

The study results cannot be considered accurate because a small population of chickens has been recruited. Only three replicate of chickens were given the three diets. The most important measurement of the experiment was that of the coccidial counts and only three birds were used for this. The number of coccidial oocytes considered in the study is not sufficient to conclude the anticoccidial effect. The vinegar was administered to the chickens through water and coccidial oocytes were administered orally, but the water and feed intakes of the chickens were not monitored.

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