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Original article

The role of high-grade Bentonite powder in coccidiosis and its effects on feed conversion ratio and blood parameters in broiler chicken

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Abstract

The aim of this study was to evaluate the effect of high doses of calcium bentonite on the blood parameters, anticoccidial activity and intestinal histology of broiler chickens. Three hundred and sixty one-day old broilers were distributed into three treatments (T_{+VE}, T_{-VE}, T_B) with three replicates. Amprolium was added to the feed of the positive control group, calcium bentonite powder was added to the T_B group, and nothing was added to the feed of the T_{-VE} group. Coccidiosis was induced on day 14, the birds were kept until day 49, measurements of the different variables started from week 3, blood samples were collected via wing vein, and fecal oocysts were counted from the intestinal contents of each individual bird using the McMaster technique. A decrease in feed consumption, body weight gain and conversion ratio was noticed in the calcium bentonite group.

Broilers in the calcium bentonite group (T_B) and negative control group (T_{-VE}) showed clinical signs of coccidiosis (blood in feces) and the number of oocysts in feces increased with time. Histopathological examinations of the affected caeca also demonstrated excessive tissue damage, hemorrhage, the presence of clusters of large schizonts and merozoites in the tissue, and coccidian oocysts in the lumen. Feed conversion was highest in the T_{+VE} group.

Key words: broiler, coccidiosis, calcium bentonite, poultry, triglycerides, protein

Introduction

Bentonite is a clay that eliminates the growth and development of a fungoid mold. The main specific feature e of bentonite clay is adsorption and the exchange of cations (Chkuaseli et al. 2016). Bentonites are white, light weight rock deposits composed mostly of salts of hydrated aluminosilicates of sodium (Na), potassium (K), calcium (Ca), and occasionally iron, magnesium, zinc, and nickel (Khander et al. 2012). The use of clay-based adsorbents has proved effective at reducing the toxic

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effects of aflatoxin-contamination in animal feeds (Fowler et al. 2015).

Coccidiosis is the most common and costly disease of the poultry industry, caused by apicomplexan parasites belonging to the genus *Eimeria* that develop in the epithelial cells of the gut. The poultry industry cannot be viable without specific prophylaxis based mainly on the use of in-feed anticoccidial drugs known as coccidiostats. Nowadays, in Europe, 12 commercial products containing chemicals and ionophores are accepted. Their extensive use has led to the development of drug resistance (Györke et al. 2016).

In Jordan, coccidiosis prevention in the broiler industry is based on coccidiostats added to feed and vaccination used only in layers and breeders (personal observation). Annual costs of coccidiosis worldwide have been estimated at two billion euro (Peek and Landman 2011).

In the poultry industry, different feed additives and growth promoters have been used to decrease the cost of production. Bentonite, a feed additive, has been used successfully without any harmful effect (Tauqir et al. 2001). The effectiveness of these additives apparently depends on their ability to bind aflatoxin in the intestine, resulting in the toxin being made unavailable for absorption (Santurio et al. 1999). Because of the unique nature of these clay feed additives, they have been increasingly incorporated into poultry diets to prevent aflatoxicosis.

Clay consumption was used for hundreds of years by animals and indigenous cultures to promote internal healing and the improvement of economic indicators and commercial use of silicate minerals is recommended as an ingredient in broiler fed. Bentonites are among the first binders to appear as supply in animal diet that contains more or less montmorillonite which is a clay of 3 sheets associated with colloidal compounds. The lubricating properties are enhanced when the montmorillonite content is elevated. Moreover, the inclusion of mineral clays as a feed additive in the broiler diet has a beneficial effect on performance, carcass yield, passage rate and chemical composition of meat (Bouderoua et al. 2016).

In this study, the effect of high-grade bentonite on feed conversion ratio, blood parameters, coccidial fecal oocyst count, and histopathological changes in the intestine is investigated.

Materials and Methods

Place of study

This research was conducted at The Jordan University. The birds were grown in an open house on floor system.

Birds

500 chicks of Lohmann broiler strain were brought from the local market and they were observed in terms of strain. The temperature of the skin was evaluated to investigate the intensity of the strain among chickens. The congruence between temperature and skin patterns was noted through physiological observations. Of the 500 chickens, 360 chickens were observed with low temperature and were considered to have strain. These chickens were distributed into three groups (120 birds in each group), which were further divided into 3 replicates (40 birds each). All animal handling procedures were conducted in accordance with the guidelines set out by the Jordanian Society for Animal Production.

Induced coccidiosis

Infectious coccidial oocysts (*Eimeria* spp.) were isolated from the intestines of naturally infected chickens, which were obtained from local farms. The oocysts were separated using sieving and sedimentation techniques according to Soulsby (1978). Oocysts 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 as described by Soulsby (1982). Fecal samples were taken from birds on day 13 and tested for the presence of oocysts to ensure that the birds were not initially infected with protozoa or worms. The sporulated oocysts were used for the induction of experimental infection of 14 day-old birds.The infectious dose of coccidian oocysts was ($3x10^5$/bird)given via oral injection) (Arabkhazaeli et al. 2011).

Experimental design and treatments

Three treatments (T_{+VE}, T_{-VE}, T_B) with three replicates/ treatment were allocated to the birds. The treatments (T_{+VE}, T_{-VE}, T_B) 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 was applied per block. The chickens were grouped into blocks according to the suspected variations that isolate them. The conditions were homogenous within each block, but large differences may exist between different blocks. The faeces of the chickens were evaluated to note the number of coccidial oocysts that appear in their faeces.

Table 1. The composition (g kg^{-1}) of the basal diets.

Ingredients	Starter (0-21 d)	Grower (22-35 d)	Finisher (36-49 d)
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-mineral premix1	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 (%)	0.9	0.86	0.81
Methionine (%)	0.54	0.51	0.5
Lysine (%)	31	1.20	1.07
Therionine (%)	0.84	0.76	0.68
Tryptophan (%)	0.29	0.27	0.23
Ca (%)	1.03	0.98	0.95
P, nonphytate (%)	0.45	0.42	0.4
Na (%)	0.18	0.18	0.18

Provided per kilogram of diet: vitamin A, 12 000 IU; vitamin D3, 1500 IU; vitamin E, 50 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; niacin, 25 mg; calcium-D-pantothenate, 12 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apo- carotenoic acid ester, 2.5 mg; colin chloride, 400 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; Se, 0.15 mg.

Management of birds

Broiler chickes were purchased from a local market and distributed into three groups with three replicates each containing 40 chickens. All the groups were fed abalanced diet, amprolium was added to the feed of the positive control group, and bentonite powder was mixed with the feed of theT_B group and nothing was added to the feed of negative control group. The infectious dose of coccidian oocysts was $3x10^5$/bird given via oral administration (Arabkhazaeli et al. 2011).

Collecting of 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 was collected in tubes, containing ethylenediamine tetraacetic acid (EDTA). Faecal samples were collected daily for the coccidial oocyst count, three birds/week per replicate were slaughtered to make investigative slides and examine the histopathological changes in the affected part of the chicken intestines.

Parasitological techniques

Wet smears of the mucosa were prepared from intestinal and caecal scrapings for microscopic examination of Eimeria spp. Eimeria spp. were identified according to the site of infection and oocyst morphology including size, shape and colour after sporulation.

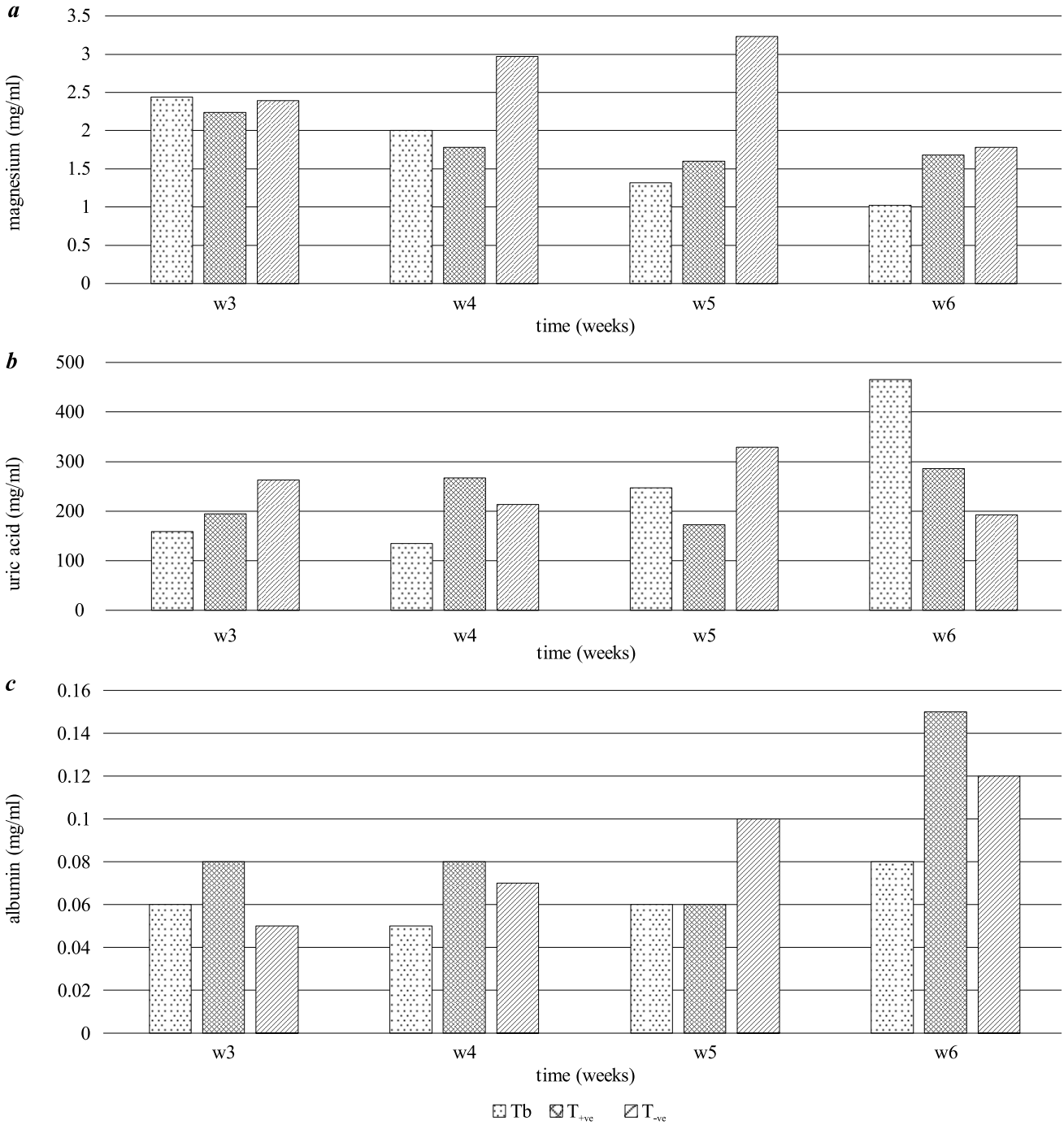

Fig. 1. Concentration of magnesium, uric acid and albumin in the blood of broiler chickens.

Five species were identified: *E. acervulina* (10%), *E. brunette* (13 %), *E. maxima* (12%), *E. necatrix* (12%), and *E. tenella* (57%) (Carvalho et al. 2011).

Measurements

- Magnesium, uric acid, albumin, cholesterol, calcium, total protein and triglycerides were measured using kits from BioSystemsusing spectrophotometry
- Feed efficiency
- Fecal oocyst count

Oocysts Output

The faecal samples were collected and stored at 4°C to determine the oocyst per gram (OPG) count and perform faecal oocyst reduction test (FORT), this method was carried out using the McMaster counting chamber technique that uses saturated NaCl as the flotation medium (Ahad et al. 2016).

Histopathological examination

Classical lesions were taken for histopathological preparation. Haematoxylin and Eosin (H&E) staining

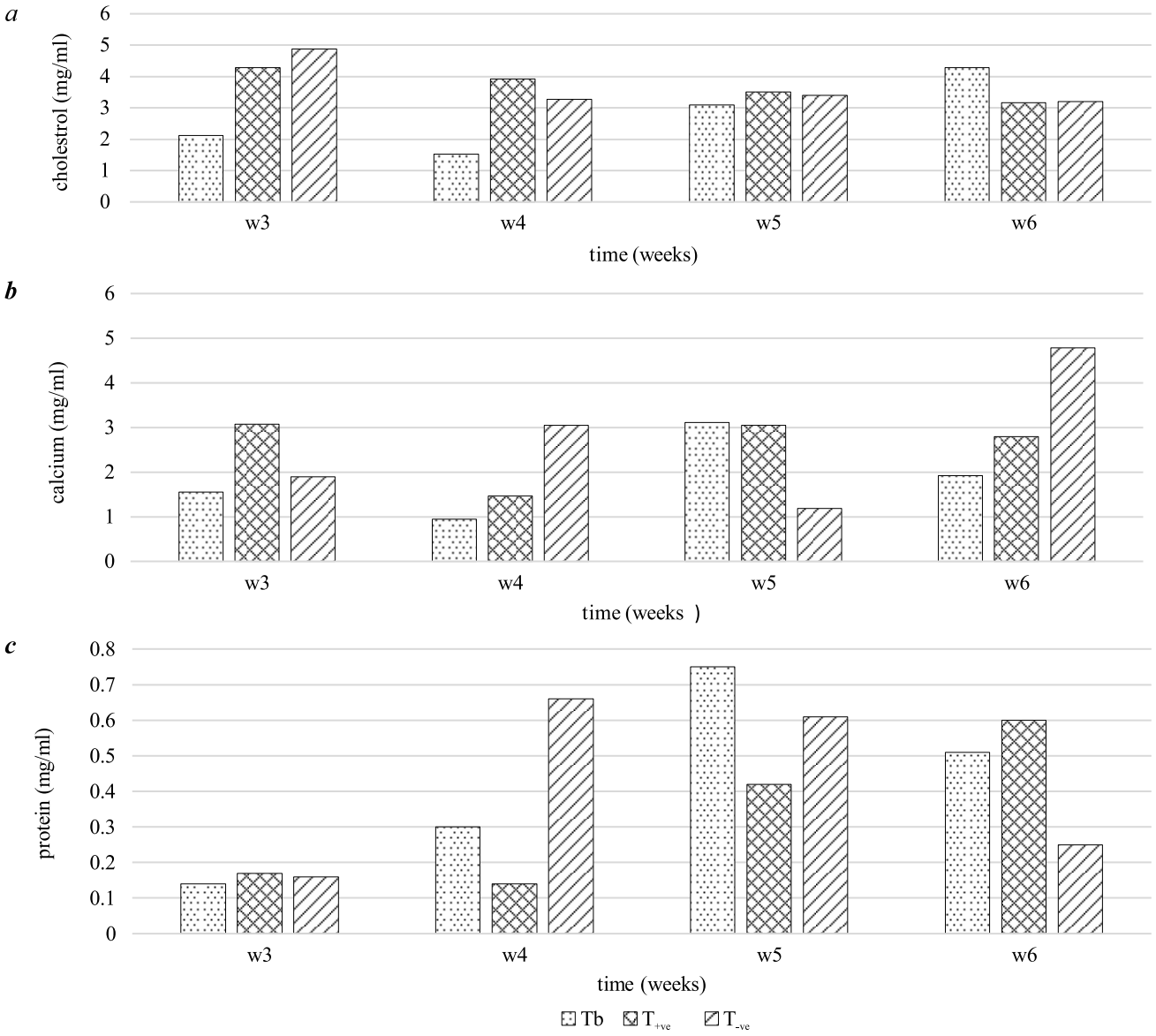

Fig. 2. Concentration of cholesterol, calcium and protein levels in the blood of broiler chickens.

was used to demonstrate the developmental stages in the cecum. Tissues sampled were fixed in 10% neutral buffered formalin, sectioned at 5-6 µm thicknesses and stained with haematoxylin eosin stain. (Kadhim et al. 2014).

Statistical analysis

The collected data were appropriately 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 was been selected as the parameter for testing. The treatments and noted variables were apparently dependent on each other. RMA is an extension of the dependent t-test and is considered ideal for such assessment. The mean values were further assessed to identify any false occurrences among the data. The differences among group means were considered significant at $p<0.05$.

Results

In the current study, Uric acid concentration was the highest in the bentonite group in the last week (Fig. 1b). Albumin concentration was lowest in the bentonite group in the last week and highest in the positive control group (Fig. 1c). Magnesium levels were the highest in the negative control group in the last week (Fig. 1a); cholesterol concentration was the highest in the bentonite group in the last week of the experiment (Fig. 2a). The lowest protein concentration was noted in the negative control group in the last week

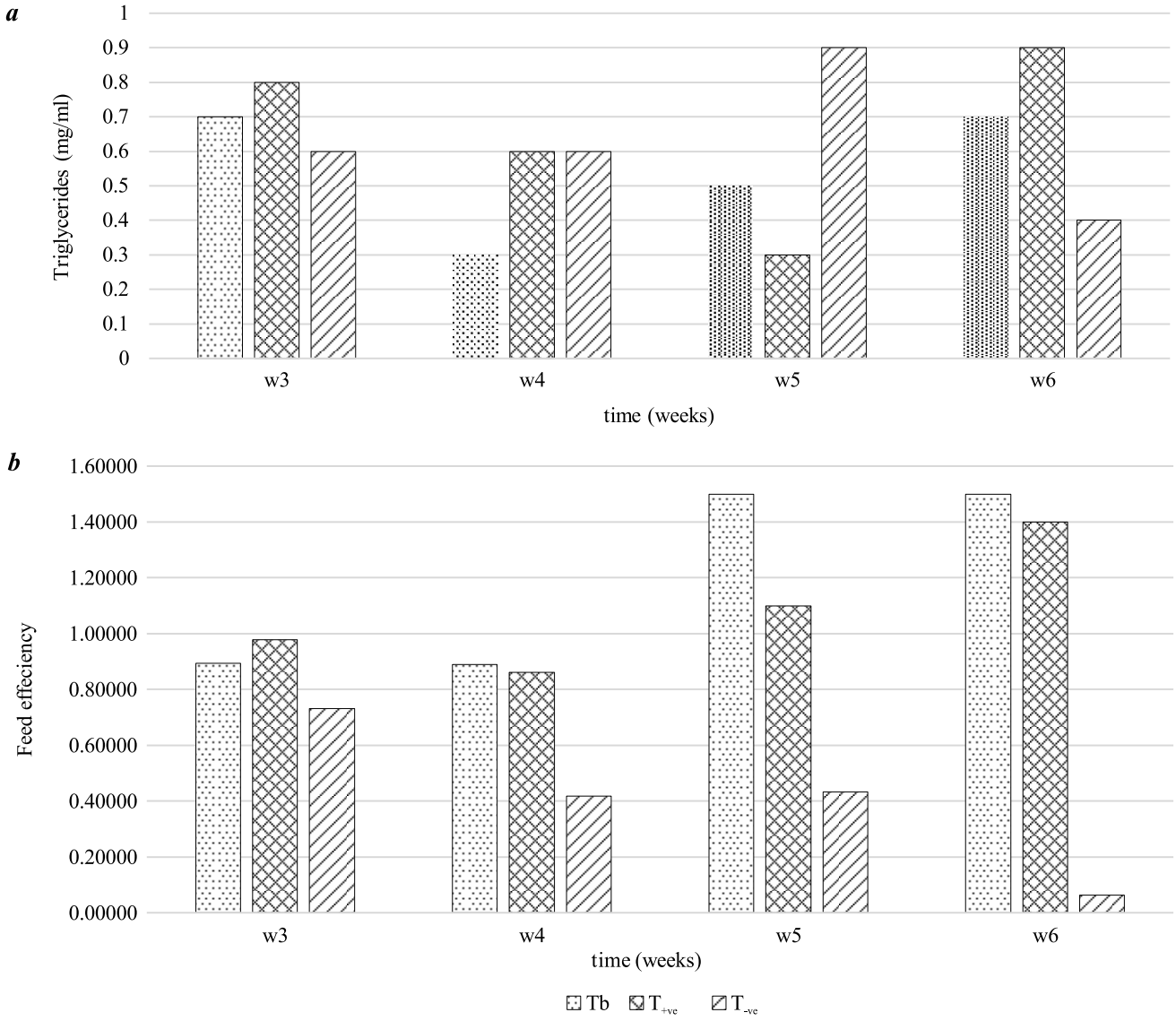

Fig. 3. Concentration of triglycerides; feed efficiency in broiler chickens.

of the experiment (Fig. 2 c). The lowest conversion ratio was noticed in the bentonite group and the highest conversion ratio was in the positive control group (Fig. 3b). The highest calcium concentration was noticed in the negative control group (Fig. 2b), the lowest triglycerides concentration was in the negative control group and the highest in the positive (Fig. 3a). The highest fecal oocyst count was noticed in the negative control group where no anticoccidial drugs were added and the lowest fecal oocyst count was noticed in the positive control group where an anticoccidial drug was added to the feed (Tables 2, 3, 4). Similar clinical histopathological lesions were observed in the negative control group and the bentonite group, no clinical signs were noticed in the positive control group (Figs. 4, 5).

Discussion

Several studies showed that poultry feed supplementation with bentonite can improve growth performance and nutrient digestibility in broilers. Bentonite treatment at 5 g/kg improved body weights at 42 d of age by 31.3%, increased food intake by 23.8% and improved productive efficiency by 40.1% (Santurio 1999); these results disagree with the current study. In the current study, a higher concentration of bentonite was added (6 g/kg), and the results of the current study agree with the results of Fowler et al (2015) who found that 1800 µg/kg was sufficient to reduce overall performance. Damiri et al (2012) used 3.75% sodium bentonite and there was an increase in the feed conversion ratio in broiler chickens. Miazzo et al. (2005) reported that carcass characteristics such as relative weight of the liver and spleen of broilers were increased

Table 2. Fecal oocyte count in Bentonite group (TB).

Bentonite group T_B (EPG)					
Week	Week	Std. Error	P value[a]	95% Confidence Interval for Difference[a]	
				Lower Bound	Upper Bound
1	2	61.02	P≤0.05	-12658.02	-11969.32
	3	115.2	P≤0.05	2996.02	5626.32
	4	45	P≤0.05	4798.30	5698.2
	5	103.69	P≤0.05	39865.3	69856.33
2	3	151.00	P≤0.05	14356	20326.32
	4	49.01	P≤0.05	16598.02	19856.23
	5	157.02	P≤0.05	16643.32	21123.35
3	4	112	P>0.05	-708.32	2036.98
	5	172	P>0.05	-10236.65	4569.33
4	5	143	P>0.05	-2001.36	2569.35

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

Table 3. Fecal oocyte count in Amprolium group (T_{+ve}).

Amprolium group T_B (EPG)					
Week	Week	Std. Error	P value[a]	95% Confidence Interval for Difference[a]	
				Lower Bound	Upper Bound
1	2	72.02	P≤0.05	0	0
	3	115.2	P≤0.05	3748	8052
	4	56	P≤0.05	4390	7677
	5	96.23	P>0.05	-27665	19998
2	3	162.3	P>0.05	-14356	20326.32
	4	98.3	P>0.05	-15623.32	18325.65
	5	211.98	P>0.05	-6325.36	7895.32
3	4	116.23	P>0.05	-608.32	-1036.98
	5	165.27	P>0.05	-10236.65	4569.33
4	5	123.36	P>0.05	-2001.36	2569.35

Based on estimated marginal means, the mean difference is significantat the 0.05 level, an Adjustment for multiple comparisons: Bonferroni. Dependent variable: coccidial oocyte count egg pergram (EPG).

by using aluminosilicates in the diet. Experimental coccidial infections decreases feed conversion ratio, feed intake, gain: feed, which is in agreement with numerous reports in the literature Hayajneh et al (2018), in this study a high concentration of bentonite was used (0.06) and the consequent effects of adding bentonite to feed was a decrease in weight gain and conversion ratio. The results of Khandeer et al. (2012) indicated that the use of each of two kinds of bentonite in diets (1%) improved the broiler performance; however, the addition of 1.5% bentonite had no significant effect. Results of the current study are in accordance with the results of Indresh et al. (2013) who indicated a significant ($p<0.05$) decrease in body weight, feed consumption, relative weights of bursa, thymus and serum protein.

Kececi et al. (1998) reported that the levels of calcium and phosphorus were decreased by aflatoxin in broiler chicks which received aflatoxin for 21 days and at a dose of 2.5 ppm. In this study the lowest concentrations of calcium were noticed in the bentonite group, which disagrees with the results of Bouderoua et al. (2016) who measured high calcium concentrations in bentonite treated broilers.

According to Khanedar et al. (2013), the addition

Table. 4. Fecal oocyte count in control group T_{-ve}.

Control group T_{-ve} (EPG)					
Week	Week	Std. Error	P value[a]	95% Confidence Interval for Difference	
				Lower Bound	Upper Bound
1	2	3041.38	P>0.05	-78350	7350
	3	1922.09	P≤0.05	-66414	-12253
	4	1589.90	P≤0.05	-70233	-25433
	5	927.96	P≤0.05	-75741	-49593
2	3	3041.38	P>0.05	-7350	78350
	4	1691.48	P>0.05	-27665	19998
	5	3929.94	P>0.05	-67702	43036
3	4	1922.09	P≤0.05	12253	66414
	5	1691.48	P>0.05	-19998	27665
4	5	1452.97	P≤0.05	-43804	-2862

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

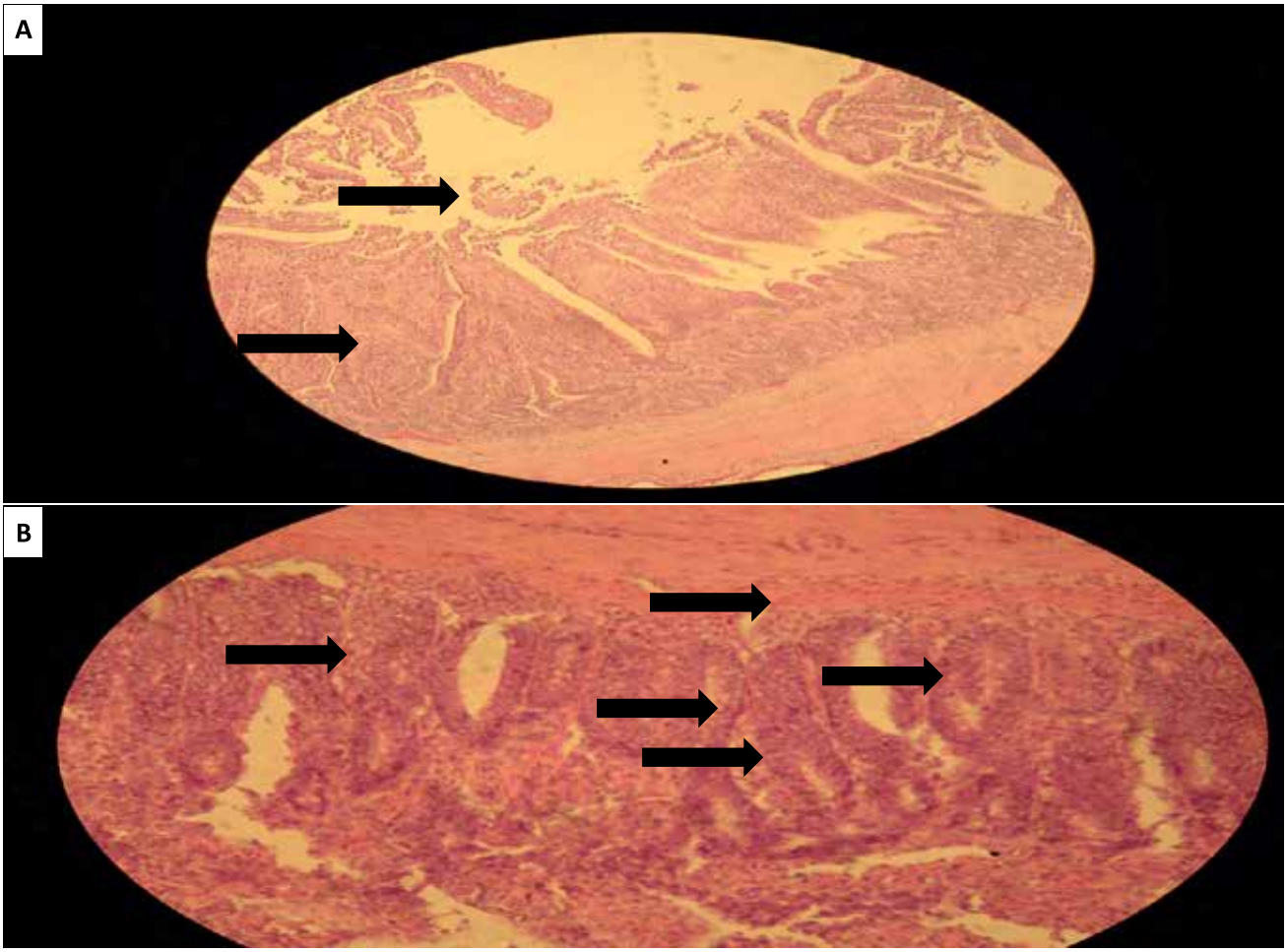

Fig. 4. Histopathology of the intestine in the negative control group; A×40, B and C×100

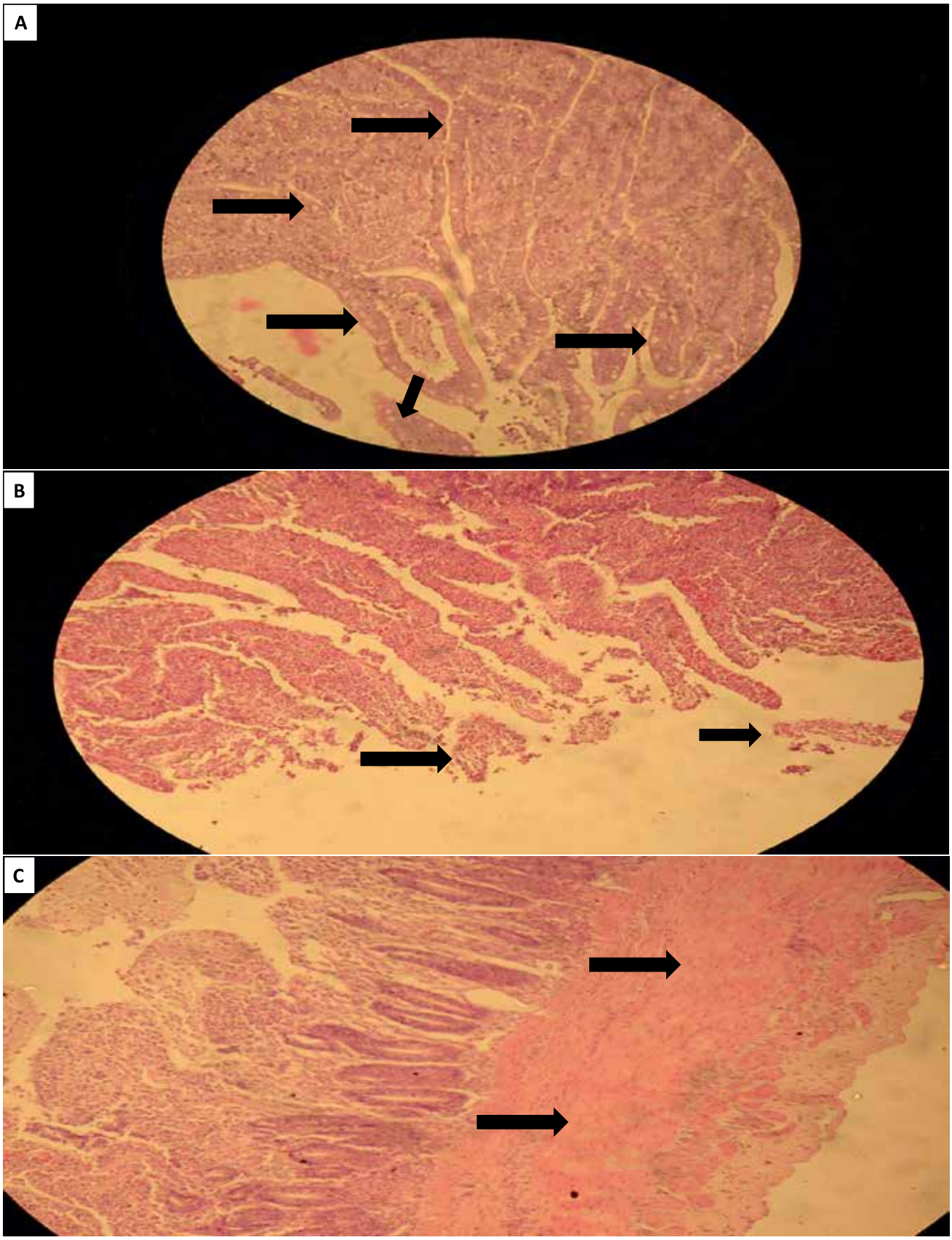

Fig. 5. Histopathology of the intestine in bentonite group, A*40, B and C *100 magnification.

of up to 1.5 %bentonite to diet had not significant effect on the blood biochemical parameters. Serum uric acid was numerically intermediate between the control and AF containing bentonite, a significant decrease in serum uric acid was seen in chickens given AF alone, and the uric acid in all the adsorbent treatments containing AF were close to those seen with the control diet (Kececi et al. 1998). Khandeer et al. (2013) showed that AF caused a decrease in blood total protein, albumin, uric acid and cholesterol levels in broiler chickens. In this study uric acid concentration was the highest in the bentonite group, protein concentrations were higher than the negative control group in the last week; this could be attributed to the fact that bentonite prevents the effects of aflatoxin present in feed that could increase uric acid absorption from the intestine.

Decreased serum albumin caused by AF was significantly ameliorated by PVPP plus BNT (Kececi et al. 1998). The AF toxicity in poultry may be manifested by decreased serum concentrations of total protein, albumin, cholesterol and glucose (Harvey et al. 1993). Protein and albumin concentrations were not affected by bentonite supplementation (Bouderoua et al. 2016). In this study albumin concentration was the lowest in the bentonite group in the last week and highest in the positive control group. These results agree with the results of Kececi et al. (1998), Harvey et al. (1993) and Bouderoua et al. (2016).

The serum level of triglycerides, total cholesterol, cholesterol HDL and sodium were significantly increased in the bentonite groups. The high triglycerides concentration observed in our trials may be attributed to the nature of the calcium bentonite which has slowed the intestinal transit and therefore induced a higher intestinal absorption of starch and there by contributing to an increase in the hepatic synthesis of lipids. The serum level of triglycerides, total cholesterol, cholesterol HDL, and sodium were significantly increased in the bentonite groups (Bouderoua et al. 2016). These results agree with the results of the current study where the highest levels of cholesterol were noticed in the bentonite group in the last week (Fig. 2a) These results disagree with the results of Yang et al (2014) who indicated a decrease in serum levels of the concentration of triglycerides when the animals received a diet supplemented with clay, The chickens receiving respectively treated and raw bentonite had an increased serum concentration of triglycerides ($p<0.05$).

The bloody enteritis caused by the intestinal species of *Eimeria* in chickens also impairs the absorption of electrolytes such as magnesium (Toledo et al. 2011).

In the present study, fecal oocyst counts were the highest in the negative control group followed by the bentonite group (Tables 2, 3, 4) Ibrahim et al. (2000) indicated that the ameliorative effect of dietary Na-bentonite on the reduced percentage and mean of phagocytosis caused by AF, could be attributed to the role of Na-bentonite as a sequestering agent against AF present in the diet through reducing its bioavailability in the gastrointestinal tract (Ibrahim et al. 2000) These results suggest that supplementation of bentonite may provide protection against a mixed *Eimeria* infection. According to Indresh et al. (2013) relative weights of bursa, thymus and serum protein are decreased which may explain the high fecal coccidial oocyst count in the bentonite group compared with the positive control group.

Conclusion

The results of the current study indicate that calcium bentonite should not be added to feed of broiler in 6% concentrations because it has adverse effects on feed conversion ratio. The high concentrations (6%) of bentonite in broiler feed cannot be used to alleviate the effects of coccidiosis. In the future, investigations should test the effects of lower concentrations of bentonite.

Acknowledgments

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