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Performance, Morphological Responses of the Small Intestine, and Humoral Immunity of Broilers Fed Oak Acorn (*Quercus brantii*) as a Substitution for Antibiotic Growth Promoters

Research Article

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ABSTRACT

This experiment was conducted to examine the effect of different levels of oak acorn (*Quercus brantii*) as a substitution for in-feed antibiotics (IFA) on growth performance, carcass traits, morphological responses of the small intestine and humoral immune responses in broiler chickens. Three hundred, 1-day-old broiler chicks (Ross 308) were allotted into 5 groups in a 6 wk study. The dietary treatments consisted of a corn-soybean coccidiostat-free basal diet as a control, control + 2 mg lincomycin/kg, or control + 10, 15 or 20 g oak acorn/kg. Dietary supplementation of lincomycin or 10 g oak acorn/kg significantly ($P<0.05$) enhanced final body weight of broilers at 42 d. Overall daily feed intake during the trial was higher ($P<0.05$) in broilers fed diets containing lincomycin or different levels of oak acorn compared with broilers fed basal diet. During the whole experiment feed:gain was significantly ($P<0.05$) better in broilers fed basal diet or basal diet supplemented with 10 g oak acorn/kg compared with other treatments. Carcass yield was significantly ($P<0.05$) higher in broilers fed diets containing 10 g oak/kg compared with other groups. In the duodenum, broilers fed diets containing antibiotic or 10 g oak acorn/kg had significantly ($P<0.05$) higher villus height:crypt depth ratio (VH/CD) compared with other groups. In jejunum and ileum the highest ($P<0.05$) VH/CD was obtained in broilers fed diets containing 10 g oak acorn/kg. Supplementation with 10 g oak acorn/kg led to higher antibody titers against Newcastle, and Influenza viruses ($P<0.05$). In summary, the results indicate that addition of 10 g oak acorn/kg could induce favorable influences on growth performance, structure of the small intestine and immune responses of broilers and it could be consumed in broiler diets as a substitution for IFA.

KEY WORDS broiler, carcass traits, immunity, intestinal morphology, performance, tannin.

INTRODUCTION

In the last decades, in-feed antibiotics (IFA) were successfully added at subtherapeutic concentrations in poultry feed to enhance growth performance, diminish pathogenic bacteria in the intestine, and maintain good t health (Kheiri *et al.* 2018; Foroutankhah *et al.* 2019; Landy *et el.* 2021; Moharreri *et al.* 2022). IFAs were generally assumed to enhance growth performance as a consequence of reduction in intestinal pathogenic bacteria population, resulting in lower incidence of enteric disease, greater nutrient utilization and reduced feed:gain (Visek, 1978). Although, in some countries inclusion of IFA have been gradually restricted because of increasing concerns on the potential of enhancing antimicrobial resistance, which can have adverse effects in human health (Toghyani *et al.* 2015).

Due to pressing need for proper substitution for IFAs some feed additives such as probiotics (Landy and Kavyani, 2014), prebiotics (Ceylan and Çiftci, 2003), phytogenic and herbal products (Nanekarani *et al.* 2012; Goodarzi *et al.* 2013; Ghanima *et al*. 2021; Moharreri *et al.* 2021), organic acids (Sobotik *et al.* 2021) and bioactive peptides (Landy and Kheiri, 2021) have widely received remarkable attention.

One of the main trees in Iran is oak acorn (*Quercus brantii*) which produces remarkable amount of oak acorn fruit. Besides nutrients, oak acorn fruit contains high amount of tannins (7.28-11.72% dry matter).

Tannin is a high-molecular-weight compound consisting of hydrolyzable and condensed tannins (Shimada, 2001). It has been reported that high tannin diets negatively affect growth performance (Armstrong *et al*. 1973), protein digestibility (Sell *et al*. 1983), mineral utilization and health (Houshmand *et al.* 2015) of poultry; although it's supplementation as an additive is under investigation.

Marzoni *et al.* (2005) investigated the effects of quebracho tannins (*Schinopsis lorentzii*) supplementation to the female pheasant's diet on productive performance of growing pheasants; the findings showed that dietary supplementation of 20 g quebracho tannins/kg of diet did not affect growth performance although examination of the fecal content showed that the pheasants fed tannin appeared free from the existence of helminths. Schiavone *et al.* (2008) reported that addition of chestnut extract in broiler chickens diet did not affect feed digestibility, carcass characteristics or nitrogen balance, although it had an affirmative impact on performance if supplemented in the diet up to 20 g/kg.

Windisch and Kroismayr (2006) suggested that low inclusion rate of tannins can promote palatability of feed and increase growth performance of monogastrics via enhancing feed use. Castillo *et al.* (2020) and Marzoni *et al.* (2020) reported that supplementation of tannins in Muscovy ducks and laying hens had no adverse effects. Abdel Tawwab *et al.* (2022) reported that dietary addition of 10 g oak acorn/kg improved performance criteria and welfare of common carp, especially under high stocking density conditions.

Starčević *et al.* (2015) and Samuel *et al.* (2017) reported the ability of tannins to improve the antioxidative traits of broiler chickens. Although tannins showed antiviral (Lupini *et al.* 2009), antibacterial (Tosi *et al.* 2013) and antiprotozoal (Cejas *et al.* 2011) activities, little is known about tannins efficacy on development of gastrointestinal tract and immunity. Despite these findings there has been dearth of information on the effect of oak acorn fruit on structure of the small intestine and humoral immunity in broiler chickens.

The goal of the current experiment was to compare the efficacy of different levels of oak acorn fruit powder as a substitution for IFAs on performance, carcass traits, structure of the small intestine and humoral immunity in broiler chickens.

MATERIALS AND METHODS

Animals and dietary treatments

This experiment was conducted in a broiler farm around the Esfahan city. Three hundred, day-old mixed mixed sex broiler chicks (Ross 308) were individually weighed and randomly allocated into 5 treatments with 5 replicate of 12 broilers each for 6 weeks. The dietary treatments consisted of a corn-soybean coccidiostat-free basal diet as a control, control + 2 mg lincomycin/kg, or control + 10, 15 or 20 g oak acorn/kg. The dietary treatments were adjusted to adequately provide the nutritional requirements specification of broiler chickens (Ross 308, Aviagen, 2019) and were fed in mash form within the experiment in 3 growth periods including, starter period: 0 to 10 d (Table 1), grower period: 11 to 24 d (Table2), and finisher period: 25 to 42 d (Table 3).

Since, the apparent metabolizable energy corrected for nitrogen for oak acorn was measured for broiler chickens (unpublished data), all formulated diets were isocaloric (De Marco *et al.* 2015). Broilers were reared based on the conditions recommended by commercial Ross 308 manual. The birds had free access to feed and water. The broiler house temperature was set at 32 ˚C within the first wk and diminished by 2 or 3 ˚C in the second and third wks, up to fetch up at 22 ˚C in wk 4 and then retained at 22 ˚C to the end of trial.

Analysis of oak acorn

Prior to formulating diets, maize, soybean meal, and oak acorn were analysed for nutrients contents (AOAC, 1995), and quantity of total amino acids (Methods 982.30E a, b, and c; AOAC, 2006). Calcium and total phosphorus (tP) were measured by inductively coupled plasma – optical emission spectrometry (Method 2011.14; AOAC, 1965). Acorns were subjected to extraction to measure phenolic contents. The extracts were analyzed as stated by Makka (2003).

Performance and carcass components

Body weights (BW) of the birds were measured at 1, 10, 24, and 42 d. Average daily weight gain (DWG), and daily feed intake (DFI) were determined in different growth phases and feed conversion ratio (DFI:DWG) was computed.

Table 1 Feed ingredients and calculated composition of experimental diets in starter period

Item	Oak acorn (g/kg)			
	0.0	10.0	15.0	20.0
Ingredients, g/kg (as-fed)				
Corn (7.5% CP)	541.4	530.8	525.3	520.0
Soybean meal (44% CP)	396.6	397.2	397.6	397.9
Oak acorn	0.0	10.0	15.0	20.0
Soybean oil	17.3	17.5	17.6	17.7
DL-methionine	3.7	3.7	3.7	3.7
L-lysine	2.2	2.2	2.1	2.1
L-threonine	1.0	1.0	1.0	0.9
Choline chloride	1.3	1.3	1.3	1.3
Mono calcium phosphate (15% Ca, 22.5% P)	16.3	16.3	16.3	16.3
Calcium carbonate	13.5	13.4	13.4	13.4
Sodium chloride	1.3	1.3	1.4	1.4
Sodium bicarbonate	3.4	3.3	3.3	3.3
Trace mineral premix[1]	1	1	1	1
Vitamin premix[2]	1	1	1	1
Calculated composition, g/kg				
Metabolizable energy, kcal/kg	2,870	2,870	2,870	2,870
Crude protein	220	220	220	220
Lysine	13.7	13.7	13.7	13.7
Methionine	6.81	6.81	6.82	6.82
Methionine + cysteine	10.3	10.3	10.3	10.3
Threonine	9.2	9.2	9.2	9.2
Tryptophan	2.7	2.7	2.7	2.7
Arginine	14.6	14.6	14.7	14.7
Valine	10.1	10.1	10.2	10.2
Isoleucine	9.2	9.2	9.2	9.2
Leucine	18.1	18.0	18.0	18.0
Calcium	9.1	9.1	9.1	9.1
Available P	4.6	4.6	4.6	4.6
Ether extract	26.5	26.5	26.5	26.6
Crude fibre	31.4	31.5	31.6	31.7
Analyzed content, g/kg				
Crude protein, g/kg	222	219	220	222

[1] Provided the following per kilogram of diet: Mg: 120 mg; Fe: 20 mg; Cu: 16 mg; Zn: 110 mg; Se: 0.3 mg and I: 1.25 mg.
[2] Provided the following per kilogram of diet: vitamin A: 12000 IU; vitamin D_3: 5000 IU; vitamin E: 80 IU; vitamin K: 3.2 mg; vitamin B_{12}: 0.017 mg; Thiamin: 3.2 mg; Riboflavin: 8.6 mg; Nicotinic acid: 65 mg; Pantothenic acid: 20 mg; Pyridoxine: 4.3 mg; Biotin: 0.22 mg and Folic acid: 2.2 mg.

Health status of the birds was monitored and mortality was monitored and recorded during the experiment.

At termination of the experiment, all birds were weighed individually and 2 male broilers/pen were selected on the basis of pen mean BW; selected birds were weighed and slaughtered thereafter by a manual neck cutter. The plucked and eviscerated carcasses were obtained and carcass yields were determined by dividing eviscerated weight by live BW. The weight of abdominal fat, liver, pancreas, spleen, empty gizzard, empty proventriculus, and empty small intestine were immediately determined and expressed as a percentage of live BW.

Morphological investigations

For morphological investigations, intestines from the two male broilers were isolated, 5 cm in the area of duodenum (loop segment), jejunum (the tract before Meckel's diverticulum), and ileum (the tract before the ileocolic junction) were cut out and cleaned to eliminate all the substances. The specimens were submerged in formalin, before fixation in Bouin's solvent and fixed in paraffin. The specimens were moved into 70% ethanol afterwards. The assessed morphometric criteria were villus height (VH), crypt depth (CD) and the VH to CD (VH/CH) ratio (Laudadio *et al.* 2012).

Table 2 Feed ingredients and calculated composition of experimental diets in grower period

Item	Oak acorn (g/kg)			
	0.0	10.0	15.0	20.0
Ingredients, g/kg (as-fed)				
Corn (7.5% CP)	545.8	535.1	529.9	524.4
Soybean meal (44% CP)	375.8	376.4	376.7	377.0
Oak acorn	0.0	10.0	15.0	20.0
Soybean oil	38.1	38.3	38.4	38.6
DL-methionine	3.2	3.2	3.2	3.2
L-lysine	1.5	1.5	1.4	1.4
L-threonine	0.6	0.6	0.6	0.6
Choline chloride	1.1	1.1	1.1	1.1
Mono calcium phosphate (15% Ca, 22.5% P)	14.8	14.8	14.8	14.8
Calcium carbonate	12.5	12.4	12.4	12.4
Sodium chloride	1.6	1.6	1.6	1.6
Sodium bicarbonate	3.0	3.0	2.9	2.9
Trace mineral premix[1]	1	1	1	1
Vitamin premix[2]	1	1	1	1
Calculated composition, g/kg				
Metabolizable energy, kcal/kg	3,027	3,027	3,027	3,027
Crude protein	210	210	210	210
Lysine	12.6	12.6	12.6	12.6
Methionine	6.24	6.24	6.24	6.25
Methionine + cysteine	9.6	9.6	9.6	9.6
Threonine	8.6	8.6	8.6	8.6
Tryptophan	2.5	2.5	2.5	2.5
Arginine	14.0	14.0	14.0	14.0
Valine	9.7	9.7	9.7	9.7
Isoleucine	8.8	8.8	8.8	8.8
Leucine	17.4	17.4	17.4	17.4
Calcium	8.5	8.5	8.5	8.5
Available P	4.2	4.2	4.2	4.2
Ether extract	26.3	26.3	26.4	26.4
Crude fibre	30.6	30.7	30.8	30.8
Analyzed content, g/kg				
Crude protein, g/kg	212	210	213	211

[1] Provided the following per kilogram of diet: Mg: 120 mg; Fe: 20 mg; Cu: 16 mg; Zn: 110 mg; Se: 0.3 mg and I: 1.25 mg.
[2] Provided the following per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 4500 IU; vitamin E: 65 IU; vitamin K: 3.0 mg; vitamin B_{12}: 0.017 mg; Thiamin: 2.5 mg; Riboflavin: 6.5 mg; Nicotinic acid: 60 mg; Pantothenic acid: 18 mg; Pyridoxine: 3.2 mg; Biotin: 0.18 mg and Folic acid: 1.9 mg.

Immunity

At 7 d of age, the birds were subcutaneously injected with an oil emulsion inactivated vaccine against Newcastle Disease virus (NDV) and Avian Influenza virus (H9N2 subtype).

At 21 d of age, vaccine recall was performed for Newcastle disease (LaSota strain) with eye drops. At 28 d of age, 2 male broilers/pen were chosen, and blood samples were taken and analyzed for antibody titers against NDV and Avian Influenza virus (AIV). Serum samples took the measurement of antibody titers against NDV and AIV by the hemagglutination inhibition test (HI), and HI antibodies were transformed to $\log_2$ afterwards. At 25 d of age, 2 male broilers were chosen and 1 mL of sheep red blood cell (SRBC 1%) was intravascularly injected in the brachial vein; blood samples were taken to obtain serum samples 6 d post immunizations.

The SRBC hemagglutination measure was executed as explained by Wegmann and Smithies (1966). The agglutination titers were represented as the $\log_2$ of the reciprocal of the highest dilution giving visible.

Statistical analysis

The obtained data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2004) (SAS Inst. Inc., Cary, NC). Differences among averages were considered by Tukey test. Statements of statistical significance are based on $P < 0.05$.

RESULTS AND DISCUSSION

Data about composition of oak are presented in Table 4. Data on performance criteria during different growth phases are presented in Table 5.

Table 3 Feed ingredients and calculated composition of experimental diets in finisher period

Item	Oak acorn (g/kg)			
	0.0	10.0	15.0	20.0
Ingredients, g/kg (as-fed)				
Corn (7.5% CP)	563.7	553.0	547.6	542.2
Soybean meal (44% CP)	339.3	339.9	340.2	340.5
Oak acorn	0.0	10.0	15.0	20.0
Soybean oil	59.1	59.3	59.4	59.5
DL-methionine	3.1	3.1	3.1	3.1
L-lysine	1.4	1.4	1.4	1.4
L-threonine	0.4	0.4	0.4	0.4
Choline chloride	1.0	1.0	1.0	1.0
Mono calcium phosphate (15% Ca, 22.5% P)	13.7	13.7	13.7	13.7
Calcium carbonate	11.7	11.6	11.6	11.6
Sodium chloride	1.7	1.7	1.7	1.7
Sodium bicarbonate	2.9	2.9	2.9	2.9
Trace mineral premix[1]	1	1	1	1
Vitamin premix[2]	1	1	1	1
Calculated composition, g/kg				
Metabolizable energy, kcal/kg	3200	3200	3200	3200
Crude protein	195	195	195	195
Lysine	11.6	11.6	11.6	11.6
Methionine	5.95	5.95	5.95	5.95
Methionine + cysteine	9.1	9.1	9.1	9.1
Threonine	7.8	7.8	7.8	7.8
Tryptophan	2.3	2.3	2.3	2.3
Arginine	12.9	12.9	12.9	12.9
Valine	9.0	9.0	9.0	9.0
Isoleucine	8.1	8.1	8.1	8.2
Leucine	16.3	16.3	16.3	16.3
Calcium	7.9	7.9	7.9	7.9
Available P	3.9	3.9	3.9	3.9
Ether extract	26.4	26.4	26.5	26.5
Crude fibre	29.4	29.5	29.6	29.6
Analyzed content, g/kg				
Crude protein, g/kg	197	195	198	197

[1] Provided the following per kilogram of diet: Mg: 120 mg; Fe: 20 mg; Cu: 16 mg; Zn: 110 mg; Se: 0.3 mg and I: 1.25 mg.
[2] Provided the following per kilogram of diet: vitamin A: 9000 IU; vitamin D_3: 4000 IU; vitamin E: 55 IU; vitamin K: 2.2 mg; vitamin B_{12}: 0.011 mg; Thiamin: 2.2 mg; Riboflavin: 5.4 mg; Nicotinic acid: 45 mg; Pantothenic acid: 15 mg; Pyridoxine: 2.2 mg; Biotin: 0.15 mg and Folic acid: 1.6 mg.

Table 4 Nutrient analysis and phenolics components of oak acorn

Composition of oak acorn, g/kg	
Ether extract	44.5
Crude fiber	37.5
Ash	53.0
Calcium	2.0
Total phosphorus	1.2
Total protein (N×6.25)	57.0
Arginine	4.7
Histidine	1.7
Isoleucine	2.8
Leucine	4.9
Lysine	3.8
Methionine	1.0
Cysteine	1.1
Phenylalanine	2.7
Threonine	2.4
Valine	3.4
Tyrosine	0.19
Tryptophan	0.7
Total phenols	65.7
Total tannins	39.5
Non tannin phenolic compounds	26.2

Within the starter period broilers fed diets containing 10 g oak acorn/kg of diet had significantly (P<0.05) greater BW compared with those fed basal diet and basal diet supplemented with 15 or 20 g oak acorn/kg of diet. During the grower and finisher phases broilers fed diets supplemented with antibiotic or 10 g oak acorn/kg had significantly greater BW compared with other groups (P<0.05). During starter period, broilers fed diets supplemented with 10 g oak acorn/kg had significantly (P<0.05) higher DFI compared with those fed basal diet, or basal diet supplemented with 15 and 20 g oak acorn/kg but did not significantly differ from the antibiotic group. During the grower phase the highest DFI obtained in the broilers fed basal diet supplemented with lincomycin (P<0.05). During finisher phase broilers supplemented with 15 and 20 g oak/kg had significantly greater (P<0.05) DFI compared with those fed basal diet or basal diet supplemented with 10 g oak/kg. Overall DFI during the experiment was higher (P<0.05) in broilers fed diets containing lincomycin or different levels of oak acorn compared with broilers fed basal diet. Remarkable distinctions amongst treatments were recognized in FCR in different growth periods. During starter period, broilers fed basal diet had better FCR compared with those fed basal diet supplemented with oak acorn. Within grower phase, broilers fed diets containing different levels of oak acorn had significantly better FCR compared with those fed basal diet or basal diet containing lincomycin (P<0.05). During the finisher period broilers fed basal diet had significantly better FCR compared with other groups (P<0.05). Overall FCR for the whole experiment was remarkably (P<0.05) better in broilers fed basal diet compared with those fed basal diet containing lincomycin or 15 and 20 g oak/kg whereas FCR of broilers fed basal diet did not significantly differ from those fed 10 g oak/kg.

Table 6 shows carcass yield, the percentage of abdominal fat and relative organ weights as a percentage of live weight at 42 d of age. The percentage of abdominal fat, and relative weight of liver, pancreas, gizzard, proventriculus, spleen and small intestine were not remarkably (P>0.05) altered by the dietary treatments. Carcass yield was significantly greater in broilers fed diets supplemented with 10 g oak/kg compared with other groups whereas it did not differ from those fed 15 g oak/kg.

The outcomes of the dietary treatments on the intestinal morphometric indices in different segments are summarized in Table 7. Duodenum and jejunum showed higher (P<0.05) VH for broilers fed diets containing 10 g oak/kg compared with other groups. Furthermore, in duodenum and jejunum, higher CD was found in broilers fed diets containing 20 g oak/kg compared with other groups (P<0.05). In duodenum, broilers fed diets supplemented with lincomycin had remarkably greater VH/CD compared with other groups; on the other hand, broilers supplemented with 15 and 20 g oak/kg showed significantly lower VH/CD compared with other groups. VH to CD ratio was significantly greater in broilers fed diets supplemented with 10 g oak/kg compared with broilers fed basal diet (P<0.05). The highest VH/CD in jejuna was found in broilers fed diets supplemented with 10 g oak/kg; on the contrary the lowest VH/Cd attained in broilers fed diets supplemented with 20 g oak/kg. In ileum, broilers fed basal diet or basal diet supplemented with 10 g oak/kg showed remarkably greater Vh compared with other groups (P<0.05). Broilers supplemented with 15 or 20 g oak/kg had remarkably (P<0.05) greater CD compared with other treatments. The highest VH/CD obtained in the group fed diets containing 10 g oak/kg compared with other treatments (P<0.05).

The outcomes of dietary treatments on antibody titers against NDV, AIV and SRBC are summarized as follows (Table 8). Treatments failed to influence any significant effects on antibody titers against SRBC (P>0.05). Broilers fed diets supplemented with 10 and 20 g oak/kg had significantly (P<0.05) higher antibody titers against NDV compared with broilers fed basal diet but did not differ from the broilers fed diets containing antibiotic and 15 g oak/kg that were intermediate. Broilers fed diets supplemented with 15 g/oak/kg had remarkably greater antibody titers against AIV compared with those fed basal diet or basal diet containing lincomycin (P>0.05).

In the present trial, dietary addition of 10 g oak acorn/kg markedly enhanced DFI of broilers as a result of sensory incitement and resultant promoted appetite via the existence of oak acorn in the diet. Similarly, Abdel-Tawwab *et al.* (2022) stated that addition of oak acorn in the diet of common carp significantly increased feed consumption as a result of an increment in the sensory characteristics of the feed. In line with our findings, Marzoni *et al.* (2005) stated that supplementation of 20 g quebracho-tannin/kg increased DFI of growing female pheasant. Windisch and Kroismayr (2006) proposed that tannins can promote palatability of feed and improve performance of poultry as a result of stimulating appetite. Furthermore, dietary addition of 10 g oak acorn/kg significantly enhanced final BW of broilers at 42 d.

Schiavone *et al.* (2008) indicated that supplementation of chestnut extract in poultry feeding affirmatively affected performance criteria if supplemented in the diet up to 20 g/kg. In line with our findings, Abdel-Tawwab *et al.* (2022) considered efficacy of oak acorn inclusion in the common carp diet in low or high density conditions; the results showed that supplementation of oak acorn can improve growth performance of common carp, although the best result obtained in the group which, were reared in low density condition.

Table 5 Effect of experimental diets on performance indices of broilers at different ages

Variables	Dietary treatments					SEM
	Control	Lincomycin	10 g oak acorn/kg	15 g oak acorn/kg	20 g oak acorn/kg	
Body weight (g)						
10 d	300^{b}	313^{ab}	320^{a}	278^{c}	247^{d}	5.58
24 d	1083^{b}	1142^{a}	1142^{a}	1062^{b}	992^{c}	27.0
42 d	2486^{c}	2635^{a}	2632^{a}	2546^{b}	2444^{c}	39.1
Daily feed intake (g/d)						
0-10 d	29.6^{c}	32.1^{ab}	33.0^{a}	31.2^{b}	29.5^{c}	0.6
10-24 d	82.0^{a}	83.0^{a}	77.8^{b}	75.3^{b}	71.0^{c}	1.8
24-42 d	150.6^{c}	173.1^{ab}	168.5^{b}	179.5^{a}	179.0^{a}	4.5
0-42 d	98.9^{b}	109.5^{a}	106.0^{a}	109.4^{a}	107.6^{a}	3.43
Feed:gain (g:g)						
0-10 d	1.12^{d}	1.16^{cd}	1.17^{c}	1.3^{b}	1.41^{a}	0.02
10-24 d	1.46^{a}	1.4^{b}	1.32^{c}	1.34^{c}	1.33^{c}	0.02
24-42 d	1.93^{d}	2.08^{bc}	2.03^{c}	2.17^{ab}	2.22^{a}	0.03
0-42 d	1.69^{d}	1.77^{c}	1.71^{d}	1.83^{b}	1.88^{a}	0.02

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of dietary treatments on carcass yield and internal relative organ weight of broilers at 42 d of age

Relative organ weight	Dietary treatments					SEM
	Control	Lincomycin	10 g oak acorn/kg	15 g oak acorn/kg	20 g oak acorn/kg	
Carcass (%)	68.6^{c}	69.7^{b}	71.0^{a}	70.4^{ab}	69.77^{b}	0.17
Abdominal fat (%)	1.90	1.91	1.83	1.77	1.83	0.08
Liver (%)	2.51	2.41	2.70	2.76	2.75	0.21
Pancreas (%)	0.21	0.23	0.27	0.28	0.27	0.05
Gizzard (%)	1.67	1.61	1.64	1.68	1.68	0.05
Proventriculus (%)	0.4	0.39	0.42	0.46	0.42	0.06
Spleen (%)	0.1	0.1	0.1	0.1	0.11	0.02
Small intestine (%)	2.73	2.68	2.96	3.00	3.08	0.19

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of dietary supplementation of antibiotic or oak acorn on the villus height (µm), crypt depth (µm), and villus height to crypt depth ratio in small intestine of broilers at 42 d of age

Variables	Dietary treatments					SEM
	Control	Lincomycin	10 g oak acorn/kg	15 g oak acorn/kg	20 g oak acorn/kg	
Duodenum						
Villus height	1643.8^{c}	1731.2^{b}	1779.0^{a}	1626.8^{c}	1568.4^{d}	15.52
Crypt depth	208.0^{b}	164.4^{d}	189.0^{c}	210.4^{b}	242.6^{a}	5.31
Villus height to crypt depth ratio	7.90^{c}	10.53^{a}	9.41^{b}	7.73^{c}	6.46^{d}	0.28
Jejunum						
Villus height	973.0^{b}	910.0^{c}	1113.0^{a}	974.8^{b}	871.2^{d}	16.84
Crypt depth	170.4^{b}	174.2^{b}	153.8^{c}	141.4^{d}	189.0^{a}	3.50
Villus height to crypt depth ratio	5.71^{b}	5.22^{c}	7.24^{a}	6.89^{a}	4.61^{d}	0.2
Ileum						
Villus height	736.0^{a}	685.4^{b}	728.4^{a}	689.4^{b}	626.4^{c}	8.07
Crypt depth	121.4^{bc}	113.2^{cd}	111.2^{d}	135.4^{a}	130.0^{ab}	20.9
Villus height to crypt depth ratio	6.07^{b}	6.06^{b}	6.56^{a}	5.09^{c}	4.82^{c}	0.14

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effect of dietary treatments on antibody titers against Newcastle and Influenza viruses at d 28 and sheep red blood cells (SRBC) at d 31

Variables	Dietary treatments					SEM
	Control	Lincomycin	10 g oak acorn/kg	15 g oak acorn/kg	20 g oak acorn/kg	
New castle (log_2)	4.1^{b}	4.3^{ab}	4.96^{a}	4.80^{ab}	4.90^{a}	0.10
Influenza (log_2)	4.20^{bc}	4.10^{c}	4.90^{abc}	5.44^{a}	5.00^{ab}	0.10
SRBC (log_2)	3.00	3.12	3.25	3.40	3.37	0.05

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In the present study, the increased final BW in broilers fed diets containing 10 g oak acorn/kg could be described by enhanced DFI and a betterment in VH/CD ratio as indicated in Table 7. Similarly, Landy *et al.* (2020) reported that an enhancement in VH/CD ratio resulted in an improvement in final BW of broiler chickens. In the current trial, addition of 10 g oak acorn could improve the FCR. As indicated in Table 7, in jejunum and ileum the highest VH/CD obtained in broilers fed diets supplemented with 10 g oak acorn/kg. In the present study the improved FCR may be due to the improved intestinal functions (Kikusato *et al.* 2016), as an improvement in morphometric indices, higher gut health and consequently better nutrient absorption (Jamroz *et al.* 2006). Similarly, Abdel-Tawwab *et al.* (2022) stated that supplementation of oak acorn in the diet of common carp resulted in better FCR. Also, Buyse *et al.* (2021) stated that, supplementation of chestnut tannins in broiler diets markedly improved meat quality, intestinal growth, and the antioxidative status. In contrary Marzoni *et al.* (2005) stated that addition of quebracho-tannin could not improve FCR in growing female pheasant. These incompatible results might be relevant to differences in the type and structure of tannins.

In the current study, relative weights of internal organ weight were not noticeably influenced by the addition of different levels of oak acorn.

Similarly, Liukkonen-Anttila *et al.* (2001) reported that carcass traits of grey partridges fed diets containing 60 g quebracho tannin/kg was not affected by the dietary treatment. Several researches indicated that dietary supplementation of antibiotic or medicinal herbs didn't influence internal organ weights (Nanekarani *et al.* 2012; Fekri Yazdi *et al.* (2014); Kheiri *et al.* 2018).

In the current trial, addition of 10 g oak acorn/kg positively affected morphometric parameters of the small intestine. Similarly, Bilić-Šobot *et al.* (2016) reported that supplementation of hydrolysable tannin in male pigs enhanced villus height, villus perimeter and mucosal thickness. In corroboration to our findings, Wang *et al.* (2020) stated that microencapsulated tannic acid supplementation positively affected intestinal morphology.

Biagi *et al.* (2010) investigated efficacy of supplementing weaned piglets diet with chestnut wood extract containing 75% tannins on performance criteria and intestinal ecosystem, the findings showed that supplementation of 1.13 g tannins/kg significantly increased CD, whereas inclusion of higher dosage reduced CD. Mannelli *et al.* (2019) reported that high inclusion rate of tannic acid in broiler chickens diet had negative effects on intestine morphology. On the other hand, Zhao *et al.* (2019) stated that tannic acid could improve intestine morphology of the Hu sheep by enhancing the duodenal, jejunal, and ileal villus height; thus it seems that its efficacy on intestine morphology depends on its inclusion rate. Since, higher villi length is very important factor to have larger surface area (Han *et al.* 2014); thus it's very important for nutritional digestion and absorption (Zhang *et al.* 2013). In summary, the better FCR which was obtained in the present study may be attributed to higher villi length and consequently larger surface area for absorption.

In the present study, supplementation of oak acorn positively affected antibody titers against NDV and AIV. Ramah *et al.* (2020) reported that dietary supplementation of tannin had affirmative and negative effects on broiler chicken immunity in a dose-dependent manner. Marzo *et al.* (1990) investigated the effects of supplementing broilers diet with tannic acid on the immune responses of broiler chickens; the results indicated that supplementation of tannic acid reduced the immune system function of broiler chickens in a dose-dependent manner. Kaleem *et al.* (2014) reported that addition of tannins derived from *Emblica officinalis* in broiler chickens' diet indicated immunostimulatory properties and increased protective immune system. Karaová *et al.* (2019), stated that tannins were effective to sustain content of mucosal immunity of broilers due to upregulating immunoglobulins A and mucin 2. It seems that supplemental tannins at proper inclusion rate can promote immune function, although higher dosage can impair the immune system function.

Supplementation of lincomycin in broiler's diet had no marked effects on antibody titers against NDV, AIV, and SRBC (Table 8).

Similarly, Landy *et al.* (2020) stated that addition of lincomycin in broiler's diet had no marked effects on antibody titers against NDV and SRBC, and hematological responses of broiler chickens.

Fekri Yazdi *et al.* (2014) reported that supplementation of broiler's diet with flavophospholipol had no significant effects on antibody titers against AIV and relative weight of bursa and spleen. Dafwang *et al.* (1985) reported that broiler fed diets containing lincomycin, oxytetracycline, penicillin, bambermycins, and tylan indicated slight responses to SRBC antigen. Therefore, the impact of intestinal conditioners and their efficacy on intestinal microbiota could be mostly restricted to the mucosal immune responses and not the systemic segment of the immune system.

CONCLUSION

In conclusion, the results indicate that addition of 10 g oak acorn/kg could induce favorable influences on performance, morphology of the small intestine and immune responses of broilers and it could be used in broiler diets as a substitution for IFA.

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