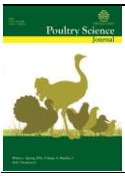

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Effects of Dietary Inclusion of Raw or Treated Iranian Oak Acorn (*Quercus brantii* Lindl.) on the Performance and Cecal Bacteria of Broilers

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Abstract

The effects of raw and sodium hydrogen carbonate-treated oak acorn were evaluated on broiler chicken performance and cecal flora. A total of 340 one-day-old broiler chicks were used in a completely randomized design with five experimental treatments and four replicates with 17 birds each. A corn-based diet served as the control and four treatment groups were diets containing 20 or 25% raw or treated oak acorn. Treatment of the acorn with sodium hydrogen carbonate significantly reduced the amount of total phenols and tannins in the feed ($P < 0.05$). Though there were no significant differences in body weight gain and final body weight between the control and treatment groups, treated oak acorn yielded greater overall weight gain than raw oak acorn. Feeding treated or raw oak acorn impaired feed conversion ratio relative to the control during the starter phase of the study. However, finisher (22-42 d) and overall (1-42 d) feed conversion ratio were similar between control and other treatments. Birds fed 25% treated oak acorn had significantly better overall feed conversion ratio than those fed raw oak acorn ($P < 0.05$). The relative weight of pancreas, liver, abdominal fat, as well as *Lactobacillus* and *E. Coli* counts, were similar between all treatments at 21 and 42 d of age. In conclusion, raw or treated oak acorn could be included in broiler diets up to 25% without negative effects on their performance. The performance may be improved by treating oak acorn with sodium hydrogen carbonate because of reducing the content of phenolic components.

Introduction

Corn is the most important energy source in poultry diets. However, due to insufficient domestic production, a huge amount of corn is imported to Iran. Thus, it is important to find new, local, unconventional feedstuffs. A native abundant tree in Iran is oak acorn (*Quercus brantii* Lindl.) and large quantities of oak acorn fruit are available. This fruit contains high levels of starch (Saffarzadeh *et al.*, 1999) and it is expected that oak acorn can be used as an energy source in poultry diets (Midilli *et al.*, 2008; Bouderoua *et al.*, 2009; Houshmand *et al.*, 2015).

Besides nutrient components, the oak acorn is considered a tannin-rich feedstuff (7.28-11.72 % DM) (Shimada, 2001). Tannins are water-soluble polyphenolic compounds with a molecular weight between 500 and 3000 Da, capable of precipitating alkaloids, gelatin, and other proteins from aqueous solutions. They are usually divided into hydrolyzable and condensed tannins. As a

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group of anti-nutritional factors, tannins have deleterious effects on animal and poultry (Jansman, 1993; Mahmood *et al*., 2006; 2007; Houshmand *et al*., 2015). For example, diet palatability and feed intake (Butler *et al*., 1984), protein and starch digestibility (Mahmood *et al*., 2007; 2008), as well as minerals and vitamins availability (Jansman, 1993) are negatively influenced by tannins. In addition, tannins adversely influence organs such as intestines, liver, and pancreas (Ortiz *et al*., 1994). Therefore, the utilization of tannin-rich feedstuffs in poultry diets is limited though different strategies are used to decrease or eliminate the tannins content and their negative effects. Chemical treatment is a proposed strategy in this field. Mahmood *et al*. (2006; 2008) reported that treatment of tannin rich salseed *(Shorea robusta)* meal with sodium hydrogen carbonate reduced tannins and overcame their negative consequences on broilers. In another study conducted by Ochanda *et al*. (2010), alkali treatment was effective in the detoxification and content reduction of tannins.

Antibacterial property of tannins has also been reported (Scalbert, 1991) and it is thought that tannins beneficially influence broiler gut bacteria. However, little is known on how sodium hydrogen carbonate-treated oak acorn impacts broiler performance and gut flora. Therefore, the current experiment was conducted to evaluate those effects.

Materials and Methods

All procedures were approved by the Institution Animal Care Committee of the Yasouj University. Using a completely random design, 340 one-day-old male and female Cobb 500 broiler chicks were randomly distributed among the five experimental treatments (diets), with 4 replicates of 17 birds each. The control group was offered a corn-based diet (without oak acorn) while the other 4 groups were fed diets containing 20 or 25% raw or treated oak acorn.

Isocaloric and isonitrogenous starter and finisher diets were formulated to meet or exceed the NRC requirements (1994) and were fed from 1 to 21 and 22 to 42 days of age, respectively. The composition of the experimental diets is shown in Table 1. Birds had free access to experimental diets and water throughout the rearing period. The birds were reared under similar management conditions in floor pens (150 cm length × 150 cm width) with rice straw as litter. Feed intake was calculated weekly on a pen basis. Birds in each pen were weighed as a group at 21 and 42 d of age and feed conversion ratio (FCR) was calculated as the ratio of feed intake to body weight gain. Mortality was recorded daily and FCR was adjusted for mortality (Bozkurt *et al*., 2014).

Table 1. Composition of the experimental diets

Ingredients (%)	*Starter			*Finisher		
	Control	20	25	Control	20	25
Corn	59.78	35.73	29.67	65.64	41.41	35.36
Oak acorn	-	20	25	-	20	25
Soybean meal	35.30	37.60	38.18	29.52	31.85	32.43
Vegetable oil	1	2.94	3.44	1.36	3.36	3.86
Limestone	1.28	1.09	1.06	1.29	1.18	1.16
Dicalcium phosphate	1.57	1.51	1.49	1.28	1.22	1.20
Common salt	0.42	0.43	0.44	0.32	0.33	0.33
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.20	0.22	0.09	0.15	0.16
Chemical composition						
ME (Kcal/kg)	2880	2880	2880	2980	2980	2980
Crude protein (%)	20.70	20.70	20.70	18.62	18.62	18.62
Calcium (%)	0.90	0.90	0.90	0.84	0.84	0.84
Available P (%)	0.41	0.41	0.41	0.33	0.33	0.33
Lysine (%)	0.99	0.99	0.99	0.93	0.93	0.93
Methionine (%)	0.45	0.45	0.45	0.35	0.35	0.35

*Control: control diet, 20: diet contained 20% oak acorn, 25: diet contained 25% oak acorn

[1]The vitamin premix supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D_3, 1,000 IU; vitamin E (dl-α-tocopherol), 30 IU; vitamin K_3, 2.5 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; d-pantothenate, 8 mg, folic acid, 0.5 mg.

[2]The mineral premix supplied the following per kilogram of diet: Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg, Se, 0.25 mg; Co, 0.2 mg.

Oak acorns were collected from the forest of Yasouj, Kohgeluyeh and Bovir Ahmad Province, Iran. In this forest, *Quercus brantii Lindl.* is the most common species. The seed coat of fruits was removed and fruits were dried in shade and then finely grounded. Oak powder was mixed with 0.67 *M* sodium hydrogen carbonate (pH=8.2) solutions at 820 mL/kg of oak DM and incubated for 12 hrs at 37°C (Mahmood *et al.*, 2006). The treated material was dried after incubation at the same temperature in the oven. Oak acorn was analyzed for contents of dry matter (DM), ether extract (EE), crude fiber (CF), crude protein (CP), Ash and nitrogen free extract (NFE) (AOAC, 1995). To measure the phenolic components of oak acorn in the first stage, 3 mL of acetone (75%) and 3 mL methanol (50%) were added to approximately 1 g of ground oak acorn. The tubes were vortexed and then centrifuged (3000 × *g* at 4°C) for 20 min. The supernatant was extracted and analyzed following the procedure of Makkar (2003).

On d 21 and 42 (end of the starter and finisher phases of the study, respectively), one bird from each pen was slaughtered by cervical dislocation and the digestive system was immediately and carefully removed to measure the weight of liver, pancreas and abdominal fat pad. Relative organ weight was expressed as a percentage of live body weight. Cecal digesta samples were also taken at d 21 and 42 and transferred to sterile tubes on ice and immediately sent to the Microbiology Lab to determine the count of *E. Coli* and Lactic acid bacteria. Each sample was serially diluted from 10^{-1} to 10^{-9}. 100 µL of diluted samples were plated on EMB (for *E. coli*) and MRS (for *Lactobacillus*) agar media which were incubated at 37ºC for 24 and 48 hrs under anaerobic and aerobic conditions, respectively. The results are shown as colony forming unit (CFU) per gram of cecal digesta.

Data were analyzed statistically using the General Linear Models (GLM) procedures of SAS software (SAS Institute, 2003). The means were compared by Duncan's multiple range test. The level of statistical significance was set at $P < 0.05$.

Results and Discussion

Proximate analysis of oak acorn (Table 2) shows that it contains a high level of NFE (72.66% DM). Consistent with this result, Shimada (2001) reported that NFE was the major component of nutrients (87.4–90.3% DM) in three species of acorn. Previously, the chemical composition of Iranian oak acorn (*Quercus branti*), from three different climates in the Zagros region of Iran was determined to contain 91.67 % DM, 3.93 % CP, 0.37 % CF, 7.7% EE, 1.5% Ash, 75.17% NFE and 58.80% starch (Saffarzadeh *et al.*, 1999).

Table 2. Proximate analysis of oak acorn (% DM)

Component	DM [1]	Ash	CP [2]	EE [3]	CF [4]	NFE [5]
Level	94.26±0.83	5.67±0.08	6.09±0.06	10.14±0.47	5.44±0.09	72.66±1.4

Data are presented as mean ± standard deviation.
[1] Dry Matter, [2] Crude Protein, [3] Ether Extract, [4] Crude Fiber, [5] Nitrogen Free Extract.

The findings indicated that the oak acorn contains high levels of phenolic components including tannins (Table 3), consistent with previous reports finding 7.28-11.72% DM in acorn (Shimada, 2001; Houshmand *et al.*, 2015). However, treatment of the acorn with sodium hydrogen carbonate significantly ($P < 0.05$) reduced the amount of all phenolic compounds (total phenols, condensed tannins, and total tannins). The highest reduction was observed in total phenols (91%), followed by condensed tannins (71%) and total tannins (70%). Previously, positive effects of chemicals treatment on the improvement of the nutritional value of tannin-rich feedstuffs have been reported (Mahmood *et al.*, 2006, 2008; Ochanda *et al.*, 2010).

Table 3. Effects of sodium hydrogen carbonate treatment on the content of phenolic components of oak acorn (% DM)

Parameter	Oak acorn		*SEM*	*P*-value	Reduction (%)
	Raw	Treated			
Total phenols	8.61[a]	0.78[b]	0.39	0.0001	91
Condensed tannins	5.22[a]	1.54[b]	0.15	0.0001	71
Total tannins	6.07[a]	1.84[b]	0.17	0.0001	70

Means within a row with different superscripts are significantly different at $P < 0.05$.

The effects of experimental diets on broiler growth performance are shown in Table 4. During the starter phase, there were no significant differences in body weight gain between the groups fed diets containing raw oak acorn (20 and 25%) and the control. However, treated oak acorn (20 or 25%) caused greater body weight gain than the other groups, leading to a greater body weight ($P < 0.05$). During the finisher phase, body weight gain was similar between the control and treatment groups though birds fed 25% treated oak acorn had significantly greater body weight gain than those fed 25% raw oak acorn ($P < 0.05$). Experimental treatments had similar overall (1-42 d) body weight gain and final body weight as the control, but overall body weight gain and final body weights were greater in birds fed treated oak acorn than those fed raw oak acorn.

Table 4. Effects of experimental diets on growth performance of broilers

Parameter	Experimental diets					*SEM*	*P*-value
	Control [1]	20R [2]	20T [3]	25R [4]	25T [5]		
Body weight (g)							
21 d	705^{b}	675^{b}	839^{a}	682^{b}	825^{a}	22	0.0001
42 d	2042^{ab}	1864^{b}	2159^{a}	1785^{b}	2281^{a}	83	0.004
Body weight gain (g)							
d 1-21	659^{b}	628^{b}	791^{a}	635^{b}	780^{a}	22	0.0001
d 22-42	1337^{ab}	1189^{ab}	1321^{ab}	1104^{b}	1456^{a}	83	0.049
d 1-42	1996^{ab}	1817^{b}	2112^{a}	1739^{b}	2236^{a}	83	0.004
Feed intake (g)							
d 1-21	1050^{b}	1345^{a}	1458^{a}	1375^{a}	1432^{a}	54	0.0006
d 22-42	3365	3278	3276	3112	3226	113	0.62
d 1-42	4451	4623	4734	4478	4659	134	0.47
Feed conversion ratio							
d 1-21	1.59^{d}	2.14^{ab}	1.83^{c}	2.17^{a}	1.90^{bc}	0.08	0.0007
d 22-42	2. 52	2.45	2.48	2.82	2.40	0.15	0.33
d 1-42	2.21^{ab}	2.58^{a}	2.24^{ab}	2.58^{a}	2.09^{b}	0.12	0.026

Means within a row with different superscripts are significantly different at $P < 0.05$.

[1] corn-based diet (without oak acorn), [2] diet containing 20% raw oak acorn, [3] diet containing 20% treated oak acorn, [4] diet containing 25% raw oak acorn, [5] diet containing 25% treated oak acorn.

Birds with diets of 20 or 25% raw or treated oak acorn significantly increased feed intake compared to the control group during the starter phase ($P < 0.05$) but not the finisher phase, which also led to insignificant differences in overall feed intake. It was expected that feeding a high-tannin feedstuff such as oak acorn reduces feed intake due to the stringent or bitter taste of tannins (Nyachoti *et al.*, 1996). The mechanisms by which tannins affect feed intake are not well understood but may be associated with reduced diet palatability (Nyachoti *et al.*, 1996). Conflicting results have been reported regarding influences of tannins on the broilers feed intake. For example, Mahmood *et al.* (2008) found that high tannin diets significantly reduced broilers feed intake but Nyachoti *et al.* (1996) found the opposite with a high tannin sorghum diet. Feed conversion ratio was significantly impaired by both concentrations of treated and raw oak acorn diets during the starter phase of the study ($P < 0.05$), but treated oak acorn had better FCR than raw oak acorn. Despite insignificant differences in FCR during the finisher stage, overall (1-42 d) FCR improved in birds fed 25% treated diets compared to 20 and 25% raw diets ($P < 0.05$).

Taken together, the present findings suggest that dietary inclusion of 20 or 25% raw or treated oak acorn had no deleterious effect on broiler performance. Feeding 15% raw oak acorn (Houshmand *et al.*, 2015) and 32.4% green oak acorn (Hamou *et al.*, 2012) have been shown to have negative effects on broilers performance. Bouderoua *et al.* (2009) found that birds fed 33.5% green oak acorn had lower body weight at 35 d of age than the control but there was no significant difference in final body weight at d 56. The findings of Midilli *et al.* (2008) indicated that different levels (5, 10, 15 and 20%) of acorn seed (*Quercus cerris*) had no negative effects on performance of Japanese quail. In another study, Kaushal and Singh (1982) indicated that feeding with diets containing 10 or 15% oak acorn had no detrimental effects on body weight gain while 20 and 25% acorn resulted in lower body weight gain. Sodium hydrogen carbonate-treated oak acorn resulted in a better performance (FCR and body weight gain) compared to raw oak acorn. This improvement could be attributed to the significant reduction in the content of phenolic components. Since tannins have deleterious consequences on broilers, reducing their concentrations in broiler diet can prevent their negative effects and thereby improve performance.

In line with our results, several reports have indicated that chemical treatment reduces the deleterious effects of tannins. Mahmood *et al*. (2007) showed that treatment of salseed meal (a tannin–rich feedstuff) with sodium hydrogen carbonate significantly reduced tannin content and improved digestive enzymatic activity. In another study (Mahmood *et al*., 2008), dietary inclusion of sale meal adversely influenced broiler performance and digestive enzymatic activity, effects that were ameliorated with treatment of the sale meal with water or alkali. The improved activity of digestive enzymes may be related to a reduction in the formation of tannin-dietary protein and tannin-enzyme complexes in the broiler gut. Treatment with wood ash, an alkaline substance, reduces the tannin content of sorghum and improves its nutritive value and broiler growth rate (Kyarisiima *et al*., 2004). In this case, the reduction in tannins in treated sorghum is probably due to polymerization of tannins to other compounds in an alkaline medium or due to reactions of tannins with cations abundant in wood ash such as potassium, sodium and calcium. Mahmood *et al*. (2006) reported that alkali (sodium hydrogen carbonate) treatment of sale meal improved protein digestibility and nitrogen retention in colostomized hens and broilers. This improvement was likely due to a reduction in tannin content of the treated saleseed meal, which may have provided more nutrients such as protein, starch, and enzymes in the digestive tract of the birds. In another study, diets of tannin-containing sorghum reduced the performance of broiler chickens but dietary supplementation with 0.25% sodium hydrogen carbonate overcame the antinutritional effects possibly due to improved electrolyte balance in the diet. This would create favorable conditions for an improvement in feed efficiency without influencing energy and apparent nitrogen retention (Banda-Nyirenda and Vohra., 1990). In the study of Muindi *et al*. (1981), anti-nutritive effects of tannin sorghum were inhibited by treatment with sodium bicarbonate (soaking for 3 days in Magadi soda solutions). It is possible that treatment hydrolyzes and converts hydrolysable tannin to smaller units which do not have tannin-like effects.

Table 5. Effects of experimental diets on relative weight (% body weight) of pancreas and liver at 21 and 42 and abdominal fat at 42 d of age

Parameter	Experimental diets					*SEM*	*P*-value
	Control [1]	20R [2]	20T [3]	25R [4]	25T [5]		
Liver							
21 d	2.83	2.47	2.45	2.38	2.62	0.14	0.22
42 d	2.21	1.86	2.24	2.09	2.03	0.18	0.58
Pancreas							
21 d	0.30	0.34	0.31	0.35	0.30	0.02	0.38
42 d	0.24	0.18	0.23	0.23	0.22	0.02	0.37
Abdominal fat pad							
42 d	3.14	2.59	3.29	2.48	2.20	0.40	0.31

[1] corn-based diet (without oak acorn), [2] diet containing 20% raw oak acorn, [3] diet containing 20% treated oak acorn, [4] diet containing 25% raw oak acorn, [5] diet containing 25% treated oak acorn.

Weights of pancreas, liver, and abdominal fat were not significantly influenced by experimental diets ($P > 0.05$) (Table 5). High tannin diets can inhibit pancreatic enzymes, which may increase enzyme production, leading to pancreatic hypertrophy (Ahmed *et al*., 1991). However, in the current study, pancreas weight was not influenced. Similar to present findings, Nyachoti *et al*. (1996) reported that tannins had no significant effect on liver and pancreas weight. In another study, 10, 20 and 30% raw or treated sorghum did not affect the weights of liver, pancreas, heart and spleen in broilers (Sharif *et al*., 2012).

Cecal bacteria population

Cecal bacteria counts at 21 and 42 d of age were not influenced by experimental treatments (Table 6). Tannins are antimicrobial and can inhabit at extracellular microbial enzymes, deprive substrates required for microbial growth, and inhibit oxidative phosphorylation in microbes (Scalbert, 1991).

Table 6. Effects of experimental diets on population of cecal bacteria at 21 and 42 d of age (CFU/g)

Parameter	Experimental diets					*SEM*	*P*-value
	Control [1]	20R [2]	20T [3]	25R [4]	25T [5]		
E. Coli							
21 d	8.79	8.60	8.17	8.99	7.94	0.33	0.23
42 d	8.31	8.43	7.04	7.47	8.74	0.60	0.27
Lactobacillus							
21 d	8.83	9.24	8.82	8.89	8.92	0.43	0.95
42 d	8.42	8.55	8.23	8.77	8.85	0.54	0.92

[1] corn-based diet (without oak acorn), [2] diet containing 20% raw oak acorn, [3] diet containing 20% treated oak acorn, [4] diet containing 25% raw oak acorn, [5] diet containing 25% treated oak acorn.
Means within a row with different superscripts are significantly different at $P < 0.05$.

Sadeghian *et al*. (2012) showed that ethanol extract of *Qercus brantii* fruits have antimicrobial activity against gastrointestinal bacterial pathogens. These antimicrobial activities, in almost all cases, were greater than standard antibiotics and may be due to the activities of tannins and phenolic compounds. Jamroz *et al*. (2009) indicated that high tannin diet reduced the count of *E. coli* and *Coliform* bacteria in broilers. However, the response to tannins may be impacted by its source and concentration, animal performance (weight gain, feed intake and feed efficiency) and factors (species, age, and production level), as well as diet composition (Jansman, 1993), which may explain differences between results.

Conclusion

This experiment show that raw or sodium hydrogen carbonate-treated oak acorn could be included in broiler diets in concentrations up to 25% without adverse effects on performance. Treatment of oak acorn with sodium hydrogen carbonate reduces the content of phenolic components, thereby improving performance.

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