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Dietary Nutrient Manipulation to Improve the Performance and Tibia Characteristics of Broilers Fed Oak Acorn (*Quercus Brantii Lindl*)

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ABSTRACT

An experiment was conducted to evaluate the effects of dietary nutrients (protein, methionine, and choline) manipulation on the performance and tibia characteristics of broilers fed diets containing 15% oak acorn. A completely randomized design with five treatments and four replicates of 15 chicks each, was used. One group was fed a diet based on corn (without oak acorn) as control and other four groups were fed diets containing oak acorn with one of the following nutrient manipulation: without nutrient manipulation, high level of methionine (100% more than NRC), high level of CP (10% more than NRC), and addition of 0.2% choline. Starter and finisher diets were fed from one to 21 and 22 to 42 d of age, respectively. The results indicated that group fed diet without nutrient manipulation presented higher feed intake and worse feed conversion ratio (p<0.05) compared with the control group throughout the experiment. Elevated levels of methionine, protein, and choline had significant beneficial effects on feed conversion ratio during starter phase. Feeding oak acorn negatively influenced tibia bone characteristics (bone ash and strength) both at 21 and 42 d of age, whereas the supplementation with protein, methionine and choline prevented those negative effects. In conclusion, dietary inclusion of 15% oak acorn had negative effects on broiler performance and tibia characteristics. Detrimental effects of oak acorn, particularly on tibia bone, were reduced or prevented by increasing the dietary levels of choline, methionine, and protein.

INTRODUCTION

In the animal and poultry production industry, the highest cost is related to nutrition. Corn is the main feedstuff of poultry diets. This feedstuff is mostly imported and thus it is expensive in importing countries, such as Iran. Therefore, looking for cheaper and local feedstuffs is of great importance in reducing production costs. Oak acorn, the seed of oak trees, is produced and available in large quantities in some countries of the world, including Iran. The nutrient composition of oak acorn is similar to that of cereal grains. Shimada (2001) reported that the main nutrients of two species of acorns were carbohydrate, protein, and fat and their main component was NFE (80-90% DM). Due to high levels of carbohydrates (47-60% starch) (Kekos & Kaukios, 1985), oak acorn may potentially be used as an energy source and therefore, replace corn in broiler diets, such as the case in North-African countries (Bouderoua & Selselet-Attou, 2003). There is limited information on the use of oak acorn in broiler nutrition. Bouderona *et al.* (2009) reported that broilers fed diets containing 35% green oak acorn presented lower body weight compared with the control group at 35 d of age, but there was no difference between two groups at the end of the experiment (56 d of age). Although oak acorn may potentially be used in poultry nutrition, some factors may limit their inclusion in the diets. High levels of tannins (7.28-11.72% on dry matter basis) are present in oak acorn (Shimada, 2001). Because of their deleterious effects on poultry, tannins can be classified as anti-nutritional factors (Jansman, 1993), and their presence in animal diets is related with numerous nutritional problems (Butler, 1989). Many studies have shown that dietary tannins have negative effects on broilers (Sharif *et al.*, 2012; Medugu *et al.*, 2012; Hassan *et al.*, 2003).

There is increasing demand for food. Thus, tanniferous plants and agro-industrial by-products have received more interest to use in animal diets (Makkar, 1999). Due to their importance, different physical and chemical methods have been used to reduce the amounts of tannins and therefore, to improve the nutritional value of tannin-rich feedstuffs (Medugu *et al.*, 2012). Also, it has been suggested that dietary nutrient manipulation can alleviate the deleterious effects of tannins. For example, beneficial effects of supplemental methionine, choline, and other methyl group donors have been reported (Rayudu *et al.*, 1970, Chang & Fuller, 1964). In addition, dietary protein level can influence the effects of tannins (Jansman, 1993), thus, deleterious effects of tannins could be reduced or alleviated by increasing the level of dietary protein (Sell *et al.*, 1983).

Tannins have adverse effects on mineral availability and absorption (Jansman, 1993). Findings of Hassan *et al.* (2003) indicated that feeding sorghum grain containing high levels of tannins, significantly decreased the apparent absorption of minerals including Ca, P, Mg, Na, K, Fe and Co. The role of dietary minerals, particularly calcium, in bone structure and health is well documented (Rath *et al.*, 2000). Considering the negative effects of tannins on mineral utilization, it is expected that high tannin diets negatively influence bone structure and health. Elkin *et al.* (1978) reported that broilers fed with tannin-rich diets had higher incidence of leg abnormalities than those fed low-tannin diets. They concluded that tannins can alter the bone formation process. To our knowledge, there is no information on the influence of dietary nutrient manipulation on the performance and tibia characteristics of broiler chickens fed diets containing oak acorn. Thus, the current experiment was conducted to evaluate above-mentioned effects.

MATERIALS AND METHODS

Birds, diets, and experimental design

All procedures used in the current experiment were approved by the Institution Animal Care Committee of the University of Yasouj (30° 39′ 6″ North, 51° 36′ 54″ East). Three hundred one-day-old male and female broiler chicks (Cobb 500) with same initial weight (around 45 g) were obtained from a local commercial hatchery. Upon arrival, birds were weighed as a group and randomly allocated to one of the five experimental diets (treatments) as follows: one group was fed diet based on corn (without oak acorn) as control (Ctrl), and other four groups were fed diets containing 15% oak acorn with one of the following nutrient manipulations: no nutrient manipulation (NM), high level of methionine (100% more than NRC) (Met), high level of CP (10% more than NRC) (HP), and addition of 0.2% choline (Cho). Each treatment had four replicates of 15 chicks each. Isocaloric diets were formulated according to NRC (1994) recommendations using the UFFDA software. The starter and finisher diets were offered as mash from 1-21 and 22-42 d of age, respectively (Table 1). Feed and water were provided *ad libitum* throughout the experiment. Birds were reared on floor pens and rice straw was used as litter. Oak acorns were collected in October 2012, from the forest of Yasouj, Kohgeluyeh and Bovir Ahmad Province, Iran. In the researched forest, *Quercus brantii Lindl* is the most common species. The seed coat of fruits were removed and fruits were dried in shadow and then fine grinded and included in the experimental diets.

Feed intake was calculated weekly on a pen basis. All birds in each pen were weighed weekly as a group and feed conversion ratio (FCR) was calculated. Mortality (around 4% throughout the experiment) was recorded daily and FCR was adjusted for mortality.

Nutrient composition of oak acorn was determined according to the AOAC (1995). Acorns were firstly submitted to extraction to determine phenolic components. About 1 g ground oak acorn was accurately weighed and put in tubes and then, 3 mL of acetone (75%) and 3 mL methanol (50%) were added to the samples. The tubes were vortexed and then centrifuged (3000 g at 4° C) for 20 min. After that, the supernatant was removed and transferred to 2 mL tubes. The extracts were analyzed according to the procedure of Makkar (2003).

Sampling and measurements

On d 21 (end of starter phase) and 42 (end of finisher phase), one bird from each pen was sacrificed by cervical dislocation and its digestive system was immediately and carefully removed. The liver, pancreas, and abdominal fat pad were weighed. Their relative weights (organ weight/live weight × 100) were used for statistical analysis. At the same time (d 21 and 42), right and left

Table 1 – Composition of the experimental diets

Ingredients (%)	[1] Starter					[1] Finisher				
	Ctrl	NM	Met	Cho	HP	Ctrl	NM	Met	Cho	HP
Corn	56.24	38.06	38.05	38.06	30.55	65.60	47.63	47.62	47.63	41.17
Oak acorn	…	15	15	15	15	…	15	15	15	15
Soybean meal (44% CP)	34.21	35.99	35.99	35.99	42.38	27.37	29.1	28.62	29.1	34.65
Meat meal (50% CP)	3	3	3	3	3	2	2	2	2	2
Vegetable oil	2.96	4.45	4.61	4.45	5.66	1.86	3.18	3.3	3.18	4.17
Limestone	1.15	1.07	1.07	1.07	1.07	13	12.3	12.3	12.3	12.2
Dicalcium phosphate	1.4	1.35	1.36	1.35	1.28	1.02	0.97	0.98	0.97	0.91
Common salt	0.42	0.43	0.43	0.43	0.43	0.3	0.31	0.31	0.31	0.31
[2]Vitamins premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
[3]Minerals premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.13	0.15	0.63	0.15	0.13	0.06	0.08	0.44	0.08	0.06
Choline chloride	…	…	…	0.2	…	…	…	…	0.2	…
Analysis results										
ME (Kcal/kg)	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Crude protein,%	21.56	21.56	21.56	21.56	23.72	18.75	18.75	18.75	18.75	20.63
Calcium,%	0.94	0.94	0.94	0.94	0.94	0.85	0.85	0.85	0.85	0.85
Available P.,%	0.42	0.42	0.42	0.42	0.42	0.33	0.33	0.33	0.33	0.33
Lysine,%	1.03	1.03	1.03	1.03	1.03	0.94	0.94	0.94	0.94	0.94
Methionine,%	0.47	0.47	0.94	0.47	0.47	0.36	0.36	0.72	0.36	0.36

[1] Ctrl: Control diet (based on corn, without oak acorn), NM: Diet containing oak acorn without any nutrient manipulation , Met: Diet containing oak acorn with high level of methionine (100% more than NRC), Cho: Diet containing oak acorn added with 0.2% choline, HP: Diet containing oak acorn with high level of CP (10% more than NRC).

[2] The vitamin premix supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D3, 1,000 IU; vitamin E (dl-α-tocopherol), 30 IU; vitamin K3, 2.5 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B6, 2 mg; vitamin B12, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; d-pantothenate, 8 mg, folic acid, 0.5 mg.

[3] 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.

tibias were removed as drumsticks. Drumsticks were boiled for 10 min. After cooling, bones were manually defleshed and dried for 24 h at room temperature. Right and left tibia bones were used to determine the tibia breaking strength and ash, respectively. Tibia length, weight, and volume were accurately determined. Tibia breaking strength (breaking force divided by bone weight) was measured using an Instron Universal Tester Machine with a 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min. Supports were placed at tibia epiphysis and the force was applied in central fraction (Park *et al.*, 2003).

In order to determine bone ash, tibia bones were dried at 105° C for 24 h and then put in a furnace at 550° C for 24 h. Bone ash was calculated based on the dry weight of the tibia. Tibia ash weight/tibia bone length index was calculated by dividing the tibia ash weight by its length (Seedor *et al.*, 1991). Tibia robusticity index was calculated using following formula: Robusticity index = tibia length/cube root of tibia weight (Reisenfeld, 1972).

Data were submitted to analysis of variance using the General Linear Models (GLM) procedures of SAS software (2005). Means were compared by Duncan's Multiple Range Test. The level of statistical significance was set at $p< 0.05$.

RESULTS

Nutrient composition of oak acorn

As expected, the results indicated that nitrogen-free extract (NFE) was the main component of oak acorn (Table 2). In addition, high levels of tannins were detected.

Table 2 – Proximate analysis and phenolics components of oak acorn

Component	ash	CP	EE	CF	NFE	TP	TT	NTP
Value (% DM)	1.06	5.03	11.67	5.20	73.52	10.58	6.06	4.52

CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, DM: dry matter, TP: total phenolic compounds, TT: total tannins NTP: non tannin phenolic compounds

Performance

The effects of experimental treatments on broiler performance (body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)) are shown in Table 3. The findings indicate that there was no difference between the Ctrl and NM group in BWG, throughout the experiment (p>0.05). On the other hand, dietary inclusion of oak acorn had no detrimental effect on BWG. Birds fed the diet containing high level of protein (HP group) were heavier than those fed the other treatment diets (except for the Cho group at 1-21 d of age) during all phases of the experiment (1-21, 22-42 and 1-42 d of age). The supplementation with choline significantly increased BWG during 1-21 and 1-42 d of age relative to the NM group. Elevated levels of methionine had no effect on BWG (p>0.05). All groups fed diets containing oak acorn presented higher FI than the Ctrl group during the starter and finisher phases and during the entire experimental period (p< 0.05). The dietary inclusion of oak acorn negatively influenced FCR; therefore, the NM group had worse FCR compared with the Ctrl group throughout the experiment.

During starter phase (1-21 d of age), FCR was not different among the Ctrl and manipulated groups (Met, Cho and HP groups) (p>0.05). These results indicate that increasing the level of these nutrients have beneficial effects and can alleviate the adverse effects of oak acorn and of its anti-nutritional factors, such as tannins. The HP group (birds fed high protein diets) had better FCR than the NM group, during 1-21 and 1-42 d of age.

Tibia characteristics

The results of experimental treatments on tibia characteristics at 21 and 42 d of age (Table 4), indicate that on d 21, differences in length, volume and robusticity index were not observed among treatments (p>0.05). Tibia ash was lower in the NM group than the Ctrl group (p<0.05), while dietary addition of all nutrients (protein, methionine and choline) increased tibia ash compared with the NM group (p<0.05) and hence, their tibia ash content was similar to that of the Ctrl group. Interestingly, same results were observed for bone strength. Birds of the NM group presented lower bone strength values than the Ctrl group, while there was no difference between the Ctrl and manipulated groups (p>0.05).

On d 42, there were no differences among treatments for tibia length, volume, weight, or and two bone indexes (p>0.05). Similarly to the results obtained at 21 d of age, the NM group had lower tibia ash content and strength compared with the Ctrl group.

Abdominal fat pad, liver, and pancreas weights

The results indicated that abdominal fat pad, liver, and pancreas weights were not influenced by the dietary treatments (p>0.05) (data are not shown).

Table 3 – Effects of experimental treatments on body weight gain, feed intake, and feed conversion ratio of broilers at different phases of the experiment

Parameter	[1] Experimental treatments					
	Ctrl	NM	Met	Cho	HP	SEM
			Body weight gain (g)			
d 1-21	489[b]	475[b]	484[b]	601[a]	597[a]	12
d 22-42	1175[b]	1107[b]	1132[b]	1147[b]	1410[a]	47
d 1-42	1663[bc]	1582[c]	1616[bc]	1748[b]	2007[a]	48
Feed intake (g)						
d 1-21	924[d]	1046[bc]	986[bc]	1179[a]	1122[ab]	36
d 22-42	2214[c]	2890[b]	3061[ab]	3182[ab]	3285[a]	109
d 1-42	3137 [c]	3639[b]	4047[ab]	4360[a]	4406[a]	126
			Feed conversion ratio			
d 1-21	1.88[b]	2.20[a]	2.03[ab]	1.97[ab]	1.87[b]	0.074
d 22-42	1.89[c]	2.60[ab]	2.70[a]	2.78[a]	2.33[b]	0.094
d 1-42	1.88[c]	2.48[a]	2.50[a]	2.49[a]	2.19[b]	0.069

Means within a row with different superscript letters are significantly different at p<0.05.

[1] Ctrl: Control diet (based on corn, without oak acorn), NM: Diet containing oak acorn without any nutrient manipulation, Met: Diet containing oak acorn with high level of methionine (100% more than NRC), Cho: Diet containing oak acorn added with 0.2% choline, HP: Diet containing oak acorn with high level of CP (10% more than NRC).

Table 4 – Effects of experimental treatments on tibia characteristics of broilers at 21 and 42 d of age

Parameter	[1] Experimental treatments					
	Ctrl	NM	Met	Cho	HP	SEM
Weight (g)						
d 21	1.8[b]	1.9[b]	2.1[ab]	2.2[a]	1.9[b]	0.09
d 42	5.7	5.9	6.1	6.5	7	0.45
Length (cm)						
d 21	6.2	6.1	6.3	6.2	6.2	0.07
d 42	9.4	9.4	9.3	9.5	9.4	0.13
Volume (mL)						
d 21	2.5	2.5	3.5	3.5	3.2	0.38
d 42	8.5	7.2	8	7.8	8.8	0.8
Ash (%)						
d 21	41.9[a]	40.7[b]	42.5[a]	41.5[a]	43.3[a]	1.1
d 42	45.3[a]	39[b]	41.7[ab]	40.8[ab]	41.6[ab]	1.36
Strength (kg/m^2)						
d 21	27[ab]	15.5[c]	28.5[a]	20.5[bc]	21.7[bc]	2.9
d 42	43[a]	25[b]	33[ab]	31[ab]	34.5[ab]	3.86
[2] W/L (mg/mm)						
d 21	9.9[b]	13.8[a]	11.9[ab]	13[a]	13.1[a]	0.71
d 42	25.2	20.3	22.9	23.4	25.6	2.8
Robusticity index						
d 21	5.4	5.6	5.4	5.4	5.3	0.13
d 42	5.3	5.2	5.1	5.2	5.1	0.08

Means within a row with different superscript letters are significantly different at $p<0.05$.

[1] Ctrl: Control diet (based on corn, without oak acorn), NM: Diet containing oak acorn without any nutrient manipulation, Met: Diet containing oak acorn with high level of methionine (100% more than NRC), Cho: Diet containing oak acorn added with 0.2% choline, HP: Diet containing oak acorn with high level of CP (10% more than NRC).

[2] W/L: tibia ash weight / tibia length index

DISCUSSION

There are limited data in literature regarding the effects of oak acorn on broilers and poultry performance. The results of the current experiment indicated that dietary inclusion of 15% oak acorn had no adverse effect on BWG. These results are in agreement with the findings of Kaushal & Singh (1982), who reported that dietary inclusion of oak acorn at the rates of 10 and 15% had no detrimental effects on body weight gain, while 20 and 25% acorn significantly decreased BWG.

There are conflicting results on the influence of tannins on feed intake. Similar with our results, Nyachoti *et al.* (1996) reported an increase in the feed intake of broilers fed a high-tannin sorghum diet. As the AMEn value of the high-tannin diet was significantly reduced, those authors concluded that the increase in feed intake may reflect an attempt to compensate for reduced energy availability. Conversely, Hassan *et al.* (2003) reported that tannic acid significantly reduced broiler feed intake. The mechanisms by which tannins affect feed intake are not well understood. Tannins can attach to salivary proteins and cause dryness in the mouth, causing a stringent or bitter taste that affects diet palatability and hence, decrease feed intake. It should be mentioned that chickens have very few taste buds (around 24) in their mouth, and therefore, diet taste has probably no important effect on bird feed intake (Nyachoti *et al.,* 1996).

Adverse effects of high-tannin diets on broiler performance have previously been reported by many researchers (Sharif *et al.*, 2012; Medugu *et al.*, 2012; Hassan *et al.*, 2003). The main effects of tannins are related to their protein-binding capacity, thereby reducing protein digestibility, particularly in monogastric animals. In addition, tannins have negative effects on starch and energy digestion in poultry and pigs (Jansman, 1993). Tannins increase endogenous amino acid loss. They also have negative effects on the parotid glands and gastrointestinal mucus, intestinal villi height, and liver cells (Ortiz *et al.*, 1994), dietary metabolizable energy and efficiency of nitrogen utilization (Nyachoti *et al.,*1996). Inhibitory effects on the activity of digestive enzymes, such as α-amylase, β-amylase, trypsin, lipase, and β- glucosidase (Jansman, 1993) have been shown.

The results of the current experiment suggest that supplementation with protein, methionine, and choline may potentially reduce the deleterious effects of tannins and therefore are in line with those reported by other researchers (Chang & Fuller, 1964; Rayudu *et al.*, 1970). Armstrong *et al.* (1973) found that feeding with high-tannin diets impaired broiler performance (growth and feed efficiency), but the dietary methionine supplementation alone and in combination with choline significantly reduced the negative effects of tannins and improved birds performance. In contrast, Wareham *et al* (1991) reported that dietary methionine levels had no significant effects on fava bean tannins. They concluded that methionine does not act as a specific detoxifier of fava bean tannins. It has been shown that tannic acid is hydrolyzed to gallic acid. A high portion of this compound is methylated and excreted in the urine as 4-0-methyl gallic acid. On the other hand, methyl groups are needed for methylation of gallic acid. This is a possible explanation why the dietary supplementation with methionine, choline, and other methyl group donors have beneficial effects on the birds fed with tannic acid-rich diets (Potter & Fuller, 1968).

The results showed that high protein diets reduced the negative effects of high-tannin diet. As mentioned earlier, tannins can attach to proteins. High levels of dietary protein can alleviate the effects of tannins by two mechanisms: 1- provide limiting amino acids, which availability is reduced by tannins, and 2- provide more protein to complex with tannins and thereby reducing some other toxic effects of tannin (Sell *et al.*, 1983). However, due to two major problems (environmental pollution and economic issues), feeding high-protein diets is not recommended (Aftab *et al.*, 2006). Thus, it seems that supplementation with choline and methionine are more practical strategies to improve the nutritional value of oak acorn and to allow its use as an alternative to corn in broiler nutrition.

Different parameters can be used to determine bone mineralization and hence bone structure and health in poultry, including bone ash, bone breaking strength, bone weight, and bone volume (Rao *et al.*, 1993). Also, bone density is evaluated by two indexes: 1- the bone ash weight/bone length index, where higher values indicate more density (Monteagudo *et al.*, 1997), and 2- robusticity index, where lower values indicate stronger bones (Reisenfeld, 1972). The current results suggest that dietary inclusion of oak acorn (containing high levels of tannins) had detrimental effects on tibia characteristics (ash and strength) at 21 and 42 d of age. Dietary minerals play important roles in bone structure and health (Rath *et al.*, 2000). Mineral availability and absorption are negatively influenced by tannins (Jansman, 1993; Hassan *et al.*, 2003). Thus, reductions in tibia bone ash and strength could be attributed to the presence of the high levels of tannins in oak acorn. The results indicated that increasing the dietary levels of protein, methionine, and choline prevented detrimental effects of oak acorn on tibia bone. As previously discussed, all these nutrients may potentially reduce or eliminate the negative effects of tannins. The negative effects of high-tannin diets on the incidence and severity of leg abnormalities have previously been reported by other researchers (Elkin *et al.* 1978; Rostagno *et al.*, 1973).

The results indicate that the pancreas, liver and abdominal fat pad were not significantly influenced by the dietary treatments. In line with our results, Nyachoti *et al.* (1996) reported that tannins had no significant effect on liver and pancreas weight. They suggested that source of tannin can be considered as an important factor. Previously, it was reported that high tannin diets have inhibitory effects on pancreatic enzymes activities. Thus, the pancreas needs to increase enzyme production, resulting in pancreatic hypertrophy (Ahmed *et al.*, 1991). It should be considered that effects of tannins are influenced by different factors such as the response parameters chosen (weight gain, feed intake, and feed efficiency), source and concentration of tannins, animal factors (species, age, and production level), and diet composition (Jansman, 1993).

In conclusion, dietary inclusion of 15% oak acorn had negative effects on broiler performance and tibia characteristics. These negative effects could be alleviated or prevented by increasing the levels of dietary choline, methionine, and protein. It is concluded that oak acorn may potentially be used in broiler diets, but the level of some dietary nutrients should be increased. In addition, more studies on the type, level, and possible role of dietary nutrients in reducing the negative effects of oak acorn are needed.

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