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Effects of Different Levels of Raw and Processed Oak Acorn (*Quercus castaneifolia*) on Performance, Small Intestine Morphology, Ileal Digestibility of Nutrients, Carcass Characteristics and Some Blood Parameters in Broiler Chickens

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Abstract

This study was conducted to determine the effect of oak acorn (*Quercus castaneifolia*) on performance, small intestine morphology, ileal digestibility of nutrients, carcass characteristics and some blood parameters in broiler chickens. A total of 504 1-d-old male chickens (Ross 308 strain) were divided into seven treatments with four replicates each. Experimental diets were: corn-soybean meal diet (control), raw oak acorn (10% and 20%), soaked oak acorn (10% and 20% oak acorn soaked in water for 24 hrs), and 10% and 20% oak acorn soaked in water for 48 hrs with twice water substitution. Chicks were fed with experimental diets from 1 to 42 days of age. Results showed that body weight, weight gain, feed intake, feed conversion ratio, ileal dry matter, protein digestibility, and small intestine morphology was significantly ($P < 0.05$) deteriorated with the inclusion of raw oak acorn in the diet. Findings showed that water-soaking of oak acorn had significant positive effects in reducing negative impacts of raw seed inclusion in diets ($P < 0.05$), although birds still had significantly deleterious performance criterion compared to the control group. The birds fed raw or water-soaked acorn seed had higher relative weight of pancreas and proventriclus compared to the control. At the end of the experiment (42 d) birds fed with raw and processed oak acorn in diet had lower ($P < 0.05$) villus height and villus : crypt depth and higher ($P < 0.05$) crypt depth and goblet cells in duodenum compared to the control diet. In conclusion, using high levels of oak acorn (up to 20%)in broiler diets has severe adverse effects on broiler performance and gut morphology ; however, water-soaking treatment of oak acorn seed has a potential to reduce its negative consequences.

Introduction

Although corn and soybean meal are the most common feed ingredients used in poultry diets in many regions of the world, several countries, including Iran, rely on its import. The introduction of alternative feed ingredients for animal and poultry nutrition would have to be nutritional and economical. Some studies have showed that applying some alternative feed

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ingredients in poultry diets resulted in lower performance usually due to anti-nutrients or inhibitor factors (Nelson *et al*., 1996; Bouderoua and Selselet-Attou, 2003; Varmaghani *et al*., 2007; Mohamed and Khadiga., 2008; Sadeghi *et al*., 2009). Therefore, determination of appropriate levels of alternative feedstuffs into poultry diets and strategies to decrease their negatives effects on animals are a considerable effort.

Oak acorn and oak tree seed (*Quercus castaneifolia*) are alternative feedstuffs used in some countries with accessibility to the plant as food drug and animal feed. Use of oak acorn for animal nutrition is most common in Mediterranean countries (Bouderoua and Selselet-Attou, 2003; Gasmi-Boubaker *et al*., 2007). Based on available data, more than 50% (16 million hectares) of Iranian forests are composed of oak tree forests which are mainly located along the ridges of Caspian Sea in the north and Zagros mountains in the west (Kafash *et al*., 2009). Oak tree belongs to the *Quercus* genus of *Fagacea* family. In Iran, the oak genus contains 8 species which exist in the west, north and north western forests. Species include *Q. brantii*, *Q. Castaneifolia*, *Q. Ilex*, *Q. Infectoria Oliv*, *Q. Libani*, *Q. Macranthera*, *Q. Petraea* and *Q. Suber* (Masodi Nejad and Rezazadeh-Azari, 2004). Because of the presence of high percentage of starch (up to 60%) in oak acorns, it has good potential to be used as animal and poultry feed. In addition, oak acorns contain about 7-14.4% lipids and 2-8% protein. Dominant fatty acids in oak acorns include oleic acid (66.8%), palmitic acid (18.4%) and linoleic acid (13.5%) (Bouderoua and Selselet-Attou, 2003; Lopes and Bernardo-Gil., 2005; Varmaghani *et al*., 2007; Rababah *et al*., 2008).

It has been well established that many of the anti-nutrients in oak acorn reduce its efficiency in animal nutrition (Bouderoua and Selselet-Attou, 2003; Varmaghani *et al*., 2007). One of the major anti-nutrients found in oak acorn are tannins (up to 9% of dry matter). Barras *et al*., (1996) indicated that metabolizable energy content of *Q. Phellos* and *Q. Nuttallii* differed and were 2,330 and 1,440 Kcal/kg dry matter of oak acorn, respectively.

Few studies have been carried out to investigate the impacts of feeding oak acorns with high levels of tannins on broiler feed consumption. Varmaghani *et al*., (2007) showed that feeding different levels of de-tannificated oak acorns (5, 10, 15 and 20% in diet) to broilers did not have a significant effect on feed intake compared to corn-soybean meal based diets. It has been shown that tannin content of different species of oak trees reduced from south to north of Alborz mountains as rainfall increased (Masodi Nejad and Rezazadeh Azary, 2004).

The objective of this experiment was to evaluate the effects of two levels of raw and processed oak acorn on broilers performance, ileal-nutrient digestibility, some blood parameters, carcass characteristics and morphology of small intestine.

Materials and Methods

Bird husbandry

All procedures used in this research were approved by Ethic committee of Sari University of Agricultural Sciences and Natural Resources, Sari, Iran. A total of 504 1-d-old Ross 308 male broiler chicks provided from a local hatchery were allocated to 28 floor pens (1.2 × 1.5 m) arranged in a well-ventilated windowless house and reared at a density of 0.08 m^2/bird. Each pen contained 18 chickens and was covered with wood shaving as litter material to a depth of approximately 5 cm and equipped with a bell-type drinker and a tube feeder. In the first two days of the experiment, lighting schedule was continuous but afterwards, a 23L: 1D lighting schedule was used until the end of the experiment. House temperature was maintained at 32°C at the start of the experiment and gradually reduced until 22°C was reached when birds were 24 d old, at which point the temperature was held until the end of the experiment (when birds were 42 d old).

Dietary treatments

Diets were formulated to meet nutritional requirements of broilers according to Ross 308 strain broiler recommendations (Table 1; Aviagen, 2014). Dietary treatments were T_1) basal corn-soybean meal diet (control); T_2) 10% raw oak acorn; T_3) 20% raw oak acorn; T_4) 10% oak acorn soaked in water for 24 hrs, T_5) 20% oak acorn soaked in water for 24 hrs, T_6) 10% oak acorn soaked in water for 48h and T_7) 20% oak acorn soaked in water for 48 hrs. For preparation of treatments T_4, T_5, T_6 and T_7, milled oak acorns (*Quercus castaneifolia*) were soaked in tap water (1:10) for 24 or 48 hrs and then air-dried. Oak acorn chemical composition was determined (expressed as %): 91.50 dry matter, 6.20 crude protein, 3.15 total ash, 0.29 calcium, 0.15 total phosphorus and 3.30 ether extract (AOAC, 1994). Anti-nutritional compounds

were also determined (expressed as %): 2.84 total phenol, 2.31 total tannin, 1.93 hydrolysable tannin, and 0.21 condensed tannin. Each treatment was replicated four times. Feed and water were provided *ad libitum*. All diets were provided in mash form.

Measured parameters

This experiment was conducted for 42 d. Bird weight and feed intake were measured by pen. Feed conversion ratio was calculated after adjusting for daily mortality. At the end of the experiment, two birds were randomly selected from each pen and the chickens were weighed and then killed by cervical dislocation. The weights of different gastrointestinal organs included liver without gall-bladder, pancreas, heart, proventriculus, gizzard, small intestine, spleen and bursa were recorded and their relative weights (g/100 g of BW) were determined. Moreover, at 42 d, about 3 mL of blood from each sampled chickens (two birds per pen) were collected in tubes containing EDTA for total hemoglobin (Hemoglobin reagent set, Cat. No. Zistchem) and glucose measurements. The blood samples for serum were clotted at room temperature for approximately 1 hr (tubes without EDTA). Blood samples were then centrifuged at 12,000 × *g* for 5 min, and serum was obtained. Serum total protein, albumin, and globulin were determined using an automated analyzer.

Ileal digestibility of dry matter and protein was determined by supplementation of 0.35% Cr_2O_3 (Merck company, Germany) to diet as the ingestible marker (Fenton and Fenton, 1979). After killing the broilers, the ileum segment of the small intestine immediately exposed and the digesta samples between Meckel's diverticulum and the cecal junction were taken for measurement of ileal dry matter and crude protein digestibility at 42 d of age. In addition, for evaluation of morphology of small intestine, the different segments of intestine including the duodenum (from the gizzard outlet to the end of the pancreatic loop), jejunum (segment between the pancreatic loop and Meckel's diverticulum) and ileum (segment between Meckel's diverticulum and the cecal junction) were identified. 5-cm samples of the middle part of the each segment of small intestine from two selected broilers of each pen were removed. The segments were flushed with physiological saline and immediately placed in 10% buffered formalin solution until further morphological evaluation.

Statistical Analysis

Data were analyzed according to the GLM procedure of SAS (1996) as a completely randomized design. Significant differences among treatments were determined at a 0.05 probability by Duncan's multiple range tests. Each pen was used as the experimental unit. Additional comparisons were also performed by subjecting the data to orthogonal contrast.

Results and Discussion

Bird performance

The effects of different levels of raw and processed oak acorn on body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) are shown in Table 2. Supplementation of diet with raw oak acorn at both 10% and 20% significantly decreased BW, BWG, and feed intake at day 21 and 42 ($P < 0.05$), and these adverse effects were higher, as level of oak acorn increased ($P < 0.05$). Similarly, supplementation of processed oak acorn significantly decreased BW, BWG, and feed intake of broilers in comparison with broilers fed corn-soybean meal based diet. However, adverse effects caused by these diets were lower than those of diets supplemented with raw oak acorns. The FCR in broilers fed with diets supplemented with 20% raw oak acorn significantly increased compared to control and 10% raw oak acorn ($P < 0.05$). Using different levels of processed oak acorn did not have a significant effect on FCR.

In general, our results suggest that using raw oak acorn, particularly at 20% in the diet, significantly decreased bird performance in comparison to control group. These results are similar to those reported by Nowar *et al*. (1994) and Bouderoua and Selselet-Attou (2003), who also found that diets supplemented with oak acorn adversely influenced bird performance. Raw oak acorn decreased feed intake and BWG which resulted in a higher FCR because of lower availability of nutrients such as energy and proteins, particularly sulfurous amino acids (Mansoori and Acamovic, 1996; Bouderoua and Selselet-Attou, 2003; Mansoori *et al.*, 2007). The reduction in bird performance is associated with tannins in oak acorns, which are inhibitors and major decreasing factors of animals' growth (Farrell and Perez-Maldonado, 2000).

Table 1. Ingredients and chemical composition of experimental diets (%)

Item	1-10d			11- 28d			29- 42d		
	Control	10% oak acorn	20% oak acorn	Control	10% oak acorn	20% oak acorn	Control	10% oak acorn	20% oak acorn
Ingredients									
Yellow corn	55.76	44.04	32.30	60.15	48.44	36.71	65.67	53.94	42.22
Soybean meal44%CP	37.36	38.55	39.74	32.06	33.25	34.44	26.73	27.92	29.11
Soybean oil	2.35	3.00	3.66	3.77	4.42	5.07	3.69	4.34	4.99
Oak acorn	00.0	10.00	20.0	00.0	10.00	20.00	00.00	10.00	20.00
Dicalcium phosphate	1.88	1.86	1.84	1.66	1.64	1.63	1.70	1.69	1.67
Oyster shell	1.22	1.14	1.07	1.12	1.04	0.96	1.13	1.05	0.98
Salt	0.34	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL-Methionine	0.30	0.30	0.31	0.22	0.22	0.23	0.14	0.15	0.15
L-Lysine	0. 29	0.26	0.23	0.17	0.14	0.11	0.09	0.06	0.03
Vitamin premix[1]	0.25	0.25	0.25	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	0.25	0.25	0.25
Chemical composition									
ME (Kcal/kg)	2,850	2,850	2,850	2, 983	2, 983	2, 983	3,030	3,030	3,030
CP (%)	21.0	21.0	21.0	19.0	19.0	19.0	17.12	17.12	17.12
SAA (%)	1.03	1.03	1.03	0.89	0.89	0.89	0.758	0.758	0.758
Met (%)	0.66	0.66	0.66	0.56	0.56	0.56	0.459	0.459	0.459
Lys (%)	1.36	1.36	1.36	1.14	1.14	1.14	0.947	0.947	0.947
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Ca (%)	0.947	0.947	0.947	0.852	0.852	0.852	0.852	0.852	0.852
Available P (%)	0.474	0.474	0.474	0.426	0.426	0.426	0.426	0.426	0.426

[1]The vitamin premix supplied the following per kilogram of diet: vitamin A, 9000 IU; vitamin D3, 2000 IU; vitamin E, 50 IU; vitamin K3, 2 mg; B1, 2 mg; B2, 7 mg; B6, 3 mg; vitamin B12, 0.015 mg; niacin, 30 mg; choline chloride, 250 mg; calcium D-pantothenate, 10 mg; folic acid, 1 mg.
[2]The mineral premix supplied the following per kilogram of diet: Mn, 100 mg; Fe, 50 mg; Zn, 85 mg; Cu, 10 mg; I, 1 mg; Se, 0. 2 mg.

Table 2. Effects of oak level, processing and interaction between level and processing on average body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens

Treatments		BW (g)			BWG (g)			FI (g)			FCR		
		1d	21d	42d	1-21	21-42	1-42	1-21	21-42	1-42	1-21	21-42	1-42
Control		46.72	743.50^{a}	2331.62^{a}	696.78^{a}	1588.12^{a}	2313.55^{a}	1032.25^{a}	3046.75^{a}	4079.00^{a}	1.48^{a}	1.92^{b}	1.89^{b}
Raw oak acorn (R)	10%	45.63	627.10^{de}	2095.25^{c}	581.48^{ed}	1468.12^{b}	2049.63^{d}	902.25^{bc}	2914.75^{bc}	3817.00^{bc}	1.55^{a}	2.01^{b}	1.88^{a}
	20%	45.78	598.69^{e}	1925.25^{d}	552.91^{bc}	1330.56^{c}	1883.47^{e}	731.25^{d}	2765.75^{d}	3497.00^{d}	1.32^{b}	2.14^{a}	1.86^{a}
Processed oak acorn (P_{24})	10%	46.88	673.72^{bc}	2169.63^{bc}	626.85^{cde}	1495.91^{ab}	2122.75^{bc}	903.00^{bc}	2924.00^{bc}	3827.00^{bc}	1.44^{ab}	1.95^{b}	1.80^{b}
	20%	46.09	638.18^{cde}	2120.25^{c}	592.09^{b}	1482.07^{b}	2074.16^{cd}	894.00^{c}	2836.50^{cd}	3730.50^{c}	1.51^{a}	1.91^{b}	1.79^{b}
Processed oak acorn (P_{48})	10%	46.11	704.32^{b}	2219.13^{b}	658.23^{bcd}	1514.81^{ab}	2173.04^{b}	1021.50^{a}	2952.25^{b}	2973.75^{ab}	1.55^{a}	1.94^{b}	1.83^{ab}
	20%	45.88	666.85^{bcd}	2156.00^{bc}	620.97^{bcd}	1489.15^{b}	2110.12^{bcd}	976.25^{ab}	2816.25^{d}	3792.50^{c}	1.58a	1.89^{b}	1.80^{b}
SEM		0.152	9.721	25.35	9.69	18.16	26.15	20.025	19.20	35.66	0.02	0.02	0.01
P-Value		0.217	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0041	0.0052	0.0085
Orthogonal contrast						Probability							
Control *vs.* R		NS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS	0.0048	0.0004
Control *vs.* P_{24}		NS	<0.0001	<0.0001	<0.0001	0.0132	<0.0001	0.0002	0.0003	<0.0001	NS	NS	NS
Control *vs.* P_{48}		NS	0.0015	0.0008	0.0018	0.0285	<0.0001	NS	0.0003	0.0034	NS	NS	NS
R *vs.* P_{24}		NS	0.0032	<0.0001	0.0040	0.0016	<0.0001	0.0034	NS	0.0397	NS	0.0021	0.0017
R *vs.* P_{48}		NS	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	<0.0001	NS	0.0003	0.0053	0.0010	0.0059
P_{24} *vs.* P_{48}		NS	0.0325	NS	0.0314	NS	NS	0.0006	NS	0.0429	0.0451	NS	NS

$^{a-d}$Means within a column with no common superscript differ significantly ($P < 0.05$).
P_{24}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 24 hrs.
P_{48}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 48 hrs.
NS: not significant ($P > 0.05$).

The presence of higher concentrations of tannin components (such as condensed and hydrolysable tannin) in diet can negatively affect feed intake and bird growth by disturbing metabolic processes (Cousins *et al*., 1981; Laurena *et al*., 1984; Bouderoua and Selselet-Attou, 2003; Mansoori and Acamovic, 2007), damaging mucosal membranes of enterocytes (Yamauchi *et al*., 1990; Koenig, 1991; Mansoori et al., 2007; Varmaghani *et al*., 2007) and eliciting astringent effects of tannins (Marzo *et al*., 1990; Potter and Fuller, 1998; Shimada *et al*., 2006).

Soaking the oak acorn in water for 24 hrs partially reduced the effects of anti-nutrients of oak acorn, though it did not completely eliminate the negative effects. Varmaghani *et al*., (2007) reported that diet supplemented with 20% detannificated oak acorn had no significant effect on broiler feed consumption.

Blood parameters

At 42 d of age, experimental treatments had no significant effects on measured parameters of hemoglobin, glucose, and albumin (Table 3). These findings are in contrast with results reported by Bouderoua and Selselet-Attou (2003) and may be due in part to the lower concentration oak acorn we used (10 and 20%) in comparison to the 60% they used. The species of oak acorn was also different.

Table 3. Effects of dietary treatments on serum glucose, hemoglobin, albumin and total protein concentrations at 42 d of age

Treatments		Glucose (mg/dL)	Hemoglobin (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Total protein (g/dL)
Control (C)		179.14	6.29	1.80	1.62	3.42
Raw oak acorn (R)	10%	201.63	5.97	1.56	1.41	2.97
	20%	192.57	6.31	1.34	1.21	2.55
Soaked oak acorn (P_{24})	10%	190.50	5.25	1.55	1.39	2.94
	20%	201.00	5.85	1.54	1.38	2.93
Soaked oak acorn (P_{48})	10%	184.14	5.62	1.69	1.42	2.21
	20%	208.25	5.94	1.27	1.15	2.42
SEM		5.688	0.10	0.053	0.048	0.102
P-Value		0.8501	0.052	0.1315	0.1315	0.1316
Orthogonal contrast				Probability		
Control *vs.* R		NS	NS	NS	NS	NS
Control *vs.* P_{24}		NS	NS	NS	NS	NS
Control *vs.* P_{48}		NS	NS	NS	NS	NS
R *vs.* P_{24}		NS	0.0036	NS	NS	NS
R *vs.* P_{48}		NS	0.0388	NS	NS	NS
P_{24} *vs.* P_{48}		NS	NS	NS	NS	NS

[a-d]Means within a column with no common superscript differ significantly ($P < 0.05$).
P_{24}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 24 hrs.
P_{48}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 48 hrs.
NS: not significant ($P > 0.05$).

Carcass characteristics

At 42 d of age, relative weights of liver, gizzard, abdominal fat, bursa of fabricious, spleen were not affected by dietary treatments (Table 4). However, relative carcass and intestine weights were affected by dietary treatments ($P < 0.05$). Relative carcass weight decreased in diets containing 20% raw oak acorn in compared with treatments containing 10% raw oak acorn or 10 and 20% processed oak acorn. There was significant difference for relative intestine weight between control group and treatments containing 10 and 20% raw oak acorn or 20% oak acorn processed for 24 h. In general, the presence of oak acorn in diet had no significant effect on other carcass characteristic parameters.

Table 4. Effects of dietary treatments on carcass yield, relative weights of pancreas, liver, proventriculus, gizzard, abdominal fat, bursa, spleen, intestine at 42 d of age (% of live body weight)

Treatments		Carcass	Liver	Pancreas	Proventriculus	Gizzard	Abdominal fat	Spleen	Intestine
Control		68.93^{ab}	2.50	0.219	0.396	1.65	1.79	0.108	2.74^{c}
Raw oak acorn (R)	10%	72.28^{a}	2.89	0.280	0.468	1.61	1.75	0.100	3.26^{ab}
	20%	64.57^{b}	2.65	0.282	0.493	1.53	1.86	0.116	3.30^{ab}
Soaked oak acorn P_{24})	10%	71.24^{a}	2.88	0.285	0.463	1.62	1.58	0.103	3.17^{abc}
	20%	69.63^{a}	3.14	0.285	0.535	1.65	1.80	0.114	3.41^{a}
Soaked oak acorn (P_{48})	10%	71.06^{a}	2.61	0.224	0.405	1.68	1.67	0.113	3.05^{abc}
	20%	71.11^{a}	2.79	0.258	0.419	1.66	1.91	0.143	2.89^{bc}
SEM		0.647	0.067	0.0052	0.010	0.021	0.051	0.004	0.061
P-Value		0.0151	0.1742	0.0838	0.4535	0.5595	0.6938	0.0662	0.0319
Orthogonal contrast		Probability							
Control *vs.* R		NS	NS	0.0202	NS	NS	NS	NS	NS
Control *vs.* P_{24}		NS	0.0189	NS	NS	NS	NS	NS	NS
Control *vs.* P_{48}		NS	NS	NS	NS	NS	NS	NS	NS
R *vs.* P_{24}		NS	NS	0.0222	NS	NS	NS	NS	NS
R *vs.* P_{48}		NS	NS	NS	NS	NS	NS	0.0420	NS
P_{24} *vs.* P_{48}		NS	NS	NS	NS	NS	NS	0.0420	NS

Values of relative weights of organs expressed as percentage of body weight.
[a-d]Means within a column with no common superscript differ significantly ($P < 0.05$).
P_{24}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 24 hrs.
P_{48}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 48 hrs.
NS: not significant ($P > 0.05$).

These results of carcass characteristics were consistent with those of Varmaghani et al., (2007). Liukkonen *et al*., (2001) reported that feeding grey partridge diets containing 6% tannic acid increased intestine length, which was caused by increased mobility of the gut in favor of detoxification of tannic acid in feed. The enhancement in pancreas size of broilers fed diets with raw or processed oak acorn was a type of hyperplasia in response to tannic acid in the gut which might be due to the secretion of secretin and cholecystokinin (CCK) into blood (Ahmed *et al*., 1991; Nyachoti *et al*., 1996). Ahmed *et al*., (1991) indicated that pancreas size and activity of trypsin and alpha-amylase increased as the level of tannic acid increased in broiler diet. They reported that the effects of tannin on gut enzymes were mediated by an increase in the release of CCK.

Nutrient digestibility

Ileal digestibility of dry matter and crude protein were significantly reduced in chikens fed raw and processed oak acorn ($P < 0.05$; Table 5). Processing of raw oak acorns by soaking in water could partly decrease negative effects of raw oak acorn on dry matter and to some extent crude protein digestibility.

Table 5. Effects of dietary treatments on dry matter and crude protein digestibility at 42 d of age

Treatments		Digestibility (%)	
		Dry matter	Crude protein
Control (C)		88.82[a]	73.92[a]
Raw oak acorn (R)	10%	87.04[c]	69.77[b]
	20%	84.56[e]	64.68[d]
Soaked oak acorn (P_{24})	10%	87.63[bc]	69.72[b]
	20%	85.96[d]	66.44[cd]
Soaked oak acorn (P_{48})	10%	88.49[ab]	70.02[b]
	20%	88.45[ab]	67.99[bc]
SEM		0.223	0.439
P-Value		<0.0001	<0.0001

Orthogonal contrast	Probability	
Control *vs.* R	<.0001	<.0001
Control *vs.* P_{24}	<.0001	<.0001
Control *vs.* P_{48}	NS	<.0001
R *vs.* P_{24}	0.0037	NS
R *vs.* P_{48}	<.0001	0.0098
P_{24} *vs.* P_{48}	<.0001	NS

[a-d]Means within a column with no common superscript differ significantly ($P < 0.05$).
P_{24}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 24 hrs.
P_{48}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 48 hrs.
NS: not significant ($P > 0.05$).

The negative impacts of oak acorn in ruminants have been previously reported (Laurena *et al*., 1984; Moujahed *et al*., 2007). There remains consistency with the effects of raw oak acorns on nutrients digestibility in non-ruminants, particularly poultry. A decline in protein, starch, and organic matter digestibility have been showed in pigs fed diets supplemented with raw oak acorn in comparison with corn-soybean meal based diet because of the existence of tannin in oak acorns (Nieto *et al*., 2002; Morales *et al*., 2003). Schiavone *et al*., (2008) reported that addition of 0.25% chestnut to broiler diet did not have any significant effect on dry matter, protein and fat digestibility, which may be due to the low concentration of condensed tannin and presence of castalagin in chestnut, which is less toxic and less potent in protein binding compared to tannins (Schiavone *et al*., 2008). Therefore, the

results of our experiment regarding the effects of oak acorns on nutrient digestibility were similar to other reports (Nieto *et al*., 2002; Morales *et al*., 2003). Lower dry matter and protein digestibility of broilers fed diets supplemented with raw and processed oak acorns may be due to the presence of higher levels of condensed tannin which can bind to proteins and some carbohydrates more than hydrolysable tannins (Koenig, 1991).

Cousins *et al*., (1981) suggested that lower crude protein, proline, glycine, and histidine digestibility might be due to increased gut endogenous secretions of pigs given diets incorporated with high tannin content sorghum. Increases in voided nitrogen components in birds fed diets supplemented with tannic acid may be due to two major reasons: 1) increased mucosal secretions of gut; and 2) increased catabolism of endogenous proteins to detoxify tannins and tannin derivatives in lumen of gut (Jansman *et al*., 1993; Mansoori and Acamovic, 1996, 2007; Hassan *et al*., 2003). Increase in mucin secretion due to the present of tannins in the gut has been well documented in broilers, which can elevate essential amino acids requirements (Yasar and Forbes, 2000; Wu *et al*., 2004). In these situations, requirements of methionine, cysteine and choline increase so these components act in detoxifying tannins as methyl donors in gut lumen (Potter and Fuller, 1998; Mansoori and Acamovic, 2007).

Small intestine morphology

The effects of raw and processed oak acorns in diet on villus height, crypt depth, villus height-to-crypt depth ratio, and goblet cell counts of duodenal segments of broilers are shown in Table 6. Raw oak acorn significantly decreased villus height and villus height-to-crypt depth ratio and increased crypt depth and goblet cell counts in the duodenum, jejunum and ileum ($P < 0.05$).

Table 6. Effects of experimental treatments on duodenum villus height (μm), crypt depth (μm), villus height-to-crypt depth ratio and goblet cell counts at 42 d of age

Treatments		Villus height (μm)	Crypt depth (μm)	Villus: crypt (μm)	Goblet cells (100 μm of villus height)
Control (C)		1781.78^{a}	187.01^{c}	9.54^{a}	124.00^{d}
Raw oak acorn (R)	10%	1421.99^{b}	230.64^{ab}	6.17^{c}	143.00^{bc}
	20%	1083.45^{c}	246.91^{a}	4.41^{d}	164.25^{a}
Soaked oak acorn (P_{24})	10%	1683.45^{a}	227.69^{b}	7.43^{b}	151.38^{ab}
	20%	1488.10^{b}	240.88^{ab}	6.28^{c}	151.00^{ab}
Soaked oak acorn (P_{48})	10%	1723.38^{a}	222.43^{b}	7.78^{b}	131.33^{cd}
	20%	1470.25^{b}	226.49^{b}	6.45^{c}	141.50^{bcd}
SEM		34.431	3.384	0.235	2.706
P-Value		<0.0001	<0.0001	<0.0001	0.0004
Orthogonal contrast		Probability			
Control *vs.* R		<0.0001	<0.0001	<0.0001	0.0001
Control *vs.* P_{24}		0.0001	<0.0001	<0.0001	0.0004
Control *vs.* P_{48}		0.0004	<0.0001	<0.0001	NS
R *vs.* P_{24}		<0.0001	NS	<0.0001	NS
R *vs.* P_{48}		<0.0001	0.0304	<0.0001	0.0064
P_{24} *vs.* P_{48}		NS	NS	NS	0.0181

$^{a-d}$Means within a column with no common superscript differ significantly ($P < 0.05$).
P_{24}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 24 hrs.
P_{48}: milled oak acorns (*Quercus castaneifolia*) samples were soaked in water (1:10) for 48 hrs.
NS: not significant ($P > 0.05$).

These negative effects worsened when oak acorn dosage increased to 20% ($P < 0.05$). Processing of oak acorn partially improved morphological parameters, and as a result, treatment of 10% processed oak acorn did not have significant effect on duodenum villus height nor a significant effect compared to control. Crypt depth and goblet cell counts of duodenum

significantly increased in broilers fed 10 and 20% of processed oak acorn ($P < 0.05$). Decrease in villus height was representative of the presence of toxicants in the lumen, which might negatively affect nutrient absorption in the small intestine (Yamauchi *et al*., 1990; Wu *et al*., 2004). As crypts are the producing places of the villus, any increases in crypt depth means a greater turnover of gut epithelium, suggestive of repair and reconstruction of villus against inflammations caused by pathogens and toxicants (Yamauchi *et al*., 1990). Therefore, it can be concluded that the decrease of villus height and increase of crypt depth could decrease nutrient digestibility, increase gut mucosal fluids and negatively affect bird performance (Yamauchi *et al*., 1990).

As shown in Table 6, with increasing levels of raw and processed oak acorn in diet, villus height and crypt depth of duodenum decreased and increased, respectively, which may be due to deleterious effects of anti-nutritional components of oak acorns on intestine morphological parameters such as hyperplasia and increased turnover of epithelial cells and subsequently, increased maintenance costs of small intestine. Goblet cells are the preservative cells of the small intestine and have a major role in the conservation of epithelial cells of the small intestine by producing and secreting mucosa (mucin) (Yasar and Forbes, 2000). The increase in goblet cell counts in broilers fed diets supplemented with raw and processed oak acorn may be due to increased mucin secretion from the intestine. Mucin secretion in the gut is a response to increased tannic acid content in the lumen which protects the brush border membrane against negative impacts of tannic acids (Marzo *et al*., 1990; Mansoori and Acamovic, 1996, 2007).

Conclusion

Based on the results of the present experiment, it can be concluded that using water-soaked oak acorns could partially reduce negative impacts of raw oak acorn on broiler performance, nutrient digestibility, and small intestine morphology, but soaking does not completely eliminate these negative effects.

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اثر سطوح مختلف دانه بلوط خام و فرآوری شده بر عملکرد، ریخت‌شناسی روده، قابلیت هضم ایلئومی مواد مغذی، خصوصیات لاشه و برخی از فراسنجه‌های خون در جوجه‌های گوشتی

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چکیده

این مطالعه به منظور بررسی اثر دانه بلوط بر عملکرد، ریخت‌شناسی روده، قابلیت هضم ایلئومی مواد مغذی، خصوصیات لاشه و برخی از فراسنجه‌های خون جوجه‌های گوشتی انجام شد. تعداد ۵۴۰ قطعه جوجه یک‌روزه گوشتی سویه تجاری راس ۳۰۸ بین هفت تیمار و ۴ تکرار در هر تیمار توزیع شدند. جیره‌های آزمایشی شامل جیره‌ی حاوی ذرت و کنجاله سویا (شاهد) جیره‌های حاوی ۱۰ و ۲۰ درصد دانه بلوط خام و جیره‌های حاوی ۱۰ و ۲۰ درصد دانه بلوط خیسانده شده در آب به مدت ۲۴ ساعت و جیره‌های حاوی ۱۰ و ۲۰ درصد دانه بلوط خیسانده شده در آب به‌مدت ۴۸ ساعت بود. جوجه‌ها از ۱ تا ۴۲ روزگی با جیره‌های آزمایشی تغذیه شدند. نتایج نشان داد که وزن بدن، افزایش وزن و مصرف خوراک، ضریب تبدیل غذایی، قابلیت هضم ماده خشک و ریخت‌شناسی روده با افزودن دانه بلوط خام به جیره به‌طور منفی تحت تاثیر قرار گرفت ($P < ۰/۰۵$). خیساندن دانه بلوط در آب تاثیر مثبت بر کاهش اثرات منفی گنجاندن دانه بلوط خام در جیره‌ها داشت، اگرچه کاهش معنی‌داری در عملکرد جوجه‌ها در مقایسه با تیمار شاهد وجود داشت. وزن نسبی پانکراس و پیش معده در پرندگان تغذیه شده با بلوط خام یا فرآوری شده افزایش یافت. در پایان آزمایش (سن ۴۲ روزگی) ارتفاع پرز و نسبت ارتفاع پرز به عمق کریپت در جوجه‌های تغذیه شده با بلوط خام یا فرآوری شده کاهش و عمق کریپت و تعداد سلول‌های گابلت در دوازدهه در مقایسه با تیمار شاهد افزایش یافت ($P < ۰/۰۵$). به‌طورکلی استفاده از دانه بلوط (تا سطح ۲۰ درصد) در جیره جوجه‌های گوشتی دارای اثرات منفی بر عملکرد و ریخت‌شناسی روده بود. خیساندن دانه بلوط خام در آب توانایی کاهش اثرات منفی گنجاندن دانه بلوط خام در جیره‌ها را نشان داد.

کلمات کلیدی

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