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The effect of adding pomegranate seed extract to fat-containing diets on nutrients digestibility, intestinal microflora and growth performance of broilers

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Summary

The present study was aimed at studying the effects of inclusion of pomegranate seed extract to fat-containing diets on nutrients digestibility, intestinal microflora and performance of broilers. A total of 320 one-day-old broiler chicks (Ross 308) were selected in a 2 × 2 × 2 factorial experiment in a completely randomized design with 4 replicates of 10 birds of each. The dietary treatments consisted of pomegranate seed extract (0 and 2%), commercial Nutriad® antioxidant (0 and 0.01%) and soybean oil without antioxidant (0 and 6% during the grower period and 0 and 8% for finisher period). The chicks were reared from 1 to 10 days in one group. On the eleventh day, 10 chicks per pen were randomly allocated to each cage per replicate. Data were performed in generalized linear model (GLM) procedures in SAS at 5% level of significance and the least square means correction after Tukey's test was used. Commercial antioxidant improved crude fat digestibility and decreased population of caecum *Lactobacillus*, but pomegranate seed extract improved crude fat digestibility and increased caecum *Lactobacillus* population. Due to positive effects of pomegranate seed extract in fat containing diets on fat digestibility and *Lactobacillus* bacteria in caecum, it could be concluded that using pomegranate seed extract in fat containing diets could be useful.

Key words: Antioxidants, Broiler, Digestibility, Performance, Pomegranate seed extract

Introduction

Dietary lipids produce a lot of calories for fast growing broilers. Today, in the formulation of high-energy rations for broiler chicks, nutritional supplements containing lipids are more than 8% (Schwalfenberg, 2006). Vegetable oils have a lot of polyunsaturated fatty acids (PUFA) which are highly digestible for chickens and show common fat sources in broiler diets (Schwalfenberg, 2006). Because chickens are monogastric, so lipids are absorbed and deposited in tissues principally in the form in which they are ingested. Therefore, the fatty acids composition of chicken tissues can be affected readily by dietary fatty acids. In humans the intake of 3-n PUFAs (particularly more than 18 carbon in their chain) is low. That's why some researchers make the effort to fortify the meat with these fatty acids (Schwalfenberg, 2006). However, expanding dietary methods in broiler chickens that improve the PUFA value of tissues are related to muscle lipid oxidation (Cherian, 2007). As well, PUFAs are highly susceptible to oxidation during storage (Açıkgöz *et al*., 2011). Intake of oxidized lipids, causes oxidation to develop in body tissues, oxidation of sensitive PUFA in body, or a low usage of nutrients including the antioxidant defense system. Lipids oxidation is commonly known as one of the principal agents lowering the meat quality (such as alteration in color, odor, flavor, texture and nutritional value) and meat production (Zhang *et al*., 2010). To improve the antioxidant resistance meat, antioxidants are included in foods, mainly alpha-tocopherol acetate (vitamin E). Alpha-tocopherol acetate addition has advantages on decreasing lipid oxidation in broiler chickens meat (Saleh *et al*., 2017), but there are difficulties on its supplementing, biological value and performance restriction when unsaturated fatty acids in the broiler chicken diet are used (Saleh *et al*., 2017). The use of artificial antioxidants in poultry diets because of conveying them to the human food chain are diminishing. It is this result of damaging effects on consumer health, such as stopping enzyme activities and the incidence of liver cancers in the body (Namiki, 1990). As a result, it is essential to recognize the natural antioxidants such as herbal extracts. Today, there is universal concern for discovering secure antioxidants from natural sources (Moure *et al*., 2001). Feeds comprising no chemical additives are progressively applied in poultry nutrition. For this reason, herbs and natural feed additives are being studied as natural sources biologically significant substances (Demir *et al*., 2003). Some biological effects such as antioxidant activity, anti-inflammatory action, inhibition of platelet aggregation, and antimicrobial activities have been demonstrated for plant phenolics (Alberto *et al*., 2001). In recent years, polyphenols have received a great deal of consideration, because of their various biological functions. Phenolic compounds may represent beneficial effects through their free radical

scavenging and antioxidant properties (Fischer *et al*., 2011). Tannins exist in many plants including pomegranate (*Punica granatum* L.) which are high molecular weight phenolic compounds. Tannins which are high in pomegranate have notable antimicrobial activity, as well as antioxidant activity (Al-Zoreky, 2009).

Iran is a native land of pomegranate, its production was reported at 665,000 tons for the year 2013 (Abbasi *et al*., 2008). The edible section of pomegranate is called aril and comprises about 52% (w/w) of the total fruit and includes of 78% juice and 22% seeds. Pomegranate seeds have high amounts of oil, in which, in some Iranian varieties the total lipid content on a dry basis ranges from 66 to 193 g per kg of the fruit. Pomegranate seed oil is composed of 65-80% conjugated fatty acids, the most substantial of which are 9-trans, 11-cis, 13-trans, octadecatrienoic acid, and so-called punicic acid. It has been reported pomegranate seed oil prevented the eicosanoid enzyme phospholipase A2 activity in human prostate cancer (Abbasi *et al*., 2008). Because of high levels of moisture and soluble sugars in fresh pomegranate biomass (Shabtay *et al*., 2012), there are some difficulties in storing them, hence, in this research the pomegranate seed extract was used. The objectives of this work were to investigate the effects of pomegranate seeds extract to fat containing diets on growth performance, nutrients digestibility and intestinal microflora in broilers.

Materials and Methods

Birds and experimental treatments

The experiment was conducted at the Experimental Research Station of Animal Science, College of Agriculture, Shiraz University, Iran. All procedures in the present work were approved by the Animal Care and Welfare Committee of our institute. A total of 320 one-day-old Ross 308 broilers (mixed sex) were used by 8 dietary treatments in a 2 × 2 × 2 factorial arrangement according to completely randomized design. Each treatment was consisted of 4 replications of 10 birds each. The dietary treatment consists of pomegranate seed extract (0 and 2%), commercial Nutriad® antioxidant (0 and 0.01%) and soybean oil (0 and 6% in grower period and 0 and 8% in finisher period). Pomegranate seed was obtained from a processing factory (Rad-Ard-Pars Co., Shiraz, Iran), the mechanical procedure was implemented for pomegranate seed extraction. Two hundred g of seed was ground by coffee mill, soaked in 1 L of distilled water and shacked by an automatic shaker for 48 h in 45°C, screened by Whatman filter paper No. 1 and stored at 4°C. The treatment diets and nutrients composition of broiler grower (11 to 25 day) and finisher (25 to 39 day) were presented in Table 1.

Table 1: Ingredients and chemical composition of broiler grower and finisher diets

Ingredients	Grower (with oil)	Grower (without oil)	Finisher (with oil)	Finisher (without oil)
Corn	61.71	46.05	63.39	40.97
Soybean meal	33.78	36.72	32.92	36.70
Soybean oil with antioxidant	0.77	0	0.44	0
Soybean oil without antioxidant	0	6	0	8
Dicalcium phosphate	1.44	1.47	1.29	1.33
Calcium carbonate	1.06	1.03	0.99	0.95
NaCl	0.42	0.43	0.37	0.38
Mineral premix *	0.25	0.25	0.25	0.25
Vitamin premix *	0.25	0.25	0.25	0.25
DL-Methionine	0.22	0.24	0.10	0.14
Lysine HCl	0.10	0.04	0	0
Rough rice	0	7.52	0	11.03
Total	100	100	100	100
ME (kcal/kg)	2900	2900	2900	2900
Crude protein (%)	20.32	20.25	19.9	19.7
Calcium (%)	0.83	0.83	0.77	0.77
Available phosphor (%)	0.41	0.41	0.38	0.38
K (%)	0.86	0.87	0.85	0.86
Cl (%)	0.31	0.30	0.26	0.26
Na (%)	0.18	0.18	0.16	0.16
Arginine (%)	1.29	1.33	1.27	1.31
Isoleucine (%)	0.84	0.85	0.83	0.84
Lysine (%)	1.14	1.14	1.05	1.1
Methionine	0.54	0.54	0.42	0.43
Methionine + Cysteine (%)	0.87	0.87	0.75	0.75
Threonine (%)	0.76	0.76	0.75	0.75
Tryptophan (%)	0.29	0.30	0.28	0.30
Valine (%)	0.95	0.94	0.93	0.92

* Each kg of vitamin and mineral supplements had: vitamin A, 7,500 IU; vitamin D_3, 3,000 IU; vitamin E, 10 IU; vitamin K, 2 mg; vitamin B_{12}, 12.5 µg; folic acid, 0.5 mg; pantothenic acid, 8 mg; pyridoxine, 1.8 mg; riboflavin, 5.3 mg; thiamine, 2 mg; biotin, 0.15 mg; iodine, 1 mg; selenium, 0.15 mg; niacin, 24 mg; choline, 350 mg; copper, 6 mg; iron, 30 mg; zinc, 50 mg; manganese, 80 mg

Sampling and data

Body weight and feed intake (FI) were measured weekly on a pen basis. The average daily gain (ADG), FI, and feed conversion ratio (FCR) were also calculated. On the 39th day of age all birds were slaughtered and the livers, hearts, kidneys, bursa of Fabricius, proventriculus, abdominal fat pad, gizzard, ileum, and pancreas of one bird from each pen were dissected out, and their weights were recorded for calculation of the relative weight of the organs. Digesta of all birds in each pen were obtained by flushing the last two third segment of the intestine, between the Meckel's diverticulum and 2 cm anterior to the ileo-ceca-colonic junction, using distilled water (Rezvani *et al*., 2008). The digesta were frozen at -20°C immediately, and then vacuum-dried and ground before analyses. The dried digesta and diets were analyzed for dry matter (DM), Ash, ether extract (EE) and CP, according to AOAC (2000). The content of Cr_2O_3 was determined in the diets and digesta using atomic absorption spectrophotometry (Shimadzu, AA 670, Tokyo, Japan) according to Williams *et al*. (1962). Apparent prececal nutrient (N) digestibility of the diets was calculated using the following equation:

$$\text{Digestibility (\%)} = 100 - 100 \times [(Cr_2O_{3Diet} \times N_{Digesta})/(Cr_2O_{3Digesta} \times N_{Diet})]$$

Where,

Cr_2O_{3Diet} and $Cr_2O_{3Digesta}$: The concentrations of Cr_2O_3 in the diet and digesta samples (g/kg)

N_{Diet} and $N_{Digesta}$: The concentrations of nutrients in the diet and digesta samples (g/kg), respectively

The microbial population of the caecum contents and the contents of the ileum biopsy were assessed according to Quinn *et al*. (1994).

Statistical analysis

The experiment was carried out in a factorial experiment 2 × 2 × 2 according to completely randomized design. The data were tested for normality, transformed where appropriate, and subjected to the PROC GLM (SAS, 2002). The means were compared by the least squares procedure adjusted for Tukey's test, and the level of significance was set at P≤0.05.

Results

Data in Table 2 showed the effect of treatments on FI, ADG, FCR, and final body weight (FW) in broilers grower, finisher and the whole period. Soybean oil decreased ADG in grower period but increased ADG in finisher period (P≤0.05) and had no effect on broilers daily weight gain in whole period (P>0.05). Average daily gain was not affected by Nutriad® antioxidant (P≤0.05). Average daily gain reduced in birds that received pomegranate seed extract in grower and the whole period. No significant differences were observed among soybean oil and Nutriad® antioxidant treatments for the FW, but the FW was lower than that of basal diet in birds that received pomegranate seed extract (P≤0.05). Feeding soybean oil however, resulted in an improvement in FCR in finisher and the whole period compared with the control group but Nutriad® antioxidant and pomegranate seed extract did not change the FCR (P>0.05).

Increases in pancreas, heart and proventriculus weight were noted in the birds receiving pomegranate seed extract. The gizzard weight was higher in the birds receiving soybean oil (Table 3; P≤0.05). Effect of Nutriad® antioxidant × pomegranate seed extract interaction on the bursa of Fabricius weight were significant (Table 3; P≤0.05) and interaction effect of soybean oil × Nutriad® antioxidants × pomegranate seed extract on proventriculus weight was significant in broilers (Table 3; P≤0.05). Data showed adding pomegranate seed extract to the diet decreased intestine

Table 2: Effect of pomegranate seed extract, Nutriad® antioxidant and soybean oil on feed intake, average daily gain, feed conversion ratio, and final body weight in Ross 308 broilers

Treatments	Grower period (11-25)			Finisher period (25-39)			Whole period (11-39)			
	FI (g/bird/d)	ADG (g/bird/d)	FCR	FI (g/bird/d)	ADG (g/bird/d)	FCR	FI (g/bird/d)	ADG (g/bird/d)	FCR	FW (g)
Main effects										
Pomegranate seed extract										
0%	103.51[a]	62.42[a]	1.66	187.70[a]	92.31	2.04	145.8[a]	77.52[a]	1.89	2494.5[a]
2%	81.60[b]	45.77[b]	1.79	150.44[b]	80.06	1.90	116.0[b]	62.95[b]	1.85	2077.1[b]
SEM	1.81	0.91	0.04	3.43	3.43	0.07	2.76	1.96	0.04	59.23
Soybean oil [1]										
0%	92.98	55.38[a]	1.70	169.32	82.44[b]	2.05[a]	131.17	68.92	1.91[a]	2248.2
6 and 8%	92.12	52.80[b]	1.75	168.82	89.93[a]	1.89[b]	130.81	71.54	1.83[b]	2323.4
SEM	0.93	0.47	0.02	1.76	1.76	0.04	1.41	1.01	0.02	30.40
Nutriad® antioxidant										
0%	91.87	53.77	1.72	168.12	85.91	1.97	130.01	69.85	1.86	2272.0
0.01%	93.24	54.42	1.73	170.02	86.46	1.98	131.97	70.61	1.87	2299.5
SEM	0.93	0.47	0.02	1.76	1.77	0.04	1.42	1.01	0.02	30.51
P-value										
Pomegranate seed extract	<0.0001	<0.0001	0.1565	<0.0001	0.0678	0.3000	<0.0001	0.0006	0.6194	<0.0001
Soybean oil [1]	0.5197	0.0007	0.1296	0.8441	0.0064	0.0034	0.8623	0.0796	0.0176	0.0718
Nutriad® antioxidant	0.3144	0.3336	0.9123	0.4576	0.8295	0.8247	0.3460	0.6015	0.7256	0.4184
Oil × antioxidant	0.3770	0.1480	0.7146	0.2172	0.2149	0.4574	0.5089	0.3790	0.6446	0.3210
Oil × extract	0.0069	0.4275	0.0314	0.1858	0.8395	0.5551	0.9499	0.6191	0.4933	0.6507
Antioxidant × extract	0.9819	0.8795	0.9240	0.1493	0.8475	0.2947	0.2838	0.9309	0.2399	0.8433
Oil × antioxidant × extract	0.9291	0.3414	0.5052	0.1272	0.3942	0.8110	0.2400	0.5073	0.7707	0.4861

FI: Feed intake, ADG: Average daily gain, FCR: Feed conversion ratio, and FW: Final body weight. [a, b] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% of the diet. SEM: Standard error of the mean

Table 3: Effect of pomegranate seed extract, Nutriad® antioxidant and soybean oil on relative weight of digestive organs in Ross 308 broilers (Least square means±SE)

Treatments	Organs						
	Liver (%)	Heart (%)	Pancreas (%)	Bursa of Fabricius (%)	Intestine length (cm) [2]	Gizzard (%)	Proventriculus (%)
Main effects							
Pomegranate seed extract							
0%	2.07	0.50[b]	0.21[b]	8.97	76.67[a]	1.75[a]	0.34[b]
2%	2.22	0.57[a]	0.25[a]	8.32	67.98[b]	1.62[b]	0.38[a]
SEM	0.07	0.01	0.008	0.77	1.44	0.04	0.007
Soybean oil [1]							
0%	2.14	0.56	0.25[a]	9.29	71.11	1.57[b]	0.37
6, 8%	2.15	0.51	0.21[b]	8.00	73.53	1.80[a]	0.35
SEM	0.07	0.01	0.008	0.77	1.44	0.04	0.007
Nutriad® antioxidant							
0%	2.20	0.54	0.24	8.38	71.46	1.72	0.37
0.01%	2.09	0.53	0.22	8.90	73.19	1.65	0.35
SEM	0.07	0.01	0.008	0.77	1.44	0.04	0.007
P-value							
Pomegranate seed extract	0.15	0.004	0.002	0.56	0.0003	0.03	0.0009
Soybean oil	0.94	0.06	0.007	0.25	0.24	0.001	0.16
Nutriad®	0.25	0.52	0.29	0.64	0.40	0.24	0.15
Antioxidant							
Oil × antioxidant	0.70	0.21	0.77	0.63	0.066	0.47	0.51
Oil × extract	0.73	0.46	0.16	0.87	0.04	0.78	0.90
Antioxidant × extract	0.74	0.47	0.81	0.04	0.54	0.49	0.18
Oil × antioxidant × extract	0.83	0.64	0.53	0.95	0.72	0.58	0.01

[a, b] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% of the diet, and [2] From Meckel's diverticulum to ileo-ceca-colonic junction. SEM: Standard error of the mean

Table 4: Interaction effect of soybean oil and pomegranate seed extract on intestine length in Ross 308 broilers

Soybean oil [1]	Pomegranate seed extract [1]	Intestine length [2] (cm)
0	0	77.66 ± 2.04[a]
1	0	75.68 ± 2.04[a]
0	1	64.57 ± 2.04[b]
1	1	71.40 ± 2.04[ab]
SEM		2.03
P-value		0.04

[a, b] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% and for pomegranate seed extract is 2% of the diet, and [2] From Meckel's diverticulum to ileo-ceca-colonic junction. SEM: Standard error of the mean

Table 5: Effect of pomegranate seed extract, Nutriad® antioxidant and soybean oil on apparent prececal digestibility of crude protein, ether extract and dry matter in Ross 308 broilers

Treatments	Digestibility		
	DM (%)	EE (%)	CP (%)
Main effects			
Pomegranate seed extract [1]			
0%	73.15[a]	69.71[b]	79.63[a]
2%	61.44[b]	81.87[a]	70.48[b]
SEM	1.14	3.11	1.57
Soybean oil [1]			
0%	66.37	81.89[a]	72.29[b]
6, 8%	68.22	69.68[b]	77.81[a]
SEM	1.14	3.12	1.57
Nutriad® antioxidant [1]			
0%	66.65	70.34[b]	74.78
0.01%	67.94	81.24[a]	75.24
SEM	1.14	3.11	1.57
P-value			
Pomegranate seed extract	<0.0001	0.0120	0.0004
Soybean oil	0.2642	0.0116	0.0206
Nutriad® antioxidant	0.4359	0.0223	0.8703
Oil × antioxidant	0.0377	0.4800	0.1507
Oil × extract	0.2157	0.0003	0.0006
Antioxidant × extract	0.2289	0.4801	0.0971
Oil × antioxidant × extract	0.0241	0.1266	0.3361

CP: Crude protein, EE: Ether extract, and DM: Dry matter. [a, b] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% of the diet. SEM: Standard error of the mean

length in broilers (Table 3; P≤0.05). Also, interaction effect of soybean oil and pomegranate seed extract on intestine length was significant (Table 4).

The effect of treatment on apparent nutrients digestibility is shown in Table 5. Adding pomegranate seed extract improved EE digestibility but decreased DM and protein digestibility (Table 5; P≤0.05). Apparent DM digestibility was not affected by soybean oil (Table 5). Apparent protein digestibility in birds which received soybean oil was higher than that of basal diet; however, inclusion of soybean oil decreased EE digestibility (Table 5; P≤0.05). Commercial Nutriad® antioxidant improved EE digestibility (P≤0.05), while it had no effect on DM and protein digestibility (Table 5; P>0.05). Interaction between soybean oil and antioxidant, and also the effect of soybean oil × antioxidant × pomegranate seed extract on the digestibility of DM was significant (Tables 6 and 7; P≤0.05). The lowest DM digestibility was when the birds received basal diet supplemented with pomegranate seed extract alone. Interaction effect of soybean oil and pomegranate seed extract on digestibility of protein and fat (percent) in Ross 308 broilers was shown in Table 8.

As shown in Table 9 the effect of treatments on population of ileum *Escherichia coli* and *Lactobacillus* bacteria was not significant (P>0.05); however, the effect of soybean oil × antioxidants × pomegranate seed extract on population of ileum *E. coli* bacteria was significant in broilers (Table 10). Also, the effect of oil × antioxidant on *E. coli* population is significant in broilers ileum (Table 11). The effects of treatments on *Lactobacillus* bacteria in caecum were shown in Table 12.

Discussion

The current study showed soybean oil increased ADG in finisher period and decreased it in grower period (P≤0.05) but had no effect in the whole period (P>0.05). This may be because of digestive enzyme secretion capacity, which is said to be relatively low in young

Table 6: Interaction effect of soybean oil and Nutriad® antioxidant on digestibility of dry matter in Ross 308 broilers

Soybean oil [1]	Pomegranate seed extract [1]	DM digestibility (%)
0	0	63.72[b]
0	1	70.91[a]
1	0	68.72[a]
1	1	67.11[ab]
SEM		1.65
P-value		0.01

[a, b] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% and for pomegranate seed extract is respectively 0.01 and 2% of the diet. DM: Dry matter, and SEM: Standard error of the mean

Table 7: Interaction effect of soybean oil, antioxidant and pomegranate seed extract on digestibility of dry matter (percent) in Ross 308 broilers

Soybean oil [1]	Nutriad® antioxidant [1]	Pomegranate seed extract [1]	DM digestibility
0	0	0	73.78[a]
1	0	0	73.23[a]
0	1	0	72.72[a]
1	1	0	72.85[a]
0	0	1	54.09[d]
1	0	1	65.50[bc]
0	1	1	64.88[ab]
1	1	1	61.30[c]
SEM			2.34
P-value			<.0001

[a, b, c, d] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% and for antioxidant and pomegranate seed extract is respectively 0.01 and 2% of the diet. DM: Dry matter, and SEM: Standard error of the mean

Table 8: Interaction effect of soybean oil and pomegranate seed extract on digestibility of crude protein and fat (percent) in Ross 308 broilers

Soybean oil [1]	Pomegranate seed extract [1]	Protein digestibility (%)	Fat digestibility (%)
0	0	81.24[a]	85.25[a]
0	1	66.17[b]	78.54[a]
1	0	78.11[a]	54.18[b]
1	1	80.54[a]	85.19[a]
SEM		1.43	4.39
P-value		<0.0001	0.0003

[a, b] Values within the same column with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% and for antioxidant and pomegranate seed extract is respectively 0.01 and 2% of the diet. SEM: Standard error of the mean

Table 9: Effect of pomegranate seed extract, Nutriad® antioxidant and soybean oil on ileal microflora in Ross 308 broilers

Treatments	*Escherichia coli* (Log CFU 10)	*Lactobacillus* (Log CFU 10)
	Main effects	
Pomegranate seed extract [1]		
0%	1.89	3.31
2%	2.38	3.21
SEM	0.18	0.20
Soybean oil [1]		
0%	1.94	3.24
6 and 8%	2.34	3.28
SEM	0.18	0.20
Nutriad® antioxidant [1]		
0%	2.37	3.39
0.01%	1.91	3.12
SEM	0.18	0.20
	P-value	
Pomegranate seed extract	0.0770	0.7436
Soybean oil	0.1445	0.8629
Nutriad® antioxidant	0.0985	0.3581
Oil × antioxidant	0.0035	0.6872
Oil × extract	0.0864	0.7382
Antioxidant × extract	0.2283	0.3759
Oil × antioxidant × extract	0.0356	0.6560

[1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% of the diet. CFU: Colony-forming unit

Table 10: Interaction effect of soybean oil, Nutriad® antioxidant and pomegranate seed extract on ileal *Escherichia coli* population in Ross 308 broilers

Soybean oil[1]	Nutriad® antioxidant [1]	Pomegranate seed extract [1]	*Escherichia coli* (Log CFU 10)
0	0	0	1.86[b]
0	0	1	1.61[b]
0	1	0	2.01[b]
0	1	1	2.29[b]
1	0	0	2.06[b]
1	0	1	3.94[a]
1	1	0	1.66[b]
1	1	1	1.70[b]
SEM			0.37
P-value			0.04

[a, b] Values within the same column values with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% and for antioxidant and pomegranate seed extract is respectively 0.01 and 2% of the diet. CFU: Colony-forming unit, and SEM: Standard error of the mean

Table 11: Interaction effect of soybean oil and Nutriad® antioxidant on ileal *Escherichia coli* population in Ross 308 broilers

Soybean oil [1]	Nutriad® antioxidant [1]	*Escherichia coli* (Log CFU 10)
0	0	1.73 ± 0.23[a]
0	1	2.14 ± 0.25[b]
1	0	3.00 ± 0.25[a]
1	1	1.68 ± 0.29[a]
P-value		0.0035

[a, b] Values within the same column values with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% and for antioxidant is respectively 0.01 and 2% of the diet. CFU: Colony-forming unit, and SEM: Standard error of the mean

Table 12: Effect of pomegranate seed extract, Nutriad® antioxidant and soybean oil on cecal microflora in Ross 308 broilers

Treatments	*Escherichia coli* (Log CFU 10)	*Lactobacillus* (Log CFU 10)
	Main effects	
Pomegranate seed extract [1]		
0%	3.31000000	5.36875000[b]
2%	3.21479167	6.18333333[a]
SEM	0.21	0.06
Soybean oil [1]		
0%	3.23729167	5.91041667[a]
6 and 8%	3.28750000	5.64166667[b]
SEM	0.21	0.06
Nutriad® antioxidant [1]		
0%	0.20325709	5.88520833[a]
0.01%	0.20325709	5.66687500[b]
SEM	0.20	0.06
	P-value	
Pomegranate seed extract	0.7436	<0.0001
Soybean oil	0.8629	0.009
Nutriad® antioxidant	0.3581	0.030
Oil × antioxidant	0.6872	0.0225
Oil × extract	0.7382	0.0184
Antioxidant × extract	0.3759	0.3586
Oil × antioxidant × extract	0.3759	0.2258

[a, b] Values within the same column values with no common superscripts differ significantly (P<0.05). [1] 0 means zero percent and 1 for soybean oil in grower and finisher period is respectively 6 and 8% of the diet. of the mean. CFU: Colony-forming unit, and SEM: Standard error

chicks (Sklan, 2001). Pomegranate seed extract lead to negative effect on broilers ADG in grower and the whole period ($P \leq 0.05$) but had no effect on it in finisher period ($P > 0.05$). In this study, inclusion of pomegranate seed extract had a negative influence on growth performance, especially in young birds. These results might be attributed to not using the suitable dose of pomegranate seed extract and it may be that the younger broilers could not accustom to the diets. These results were in agreement with Schiavone *et al*. (2008) who showed the negative effects of tannin on bird feeding. Tannins have been thought detrimental for many years containing their negative effects on N digestibility, N retention, and productive features. It has also been observed pomegranate seed extract reduced FI ($P \leq 0.05$). These results were consistent with our assumption. Interaction of soybean oil and pomegranate seed extract on daily FI in grower period was significant ($P \leq 0.05$).

As shown in Table 2, the highest daily FI was observed in the grower period of the group who received Nutriad® antioxidants. The lowest daily FI in the grower period of the group that received pomegranate seed extract. It is possible that terpene and phenolic compounds are excreted as a conjugate with other components, which may enhance the endogenous losses from the bird and reduce the consumption of nutrients (Cross *et al*., 2007). It is speculated that the inclusion of phytogenic substances to the diet may lead to a change in the taste of diet, and reduce feed utilization by animals (Amad *et al*., 2011). Halle *et al*. (2004) and Amad *et al*. (2011) reported that phytogenic feed additives reduced daily FI of broilers and significantly amended feed conversion compared with that of control birds. The data further indicated no changes in FCR by including pomegranate seed extract to the diet. Soybean oil improved feed conversion ratio in the finisher and the whole period. However, the results of the present study were in agreement with previous observations that indicated herbs, plant extracts, essential oil and/or the main components of the essential oil did not affect body weight gain, FI or feed efficiency in broilers (Demir *et al*., 2003; Hernandez *et al*., 2004). The effects of plant extracts on performance are clearly variable. This may be attributed to diversities in composition of the various phytogenic additives and the concentrations of the active substances and their biological activity, respectively. In addition, the response of chickens to a phytogenic feed additive might be influenced by other agents, such as the diet type, animal age, hygiene, environmental factors, and product quality (Amad *et al*., 2011).

The results of the present study showed that the relative weights of the pancreas, proventriculus and heart were increased, and gizzard decreased by using extract. These results were in contrast with those of Hernandez *et al*. (2004), who concluded that herbal powders and essential oils did not affect the relative weights of internal organs. In addition, feeding soybean oil increased the relative weight of gizzard. Presence of lipid in gizzard may reduce the pH of gizzard digesta ($P \leq 0.001$) and increased the relative weight of gizzard (Gonzalez-Alvarado *et al*., 2007).

Binding polyphenolic compounds to both dietary and endogenous protein such as digestive enzymes and proteins placed at the luminal side of the intestinal tract have been used to describe the reduced apparent digestibility of protein in polyphenol-containing diets. Polyphenols are known to form complexes with protein due to the interaction of their reactive hydroxyl groups with the carbonyl group of protein. Due to these complexities, protein and amino acid digestibility were reduced by the incorporation of polyphenols in chicken diets (Brenes *et al*., 2008). Soybean oil reduced EE digestibility, but antioxidant and pomegranate seed extract increased EE digestibility. By addition of soybean oil to the diet of birds, EE digestibility decreased due to oxidation. While antioxidant and pomegranate seed extract prevented fat oxidation and thus increased the EE digestibility. These results do not agree with findings of Jamroz *et al*. (2005) who indicated no effect of phytogenic feed additive on digestibility of nutrients. Kamel (2001) mentioned that there is proof to proposed that herbs and plant extracts have appetite and digestion-stimulating properties and antimicrobial effects. Also, positive effects of essential oils on the digestive system have been previously reported (Hernandez *et al*., 2004), supposing exciting effects on the output of digestive enzymes from the pancreas, gut mucosa, and increasing bile flow (Jamroz *et al*., 2005).

As a consequence, such effects might lead to improved N utilization in the small intestine. Amad *et al*. (2011) reported phytogenic feed additives have a possible effect on microbial communities and could therefore be considered as an alternative in controlling the intestinal microbial population. But in this study the effects of treatments on number of ileum and caecum *E. coli* and ileum *Lactobacillus* were not significant. Due to the positive effects of pomegranate seed extract in fat containing diets on fat digestibility and *Lactobacillus* bacteria in caecum, it could be concluded that using pomegranate seed extract in fat containing diets could be useful. However, because the same performance of the birds receiving Nutriad® or pomegranate extract in fat containing diets, using extract in fat containing diets could be more acceptable presumably because of fewer side effects of natural antioxidant residuals for human, but more research in this area is needed to find the lowest dose of this extract in commercial broiler diets.

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Conflict of interest

The authors declare that there is no conflict of

interest.

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