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Effect of Adding Grape Pomace into the Diet, Grape Pomace Extract and Vitamin E-Selenium into the Drinking Water on Growth Performance, Internal Organs' Weight, Cecum Bacterial Population and Prececal Nutrient Digestibility in British United Turkeys

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Abstract
This study aimed to use grape pomace and pomace extract to feed British United Turkey Big 6 (B.U.T.6) and compare its effect with vitamin E-selenium on growth performance, internal organs, and bacterial population of the cecum, and prececal nutrient digestibility. For this purpose, 80 hatched day-old B.U.T.6 were placed in a completely randomized design, including four treatments, four replicates for each treatment, and five birds in each replicate. The turkeys underwent experimental treatments from 31 to 127 days, including control, 3% dietary grape pomace, 150 mL/L aqueous extract of grape pomace, and 0.5 mL/L vitamin E-selenium in the drinking water. The turkeys treated with grape pomace had the highest daily weight gain and final body weight. Grape pomace extract treatment had a negative effect on daily feed intake. Experimental treatments throughout the course did not have a significant effect on the feed conversion ratio. The bacterial population of *Escherichia coli* in the cecum was lower in the vitamin E-selenium treatment than in other treatments. The population of *Lactobacillus* bacteria in all experimental treatments was higher than in the control treatment and the highest in the treatment of grape pomace extract. Digestion of dry matter, crude fat, and protein in turkeys treated with grape pomace extract and control were more than in the other treatments. The digestibility of crude fat in turkeys treated with grape pomace was significantly lower than in other treatments. The use of grape pomace in turkey diets is recommended because it increases the overall daily weight gain, final body weight, and the number of beneficial bacteria**.** In turkeys treated with grape pomace extract, daily weight gain and final body weight can be considered helpful in reducing feed costs.

Introduction

British United Turkey Big 6 (B.U.T.6) has proper growth rate, and they reach suitable weight in 4-6 months. The increase in weight for male turkeys is 180 g per day and 80-90 g per day for female turkeys. This weight increases outstandingly and is noticeable compared to broiler chickens (Leeson and Atteh, 1995). According to satisfactory growth aspects like fast body weight gain and final live weight (10.74 kg weight for females in the 16th week and 20.39 for males in the 20th week of age), suitable feed conversion ratio (2.51 on the 140th day of age), low carcass percentage losses (23% in the 140th day of age) and the value of its flesh nutrient like protein, minerals, rich essential amino acids, low cholesterol, compared to other birds, the industrial raising of turkeys have rapidly increased throughout the whole world including Iran (Haghighi Khoshkho *et al.*, 2010).

One of the poultry industry's major issues is carcass fat oxidation and reduced carcass quality (Choi *et al.*, 2010). Due to polyunsaturated fatty acid (PUFA) content, the birds' meat is sensitive to oxidation (Min *et al.*, 2008). Additionally, raising

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birds on a large scale, warm weather, high density, and the use of oxidized food have intensified oxidative conflict (Ismail *et al*., 2013). Also, choosing birds for faster and better growth causes conflicts in their immunization system, making them incapable of giving suitable responses to environmental stress (Van der Most *et al*., 2011). Therefore, developing strategies to control of oxidative reactions in the body and chicken meat are inevitable. Vitamin E and selenium have been identified as the most effective fat-soluble antioxidant (Bjelakovic *et al*., 2004). It has been shown that the oxidative stability of poultry is highly dependent on Vitamin E added to the diet (Wen *et al*., 1997).

The interest of using natural antioxidants of plant origin for health reasons has increased (Fellenberg and Speisky, 2006). All parts of the grape cluster have also been shown to be rich sources of tocopherols with antioxidant function (Tangolar *et al*., 2011). Due to food shortages and the growing need for livestock products, the use of factory wastes, including fruit pulp in livestock and poultry feed, has received much attention. In addition to reducing the dependence of livestock on human grains, environmental pollution is prevented and sustainable agricultural practices are also established. Considering nutrient bioavailability, fruit pulp also contains a lot of crude fiber, antioxidants, minerals, and vitamins and is less expensive than the main product (Grasser *et al*., 1995; Martins *et al*., 2011). Grape (*Vitis vinifera*) is the fourth largest fruit crop globally (FAO, 2015). In Iran, according to the agricultural statistics of 2016, the production of grapes is about three and a half million tons per year. In the juicing process, 15% of grapes' dry weight and 25 to 45% of their fresh weight are converted into pulp (Wadhwa and Bakshi, 2013). Grape pomace production in Iran is estimated at more than 50,000 tons per year (Alipour and Rouzbehan, 2007), which generally has relatively high amounts of sugar (mainly glucose and fructose), tartrate, anthocyanin, and crude fiber (Bakhshizadeh *et al*., 2012). It is also a rich source of flavonoids such as catechins, epicatechins, and a variety of procyanidins (Goni *et al*., 2007), known as antioxidant sources (Brenes *et al*., 2010) and has antimicrobial, antioxidant, and immune-boosting effects. (Adams *et al*., 2006). Grape pomace has not been welcomed as bird feed due to its high fiber, low protein, lignin, and anti-nutrient content such as tannin (Alipour and Rouzbehan, 2007).

On the other hand, due to the pulp's low dry matter, it is difficult to store and transport it. Considering its economic aspects, drying and extracting of grape pomace can be possible solutions to this problem (De Pina and Hogg, 1999). Grape pomace extract contains significant amounts of flavonoids in phenolic compounds and can absorb more potent oxygen radicals (Hogan *et al*., 2010). The results of Brenes *et al*. (2010) studies showed that grape seed extract is a concentrated source of polyphenols with antioxidant capacity. Compared to the control diet, the diet containing grape pulp extract had significantly higher free radical scavengingcapacity at 21 and 41 days of age in broilers (Brenes *et al*., 2010). However, another study showed that natural dietary antioxidant extracts were less effective in protecting against oxidation than treatment with synthetic antioxidants combined with vitamin E acetate (Smet *et al*., 2008). Furthermore, feed conversion ratio improved in broiler chickens fed diets containing 30 g/kg (Sadeghi and Nobakht, 2015) and 60 g/kg (Viveros *et al*., 2011) grape pomace compared to those of control and grape seed extract groups. Grape polyphenolic extract and its by-products have been shown to affect the intestinal microflora, reduce the number of Propionibacteria, Bacteroids, and Clostridia, increase the number of *Lactobacillus* and *Bifidobacteria*, and ultimately increase the biodiversity of intestinal bacteria (Ozkan *et al*. 2004; Dolara *et al*., 2005; Papadopoulou *et al*., 2005). It has also been shown that in birds fed grape pomace compared to birds fed vitamin E, the digestibility of some amino acids such as arginine, leucine, phenylalanine, glutamine, prolamine, tyrosine, histidine, and cysteine decreased (Goni *et al*. 2007). Studies evaluating the effect of natural antioxidants like grape byproducts on turkeys are lacking in the literature, thus this study aimed to use grape pomace and aqueous extract in turkey nutrition and evaluate its effect on growth performance, prececal nutrient digestibility, internal organs, and cecum bacteria population compared with vitamin E-selenium, a rich source of antioxidants.

Materials and Methods

This research was conducted in the educational-research complex of the Department of Animal Sciences, Faculty of Agriculture, Shiraz University. All procedures in the current study were approved by the Animal Care and Welfare Committee of the Department of Animal Science, School of Agriculture, Shiraz University (Shiraz, Iran). The raw materials for preparing diets in each period were purchased from Rouhani Livestock and Poultry Feed Factory (Rad-Ard-Pars) located in Marvdasht, Shiraz, and ground and mixed in the livestock station of Faculty of Agriculture, Shiraz University. Marivan black grape pulp was prepared from Alifard Company located at 15 km of Saveh-Isfahan road, and after drying and grinding with a 2.5 mm sieve, it was transferred to the experiment site. Extraction of grape pomace was carried out in a ratio of one to ten grape pomace to water at a temperature of 40 to 45 ºC for six hours (López *et al*., 2011). Finally, the obtained extract was passed through a filter three times. Diets for different age periods were balanced with the WUFFDA software version 1.0 according to the ordered needs of the desired strain (B.U.T.6, Table 1), and the chemical composition of grape pulp is shown in Table 2. Table 3 also shows the polyphenolic compounds of aqueous and methanolic grape pomace extracts using the HPLC method (López *et al*., 2011).

Table 1: Ingredients and chemical composition of turkey basal diet of B.U.T.6 strain in different age periods (day)

Ingredients (%)	**0-30**	**31-42**	**43-75**	**76-105**	**106-126**
Corn	45.91	52.00	53.71	66.93	65.18
Soybean meal (44% protein)	40.00	40.00	38.11	27.60	28.33
Soybean oil	0.00	2.16	1.73	1.00	2.79
Threonine	0.11	0.12	1.00	0.05	0.00
DL-Methionine	0.34	0.28	0.27	0.17	0.13
Dicalcium phosphate	3.68	3.00	2.28	1.54	1.29
Limestone	1.13	1.12	1.43	1.27	1.21
Bentonite	0.14	0.16	0.00	0.00	0.00
NaCl	0.26	0.32	0.33	0.30	0.30
NaHCO3	0.12	0.00	0.00	0.00	0.00
[1]Premix/Vitamin	0.20	0.20	0.30	0.30	0.30
[1]Premix/Mineral	0.20	0.20	0.30	0.30	0.30
Lysine Sulfate	0.53	0.44	0.44	0.44	0.07
Anzymite (natural zeolite)	7.38	0.00	0.00	0.00	0.00
Sanbiosyme Synbiotic	0.00	0.00	0.10	0.10	0.10
Sum	100	100	100	100	100
Calculated analysis (as fed basis; %)					
ME (kcal/kg)	2650	2835	2835	2925	3030
Dry matter	96.74	96.74	96.74	96.74	96.74
Crude protein	24.32	21.46	20.81	17.14	17.04
Crude fiber	3.32	3.16	3.12	2.88	2.87
Lysine	1.52	1.28	1.24	0.95	0.84
Methionine	0.78	0.70	0.67	0.50	0.46
Methionine + cystine	0.98	0.86	0.83	0.66	0.62
Threonine	0.88	0.77	0.74	0.59	0.54
Tryptophan	0.28	0.23	0.23	0.18	0.18
Arginine	1.54	1.32	1.27	1.01	1.02
Isoleucine	0.94	0.80	0.78	0.63	0.64
Leucine	1.75	1.56	1.53	1.34	1.35
Valine	1.02	0.89	0.86	0.71	0.72
Histidine	0.50	0.51	0.50	0.42	0.42
Calcium	1.31	1.12	1.12	0.88	0.81
Available phosphorus	0.67	0.56	0.56	0.44	0.41
Potassium	1.08	0.95	0.92	0.75	0.76
Sodium	0.15	0.15	0.15	0.13	0.13
Chloride	0.18	0.22	0.22	0.20	0.20
[2]DCAD (meq/kg)	293.78	245.67	237.79	193.80	195.93

[1] Premix: Provided per g: vitamin A, 7500.0 IU; vitamin D_3, 3000.0 IU; vitamin E, 10.0 IU; vitamin K, 2.0 mg; riboflavin, 5.3 mg; pantothenic acid, 8.0 mg; niacin, 24.0 mg; choline, 350.0 mg; vitamin B_{12}, 12.5 mg; folic acid, 0.5 mg; thiamine, 2.0 mg; pyridoxine, 1.8 mg; biotin, 0.15 mg; Cu, 6.0 mg; Fe, 30.0 mg; I, 1.0 mg; Mn, 80.0 mg; Se, 0.15 mg; Zn, 50.0 mg.
[2] DCAD = Na + K – Cl

Table 2: Chemical composition of Marivan black grape pomace (percentage in dry matter)

Grape pomace nutrients	Amount (%)
Crude protein	13.77
Ether Extract	3.93
Ash	4.05
Crude fiber	30.27
Dry matter	91.75

After disinfection and preparation of the rearing hall and feed and supplements, 80 hatched one-day-old of B.U.T.6 strain with an average weight of 80 ± 2 g were purchased and transferred to the rearing site. The chicks were kept on the bed in groups until 30 days and fed from the basal diet. At the beginning of day 31, feather trimming and wing numbering were performed for all chickens to prevent the flight and mixing of the treatments. They were then weighed and randomly placed in 16 pens in four treatments, four replications, and five birds per replication. Treatments included a control diet based on corn and soybean meal, 3% grape pulp added to the control diet based on Sadeghi and Nobakht (2015) studies, 150 mL/L aqueous grape pomace (equivalent to 3% grape pomace) added to the control diet, and 0.5 mL/L vitamin E-selenium (E-Selen®, Afagh Pharmaceutical Company, Iran at 0.5 mL/L in drinking water based on manufactured company advice). During the rearing period, water and food were provided ad libitum to the chickens, and the environmental conditions were the same for all treatment groups. The light and temperature programs were adjusted according to the ordered needs of BUT management manual described in Tables 4 and 5. The vaccination schedule was adjusted according to the recommendations of the local veterinarian and joint diseases, according to Table 6.

Table 3: Polyphenolic composition of Marivan black grape pomace (mg / ml extract)

Polyphenolic composition	Methanol extract	Aqueous extract
Gallic acid	36.15	55.94
Protocatechuic	13.50	17.10
Catechin	7.15	6.78
Vanillic acid	2.96	-
Epicatechin	2.68	-
Syringic acid	2.28	-
Coumaric acid	7.89	2.54
Ferulic acid	11.29	3.28

Table 4: The light program of turkey B.U.T.6 strain

Age (day)	Darkness (hour)	Light intensity (Lux)
1-4	1	80-100
5-10	Increase the dark period gradually	80-100
11 until depletion	8	Minimum 40

Table 5: The temperature program of turkey B.U.T.6 strain

Age	Under the brooder (˚C)	Whole house brooding (˚C)
day 1	36-40	36-37
day 2	36-40	35-36
day 3	35-36	34-35
days 4-7	34-35	Decrease 1℃ per day
Week 2	-	27-28
Week 3	-	25-26
Week 4	-	23-24
Week 5	-	21-22
Week 6	-	20-21
Week 7	-	19-20
Week 8	-	18-19
Week 9	-	17-18
Week 10 until depletion	-	16-17

Table 6: Vaccination and medicament schedule of turkey B.U.T.6 strain

Age (day)	The name of the vaccine	Method of vaccination
8	Dual flu and Newcastle	Subcutaneous injection
8	Newcastle B1 strain	eye drop
18	Newcastle, the Lasota strain	eye drop
21-23	Doxycycline antibiotic	Beverage
38	Newcastle, the Lasota strain	eye drop
40	Fowl pox	Inoculation in the thigh membrane
58	Newcastle, the Lasota strain	eye drop
78	Newcastle, the Lasota strain	eye drop
98	Newcastle, the Lasota strain	eye drop

In each period, to evaluate the daily feed intake, daily weight gain, and feed conversion ratio of each bird, body weight, feed intake, and residuals were measured weekly. At the end of 127 days, one male bird was randomly selected from each experimental unit and slaughtered to weigh the carcass parameters. During this measurement, the length and weight of the intestine between the Meckel's diverticulum and the junction of the ileum with the cecum, the percentage of carcasses without viscera, the relative percentage weight of intestine, heart, foregut, full gallbladder, spleen, liver, gizzard, and pancreas in comparison to living weight of the bird were calculated.

The count of *Escherichia coli* and *Lactobacillus* population of of cecum were measured at the end of 127 days after five hours of starvation. One gram of its contents was removed with sterile forceps, transferred to a microbiology laboratory under completely sterile conditions, and refrigerated at room temperature. After serial dilution of each sample, for sterilization of each bacterium, two sterile plates containing suitable culture medium of the desired bacterium (for *Escherichia coli* bacterial mechanical agar medium and lactic acid bacteria MRS agar medium) Falcon was sampled with 5-10 and 6-10 dilutions of one-tenth of cc and spread evenly on the surface of the plate with sterile glass. Finally, all plates were placed in a 37 °C incubator for 48 hours. Then, the number of colonies that grew on the plate surface and represented the number of bacteria were counted and multiplied by the plate dilution image (Wang *et al*., 2008).

To measure the prececal digestibility of nutrients, three percent of rice hull was added to all diets except the diet containing grape pomace as a marker and source of acid-insoluble ash (AIA). On the day of slaughter, intestinal digesta were collected from birds in the distance between the Meckel's diverticulum and the ileum's junction with the cecum and stored at -21 °C. Prececal digesta and feed samples were dried in a 100 °C oven for 24 hours and then ground. Finally, the samples of each experimental unit were mixed well and stored in suitable containers. To calculate the prececal nutrient digestibility of each nutrient, dry matter, crude ash and acid insoluble ash (Coon *et al*., 1990), crude fat (Jee, 1995), and crude protein (Wendt Thiex, 2000) of feed and digesta samples were analyzed and multiplied by the inverse of the dry matter. The prececal nutrient digestibility (Pc D) of dietary nutrients was calculated based on equation 1 (Scott and Kennedy, 1976).

Equation 1

$$\text{Pc D} = 100 - (100 \times ((\text{diet nutrient} / \text{ileal nutrient}) \times (\text{ileal AIA} / \text{diet AIA}))$$

Statistical analysis

Statistical analysis was performed using the GLM procedure of SAS software version 9.4 (SAS, 2013), and the mean of the least-squares of the groups were compared at the level of 5%. For all measured traits, 31-day-old weight was considered as the covariate factor, and the data were normalized after removing the normality by deleting the outlier data. The ststitical model of the experiment was as follow:

$$y_{ijk} = \mu + T_i + \beta (W_{ij} - \overline{W}) + e_{ijk}$$

Where: y_{ijk} = y^{th} observation in the i^{th} level of treatment and j^{th} level of replication, μ = overall mean, T_i = effect of i^{th} level of treatment, β = regression coefficient of the studied traits on body weight at 30th d, w_{ij} = body weight of i^{th} level of treatment and j^{th} level of replicate, $\overline{W}$= average body weight of birds at 30th d and e_{ijk} = residual effect with mean of 0 and normal distribution.

Results

The effect of treatments on daily weight gain, daily feed intake (FI), feed conversation ratio (FCR), final live body weight (BW) and internal organs weight of Turkeys are shown in Table 7. The effect of treatments in the whole period on turkeys' daily feed intake was significant. Daily feed intake in turkeys fed with control, grape pomace, and vitamin E-selenium treatments was similar and higher throughout the rearing period than grape pomace extract treatment. The results show that the treatments in the whole period had a significant effect on daily weight gain. In the whole rearing period, birds treated with grape pomace had the highest daily weight gain and final weight, and birds treated with grape pomace extract showed the lowest daily weight gain and final body weight. There was no significant difference in turkeys' feed conversion ratio in the experimental treatments during the whole rearing period. The effects of treatments on the relative weight of carcass, heart, proventriculus, spleen, liver, gizzard, and length and weight of intestine were not significant, However, the relative weight of pancreatic and gallbladder was affected significantly by treatments. The relative weight of the pancreas in birds fed with grape pomace extract and gallbladder in birds fed with grape pomace treatment was higher than other treatments.

The results in Table 8 show that the population of cecum *Escherichia coli* in vitamin E-selenium treatment was lower than the other treatments. Lactic acid bacteria had the highest population in the cecum of turkeys fed with grape pomace and grape pomace extract treatment. In contrast, the population of this bacterium was lower in the control treatment than in the other treatments.

According to the results shown in Table 9, the digestibility of dry matter, ether extract, and crude protein in turkeys of control and grape pomace extract treatments were similar to each other but more than grape pomace and vitamin E-selenium treatments. Moreover, digestibility of ether extract and crude protein in bird under vitamin E-selenium treatment was greater than grape pomace treatment. The digestibility of ash in control group was improved compared to those of other treatments. Also, birds received vitamin E-selenium treatment showed greater ash digestibility than those of grape pomac and grape pomac extract treatments.

Table 7: Effect of dietary inclusion of grape pomace aqueous extract, dried grape pomace and vitamin E-selenium on performance, carcass percentage, intestine length, and relative organ weight in B.U.T.6 turkeys

Measurement	Treatments [2]				*SEM*	*P*-value
	Control	Vitamin E-Selenium	Grape pomace	Grape pomace extract		
Daily feed intake (g/bird)	336.4^{a}	326.5^{a}	351.9^{a}	306.6^{b}	8.49	0.027
Daily weight gain (g/bird)	116.8^{bc}	119.9^{ab}	125.5^{a}	110.2^{c}	2.73	0.028
Final body weight (g)	10086.3^{bc}	10319.9^{ab}	10755.3^{a}	9576.1^{c}	210.00	0.029
Feed conversion ratio	2.8	2.7	2.8	2.8	0.05	NS
Carcass percentage	71.7	71.9	72.7	70.5	0.68	NS
Intestine length[1] (cm)	124.6	116.5	113.8	103.5	4.81	NS
Intestine weight[1] (%)	2.8	2.6	2.4	2.8	0.15	NS
Heart weight (%)	0.34	0.37	0.45	0.41	0.03	NS
Pre-gastric weight (%)	0.17	0.16	0.15	0.18	0.01	NS
Gallbladder weight (%)	0.03^{b}	0.04^{a}	0.04^{a}	0.03^{b}	0.004	0.006
Spleen weight (%)	0.14	0.17	0.14	0.19	0.02	NS
Liver weight (%)	1.3	1.5	1.5	1.6	0.15	NS
Gizzard weight (%)	2.6	2.1	2.1	2.3	0.16	NS
Pancreas weight (%)	0.09^{b}	0.09^{b}	0.09^{b}	0.13^{a}	0.01	0.013

[a-c] Within each row, means with different superscript (s) are significantly different ($P < 0.05$).

[1]From Meckel's diverticulum to ileo-ceca-colonic junction.

[2] Grape pomace aqueous extract (150 mL / L), dried grape pomace (3%), and E selenium (0.5 mL / L) were added to the basal diet.

Table 8: Effect of dietary inclusion of grape pomace aqueous extract, dried grape pomace and vitamin E-selenium to the basal diet on bacterial population ($\log_{10}$ CFU/g) of the cecum in turkey B.U.T.6 strain

Measurement	Treatments [1]				*SEM*	*P*-value
	Control	Vitamin E-Selenium	Grape pomace	Grape pomace extract		
Escherichia coli	7.55^{a}	7.21^{b}	7.45^{a}	7.43^{a}	0.052	0.011
lactic acid bacteria	7.39^{c}	8.32^{b}	8.44^{ab}	8.56^{a}	0.042	<0.0001

[a-c] Within each row, means with common superscript (s) do not differ ($P < 0.05$).

[1] Grape pomace aqueous extract (150 mL / L), dried grape pomace (3%) and E selenium (0.5 mL / L) were added to the basal diet.

Table 9: Effect of dietary inclusion of grape pomace aqueous extract, dried grape pomace and vitamin E-selenium to the basal diet on apparent prececal digestibility of dry matter (DM), ash, ether extract (EE) and crude protein (CP) in turkey B.U.T.6 strain diet

Prececal nutrient digestibility (%)	Treatments [1]				*SEM*	*P*-value
	Control	Vitamin E-Selenium	Grape pomace	Grape pomace extract		
DM	81.6^{a}	79.4^{b}	79.5^{b}	81.5^{a}	0.33	0.0006
Ash	39.4^{a}	23.8^{c}	32.1^{b}	31.0^{b}	1.79	<0.0001
EE	61.9^{a}	52.4^{b}	33.3^{c}	66.3^{a}	1.85	<0.0001
CP	48.3^{a}	38.4^{b}	31.4^{c}	51.6^{a}	1.25	<0.0001

[a-c] Within each row, means with common superscript (s) do not differ ($P < 0.05$).

[1] Grape pomace aqueous extract (150 mL / L), dried grape pomace (3%) and E selenium (0.5 mL / L) were added to the basal diet.

Discussion

Daily feed intake in treatments with grape pomace, vitamin E-selenium, and control treatment was similar and significantly higher than grape pomace extract treatment. The reason for less feed intake in birds treated with grape pomace extract compared to the other treatments may be the overdose or higher concentration of some water-soluble compounds like polyphenols present in the concentrated aqueous pomace extract. Pascariu *et al*. (2017) reported an unpleasant taste in grape byproducts that can avoid water consumption and harm feed intake in broilers. Of course, it seems that with an increase of age in turkeys, this decreasing effect would disappear. This effect may be related to the compatibility of gastrointestinal enzymes and microflora of the gastrointestinal tract to grape pomace ingrown turkey (Sadeghi and Nobakht, 2015). Grape pomace-treated birds had the highest daily weight gain and final body weight in the whole breeding period. While the vitamin E-selenium treatment did not differ significantly from the control treatment, birds in grape pomace extract showed the lowest daily weight gain and final body weight. The lower daily weight gain and final body weight in birds treated with grape pomace extract can be related to lower feed intake in this treatment. Pascariu *et al*. (2017) showed that the lowest average body weight gain in broilers belonged to the grape pomace extract, which may have a negative effect on chickens' growth due to the increase of polyphenols or other effective compounds in grape byproduct extract. Brenes *et al*. (2010) reported that lower concentrations of grape pomace extract up to 3.6 g/kg in a broiler diet did not reduce growth performance. Viveros *et al*. (2011) also reported that using grape pomace extract in broiler diets did not alter the bird's daily weight gain and final body weight. There was no significant difference in turkey feed conversion ratio in experimental treatments throughout the breeding period. Studies have shown that adding 200 mg/kg vitamin E to the diet reduces the feed conversion ratio in broilers compared to grape pomace (Brenes *et al*., 2008). However, in other studies, the use of diets containing grape byproduct extract in broilers was associated with reduced growth performance and a higher conversion ratio than the control group (Pascariu *et al*., 2017; Chamorro *et al*., 2013; Viveros *et al*., 2011). The reason for these discrepancies may be related to the level of polyphenols in grape pomace and extract byproducts. The effects of polyphenols in broilers have been studied by adding rich polyphenols compounds such as sorghum and fava beans, which indicates a decrease in performance. Dietary polyphenols are commonly associated with decreased feed efficiency and especially proteins and amino acids digestibility, increased excretion of endogenous proteins, and inhibition of digestive enzymes by binding to proteins (Pascariu *et al*., 2017). Also, the crude fiber in grape pomace is unusable for monogastric, especially at a young age, but may also interfere with the absorption of other nutrients in the diet. Thus, despite the beneficial properties of grape pomace and its beneficial nutrients, young chickens have not been able to use it, which has been overcome with age and the development of the gastrointestinal tract and its bacteria in Turkeys. Grape pomace, especially its polyphenols content, protected nutrients from oxidation, and improve nutrient digestion and absorption (Sadeghi and Nobakht, 2015). It was shown that in broiler chickens fed grape pomace, the ratio of villi height to crypt depth in the intestinal tract increased, which can lead to better nutrient absorption, reduced endogenous gastrointestinal losses, increased disease resistance, and improved growth performance, especially when using in suitable dose, proper age and spices (Viveros *et al*., 2011). The relative weight of the pancreas in birds fed with grape pomace extract and gallbladder in birds fed with grape pomace was higher than in control treatments. Due to its high fiber content, the grape pomace may increase the feed passage rate in the gastrointestinal tract. Increasing the rate of material passage may not provide the opportunity for the pancreatic enzymes to come into contact with intestinal digesta. Also, the interaction of polyphenols in grape pomace with some amino acids, such as methionine, and in grape pomace extract with crude fat, may cause the secretion of more enzymes in the pancreas and more bile acids, which in turn increases the weight of the pancreas and gallbladder (Quan *et al*., 2019). Research has shown that replacing grape pomace up to 60 g/kg in broiler diets does not affect the spleen, liver, abdominal fat, jejunum length, ileum, duodenum, and cecum. However, in birds fed grape pomace compared to vitamin E acetate, spleen weight increased by 20% and ileum length by 8% compared to the control (Brenes *et al*., 2008). Using 3% grape pomace in broilers' diet compared to the control has increased the relative weight of carcasses and decreased the relative weight of gizzard and liver (Sadeghi and Nobakht, 2015).

The stimulatory activity of lactic acid bacteria from grape pomace and grape pomace extract in cecum can be attributed to their extractable polyphenolic composition and content, as polyphenols have been shown to alter intestinal bacterial populations. Although the exact mechanism of action of polyphenols in the body is unknown, few studies have considered some evidence. they show a bactericidal activity via inhibiting the adhesion of bacteria that cause infection to intestinal cells (Viveros *et al*., 2011). Another possible effect is that phenolic compounds act as nutrient substrates for some microorganisms depending on their chemical structure (substitutions in the phenolic ring) and concentration. For instance, *Lactobacillus* can metabolize phenolic compounds and bring energy to the cell (García-Ruiz *et al*., 2008). It has also been reported that grape products may act as prebiotics in favor of beneficial bacteria such as Bifidobacterium and *Lactobacillus* (Dolara *et al*., 2005; Tzounis *et al*., 2008). In vitro studies reproted the antibacterial activity of phenolic compounds against harmful bacteria such as *Escherichia coli* (Baydar *et al*., 2006; Vaquero *et al*., 2007; Papadopoulou *et al*., 2005) , butstimulatory effect on the growth of *Lactobacillus acidophilus* (Hervert-Hernández *et al*., 2009). Grape polyphenolic extract and its by-products have been shown to affect the intestinal microflora, reducing the number of Propionibacteria, Bacteroids, and Clostridia, increasing the number of *Lactobacillus* and *Bifidobacteria*, and ultimately increasing the biodiversity of intestinal bacteria (Ozkan *et al*. 2004; Dolara *et al*., 2005; Papadopoulou *et al*., 2005). In cecal feces of birds fed diets containing pulp and grape seed extract, more populations of Lactobacillus, *Escherichia coli*, Enterococcus and Clostridium were present compared to the control group. Birds fed grape seed pomace had a significantly lower *Escherichia coli* and Enterococcus than grape seed extract. Birds fed grape seed extract had higher numbers of *lactobacilli Clostridium* than grape seed extract (Viveros *et al*., 2011). Hedayati *et al*. (2017) also showed that methanolic grape extract in broilers' diet had reduced the population of Salmonella, *Escherichia coli*, and coliforms. In the current study, *Escherichia coli* population was not influenced by the grape extract. It may be due to the usage of aqueous grape pomace extract in present treatments with different content and concentration of phenolic compounds.

Nutrient digestibility results agree with a study in birds fed with grape pomace in which fat digestibility was reduced compared to a diet containing vitamin E acetate (Brenes *et al*., 2008). Increasing the amount of crude fiber in a diet containing grape pomace can reduce fat absorption because crude fiber inhibits the bile acids responsible for fat absorption and leads to lower fat and cholesterol absorption. The inhibitory effect of grape seed extract on fat metabolizing enzymes and lipoproteins has also been shown in in vitro studies (Goni *et al*., 2007). Some investigations have suggested that the substitution of grape pomace in broiler have no effect on the apparent intestinal digestibility of proteins and amino acids. This fact can be attributed to the low content of polyphenols in experimental diets (Brenes *et al*., 2008). Studies have shown reduced digestibility of some amino acids in birds fed grape pomace than diets containing vitamin E acetate. A secondary effect on the digestibility of amino acids like lysine, tryptophan, glutamine, and serine has also been observed with increasing dietary grape pomace. The differences observed may be due to probability of polymerization between polyphenols and substances such as amino acids and sugars (Goni *et al*., 2007). In general, the effect of a polyphenolic compound on absorption and composition of nutrients depends on its type, dose, and binding with other compounds (Martel *et al*., 2010). Also, in explaining the decrease in digestibility of proteins in treatments containing grape pomace, it has been stated that binding of the reactive hydroxyl group of polyphenols, especially tannins, to the carbonyl group of proteins creates a complex that can reduce the digestibility of proteins (Pascariu *et al*., 2017). Another mechanism that leads to less digestion of feed is the lower performance of digestive enzymes such as trypsin, alpha-amylase, and lipase by natural polyphenols, mainly related to the ability of tannins to form insoluble compounds with gastrointestinal proteins (Goni *et al*., 2007). The use of grape pomace in broilers' diet reduced the apparent ileal digestibility of fat but did not affect the digestibility of proteins and amino acids (Brenes *et al*., 2008). It has also been shown that in birds fed grape pomace compared to birds fed vitamin E, the digestibility of some amino acids such as arginine, leucine, phenylalanine, glutamine, prolamine, tyrosine, histidine, and cysteine decreased (Goni *et al*. 2007). The raw fibers in grape pomace are not only unusable for monogastric, especially at a young age, but may also interfere with the absorption of other nutrients in the diet, and young chickens may not be able to use it. As they age and develop the gastrointestinal tract and microflora, they may overcome it and use the beneficial nutrients of grape pomace (Sadeghi and Nobakht, 2015).

Conclusion

Using 3% of grape pomace in the turkey diet increased daily weight gain, final body weight, and the number of beneficial cecal bacteria without any adverse effect on feed conversion ratio. Therefore, these natural antioxidants can be considered in turkey diets. The use of grape pomace extract in the turkey water positively affected the beneficial cecal bacterial population and decreased daily feed intake. In birds treated with grape pomace extract, daily weight gain and final body weight were the same as the control diet, so because feed intake decreased, using grape pomace extract in the water can be considered helpful in reducing feed costs. It is recommended that the harmful or beneficial compounds in grape pomace extract and its effective dose be accurately identified in future research.

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