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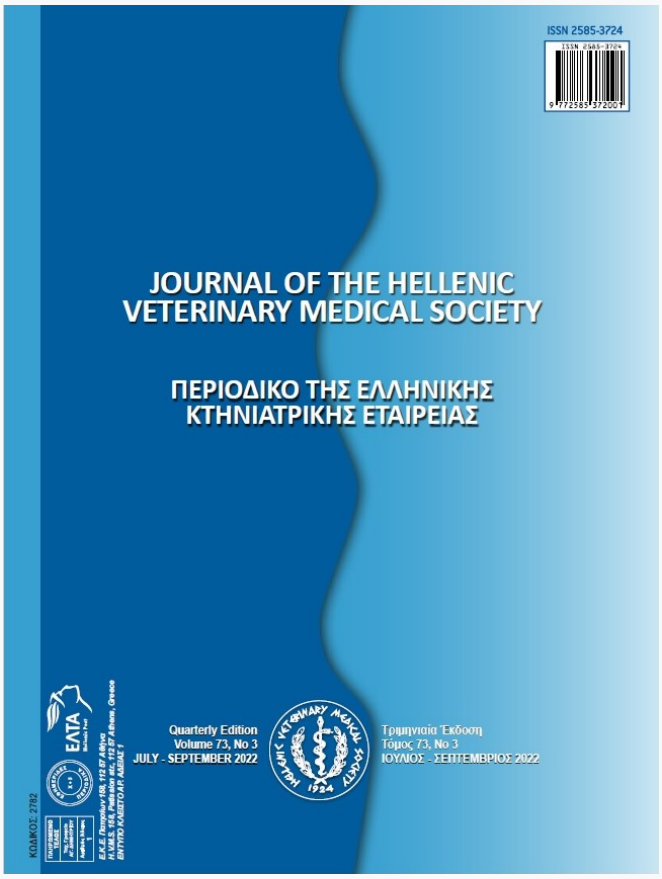

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G Şen, M Başalan

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Effects of Using Inulin and Grape Pomace in Broiler Diets on Performance, Carcass Yield, Intestinal Viscosity, Immunity, and Antioxidant Status

G. Sen*, M. Basalan

Kirikkale University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kirikkale, Turkey

ABSTRACT: This study was carried out to investigate the effects of inulin and grape pomace addition to broiler diets on broiler chicken performance, carcass yield, intestinal viscosity, immunity, and antioxidant status. In the study, a total of 160 unsexed 0-day-old Ross 308 broiler chicks were divided into 4 groups:(1) control (CON) group, (2) 10 g/kg inulin (IN), (3) 50 g/kg grape pomace (GP),and(4) 10 g/ kginulin + 50 g/kg grape pomace (INGP). At the end of the 42-day experiment, 48 animals were exsanguinated to obtain blood for the analysis of immunity and antioxidant parameters. At the end of the study period, while body weight gain, carcass yield and intestinal viscosity values between the groups was not significant (P>0,05), in grape pomace-containing groups, negative effects on feed consumption and feed conversion ratios were found (P<0.05). Immunoglobuline (Ig)G levels of the animals in the INGP group were significantly lower than thosein the other groups (P<0.05). Catalase (CAT) activity increased in groups consuming diets containing grape pomace (P<0.05). While inulin administration produced an increase in β carotene level,vitamin C and E levels were significantly increased consumption of inulin and grape pomace (P<0.05). As a result, grape pomace up to 5% and inulin up to 1% in broiler diets can be used separately as antioxidants. However, grape pomace may adversely affect FCR depending on the polyphenol level. In addition, it should be consider that they may have a negative effect on immunity when used in combination.

***Keywords*:** Antioxidant Status; Broiler Performance; Grape Pomace; Immunity; Inulin.

Corresponding Author:
G. Sen, Kirikkale University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kirikkale, Turkey
E-mail address: gokhansen@kku.edu.tr

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INTRODUCTION

Using agricultural wastes and residues from farming and horticulture in poultry diets is a long-term practice in broiler farming. The correct management of these waste products released in the agriculture and food industry can provide cheap and suitable feed materials for poultry animals without competion for human food. In addition, use of these productsin poultry feeds will reduce waste and prevent environmental pollution (Azizi, 2018). Feed-related expenses constitute the biggest costs in the poultry industry. For this reason, various studies are being conducted to meet the increasing feed requirement and reduce feed costs (Iqbal et al., 2015; Abdel-Hafeez et al., 2017; Şengül et al., 2019).

Grapes are one of the most produced fruits in the world. After being squeezed for wine or fruit juice production, the remaining part consists of seeds, skin, and stems, and approximately 20% of the total amount of grapes in the remaining part is called grape pomace. Grape pomace contains proteins, fats, vitamins, minerals, fibers, and phenolic compounds, such as (+)-catechins, (–)-epicatechins, (–)-epicatechin-3-O-gallate, and proanthocyanidins, which are bioactive substances. Phenolic compounds, which are bioactive substances found in grapes, were first described as antinutritional, but later it was determined that they had antibacterial and antioxidant effects. As an alternative substance to antibiotics (antibiotics leave residues in broiler meat) with antimicrobial features, phenolic compoundsare products without the risk of leaving residues in broiler meat. Phenols also show antioxidant effects against the oxidative substance-induced damage (Viveros et al., 2011; Brenes et al., 2016; Pascariu, 2017; Kumanda et al., 2019). Recently, phytochemicals are a more reasonable choice as natural antioxidantsas opposed to synthetic antioxidants due to their possible toxic and mutagenic effects on the body (Aditya, 2018). Reports that phenolic compounds can cause improvements in immune responses for broilers have been published (Iqbal et al., 2015; El-Kelawy et al., 2018).

Inulin is a type of fructose obtained from plants, such as chicory and Jerusalem artichoke, and is formed by linear binding of D-fructose molecules with β–(2→1) bonds. While it cannot be digested by the digestive enzymes of monogastric animals due to the type of bond in its structure, it can be used by beneficial bacteria in the large intestine (Shoaib, 2016; Shang, 2018). Inulin is classified as a prebiotic and benefits the host by causing an improvement in gut microbiota, increase in mineral absorption, and stimulation of immune function in addition to affecting gut health (Ahmed and Rashid, 2019). In addition, recent studies with inulin demonstrate that it also has antioxidant effects (Liu et al., 2015; Shang et al., 2018; Guaragni et al., 2020; Shang et al., 2020).

The aim of this study was to investigate the individual or combined effects of grape pomace, a waste product rich in penolic compounds, and inulin used as a prebiotic with reported antioxidant and immune response-improving effects on broilers.

MATERIALS AND METHODS

Feed supplements

Inulin was obtained from a commercial company. Grape pomace was kindly supplied by several wine factories in the Kalecik district of Ankara. Moist grape pomace was dried in anairy atmosphere until the dry matter was about 90% after which it was groundusing a grinder with 1mm screens. Proximate analyses of diets and grape pomace were done according to the AOAC (2005). Nutrient composition of grape pomace is shown in Table 1.

Animal care and experimental design

The experiment was carried out with 160 heads of 0-day old broiler chicks (Ross 308)for 42 days. Groups were hosted in an environmentally controlled room lit for 24 h with constant fluorescent lighting and heated by electrical heaters. The room temperature was initially set at 33 °C and then gradually reduced to 21 °C by the sixth week after which time it was maintained at 20 ºC there after. The chicks were allocated to 16 pens, each pen containing 10 chicks, that receivedfour dietary treatments with four replicates of each treatment. The experimental groupsconsisted of chicks fed different diets:(1) the control (CON) group fed with basal diet containing neither inulin nor grape pomace,(2) the inulin (IN) group fed with diet containing 10 g/kg inulin, (3) the grape pomace(GP) group fed with diet containing 50 g/kg grape pomace, and (4) the inulin + grape pomace (INGP) group fed with diet containing 10 g/kg inulin + 50 g/kg grape pomace. The diets were formulated as starter (1-10 days), grower (11-28 days), and finisher (29-42 days)in order to supply necessary nutritional requirements for broiler chickens. Ingredients and nutrient compositions of dietsare shown in Table 1. Diets in mash form and water were provided *ad libitum*. The experimental procedures were approvedwith 15/68

Table 1. Ingredients, nutrient compositions of the diets and nutrient composition of the grape pomace

	Starter				Grower				Finisher				Grape
	CON	IN	GP	INGP	CON	IN	GP	INGP	CON	IN	GP	INGP	Pomace
Corn, %	44,60	44,15	42,37	41,92	49,00	48,51	46,55	46,06	48,20	47,72	45,79	45,31	-
Soybean meal, %	41,25	40,84	39,19	38,78	37,35	36,97	35,49	35,11	30,00	29,70	28,50	28,20	-
Vegetable oil, %	5,70	5,64	5,42	5,36	5,05	5,00	4,80	4,75	5,95	5,89	5,65	5,59	-
DDGS, %	-	-	-	-	5,35	5,30	5,09	5,03	7,90	7,82	7,51	7,43	-
Corn bran, %	5,00	4,95	4,75	4,70	-	-	-	-	5,00	4,95	4,75	4,70	-
Limestone, %	1,30	1,29	1,24	1,22	1,55	1,53	1,47	1,46	1,25	1,24	1,19	1,17	-
Salt, %	0,25	0,25	0,24	0,24	0,25	0,25	0,24	0,23	0,25	0,25	0,24	0,24	-
DCP, %	1,40	1,39	1,33	1,31	1,05	1,04	0,99	0,99	1,15	1,14	1,09	1,08	-
Vit.-Min., %	0,25	0,25	0,23	0,24	0,25	0,25	0,23	0,23	0,25	0,25	0,23	0,24	-
Metionin, %	0,15	0,15	0,14	0,14	0,15	0,15	0,14	0,14	0.05	0,04	0,04	0,04	-
Na_2HCO_3, %	0,10	0,09	0,09	0,09	-	-	-	-	-	-	-	-	-
Grape Pomace, %	-	-	5,00	5,00	-	-	5,00	5,00	-	-	5,00	5,00	-
Inulin, %	-	1,00	-	1,00	-	1,00	-	1,00	-	1,00	-	1,00	-
DM (g/kg)	910.7	906.8	906.7	912.4	902.6	900.5	906.9	909.6	897.9	897.4	893.2	896.1	931.3
CP (g/kg)	219.1	218.6	220.9	217.8	205.0	204.5	205.6	207.3	187.0	184.3	184.5	187.8	59,2
Ash (g/kg)	67.5	57.8	62.2	66.6	103.7	72.4	80.8	73.7	58.6	83.2	66.6	78.5	93,8
Fat (g/kg)	88.0	82.8	92.3	78.4	80.8	81.4	93.4	85.0	83.3	87.5	85.8	84.2	321,6
CF (g/kg)	33.4	30.8	48.7	45.7	46.7	44.8	60.5	60.5	39.5	38.4	61.8	56.6	102,2
ME* (Kcal/kg)	3229	3291	3268	3248	2839	2945	2914	2952	3272	3244	3056	3144	1270

*ME (Metabolizable Energy) contents were calculated according to the formula of Anonymous (2004)
DDGS: Dried Distillers Grain with Solubles, DCP:Dicalciumphosphate, Na_2HCO_3: Sodium bicarbonate, DM: Dry Matter, CP: Crude Protein, CF: Crude Fiber

decision number by the Kirikkale University Animal Experiments Local Ethics Committee.

Sample collection and analysis

During each week of the experimental period, body weights of chicks and remaining feeds were weighed and, mean body weights and feed consumptions of every group were determined. Feed conversion ratios were calculated weekly with total feed consumption divided by body weight gain. At the end of study, threechicks from each subgroup were randomly selected and body weights were weighed. Blood samples were obtained from vena jugularis for determination of immunity parameters and antioxidant status. A total of 48 chickens with, 12 randomly selected from each group weresacrificed. Hot carcass weights of sacrificed animals were immediately recorded. Carcass yields were obtained by dividing hot carcass weights by live body weights.While internal organs were removed,the contents of the duodenum in which, chemical and enzymatic digestion occurs,wasused for viscosity determinations. The collected duodenal contents were prepared according to the method of Günal et al. (2004) and were measured with Brookfield DV-II viscometer at 40 °C and 100 rpm.Plasma superoxide dismutase (SOD), catalase (CAT), glutation peroxidase (GPx) activities and plasma IgG and Ig M levels were determined by using kits according to the manufacturer's instruction.Plasma malondialdehyde (MDA) levels were measured with microplate reader (Thermo Scientific Multiscan Go) according to the method by Buege and Aust (1978). Plasma vitamin A and β carotene levelswere determined via spectrophotometric measurements(Shimadzu UV-1700) according to Suzuki and Katoh (1990). Plasma vitamins C and E analyses were done with spectrometer (Shimadzu UV-1700) according to Haag (1985) and Martinek (1964), respectively.

Statistical analysis

The study was designed as a trial pattern of random blocks. Statistical analysis of the data obtained were done using the one-way analysis of variance (ANOVA)using the "SPSS 15.0 for Windows". Descriptive statistics were expressed as means and standard errors. The differences between the groups were indicated by Duncan's means separation test. AP value <0.05 was considered as the criterion for statistical significance.

RESULTS

Growth Performance, Feed Consumption and Feed Conversion Ratio

The values of the body weight gains in the research

Table 2. Body weight gains of groups (g/chicken)

Age (weeks)	CON	IN	GP	INGP	P
0	43.10 ± 0.40	42.58 ± 0.40	43.10 ± 0.40	42.90 ± 0.40	0.762
1	186.78 ± 2.34^{b}	185.00 ± 2.93^{b}	188.71 ± 3.07^{b}	172.59 ± 5.14^{a}	**0.029**
2	241.03 ± 5.62	235.77 ± 8.96	227.87 ± 9.53	257.49 ± 9.38	0.146
3	276.52 ± 8.30	284.46 ± 5.14	267.55 ± 9.37	282.97 ± 6.89	0.415
4	334.42 ± 11.51	321.58 ± 13.72	304.70 ± 11.12	343.59 ± 2.55	0.107
5	463.70 ± 8.83	427.86 ± 5.71	471.11 ± 34.79	476.64 ± 7.42	0.290
6	478.31 ± 29.33	453.11 ± 9.58	454.53 ± 6.48	488.99 ± 9.51	0.363
0-3	704.32 ± 5.06	705.23 ± 12.06	684.12 ± 16.42	713.05 ± 19.96	0.555
3-6	1276.42 ± 39.23	1202.56 ± 19.08	1230.34 ± 47.64	1309.22 ± 10.10	0.153
0-42	1980.73 ± 41.75	1907.79 ± 25.96	1914.46 ± 61.42	2022.27 ± 11.37	0.182

$^{a\,b}$: Different letters in the same row indicate significant differences (P<0.05) n=4

Table 3. Feed consumptions of groups (g)

Age (week)	CON	IN	GP	INGP	P
1	163.10 ± 3.10	157.51 ± 3.66	165.74 ± 3.85	166.53 ± 5.57	0.441
2	349.54 ± 4.26	342.22 ± 6.24	360.29 ± 6.96	367.38 ± 6.23	0.051
3	586.01 ± 9.76	573.68 ± 12.11	595.78 ± 11.48	607.51 ± 10.27	0.215
4	546.97 ± 17.01	599.28 ± 28.98	585.28 ± 22.94	634.42 ± 25.07	0.130
5	813.97 ± 13.41^{a}	791.78 ± 15.50^{a}	832.03 ± 40.50^{ab}	894.24 ± 11.20^{b}	**0.048**
6	1112.78 ± 17.97^{b}	1031.00 ± 31.19^{a}	1123.78 ± 14.70^{b}	1128.88 ± 17.61^{b}	**0.023**
0-3	1098.66 ± 13.70	1073.41 ± 19.51	1121.82 ± 21.47	1141.42 ± 20.31	0.119
3-6	2473.72 ± 31.75^{a}	2422.06 ± 51.01^{a}	2541.08 ± 74.36^{ab}	2657.54 ± 9.36^{b}	**0.025**
0-42	3572.38 ± 25.43^{a}	3495.47 ± 68.08^{a}	3662.90 ± 90.49^{ab}	3798.96 ± 27.49^{b}	**0.020**

$^{a\,b}$: Different letters in the same row indicate significant differences (P<0.05) n=4

Table 4. Feed conversion ratios of groups (g/g)

Age (week)	CON	IN	GP	INGP	P
1	0.87 ± 0.01	0.85 ± 0.02	0.88 ± 0.03	0.97 ± 0.06	0.146
2	1.45 ± 0.05	1.46 ± 0.04	1.59 ± 0.04	1.43 ± 0.05	0.128
3	2.12 ± 0.05	2.02 ± 0.05	2.23 ± 0.08	2.15 ± 0.05	0.149
4	1.64 ± 0.08^{a}	1.86 ± 0.05^{b}	1.92 ± 0.02^{b}	1.85 ± 0.07^{b}	**0.032**
5	1.76 ± 0.04	1.85 ± 0.04	1.78 ± 0.10	1.88 ± 0.01	0.446
6	2.35 ± 0.16	2.28 ± 0.04	2.47 ± 0.04	2.31 ± 0.03	0.410
0-3	1.56 ± 0.01	1.52 ± 0.02	1.64 ± 0.03	1.60 ± 0.05	0.103
3-6	1.94 ± 0.05	2.01 ± 0.01	2.07 ± 0.03	2.03 ± 0.02	0.106
0-42	1.81 ± 0.03^{a}	1.83 ± 0.01^{ab}	1.91 ± 0.02^{c}	1.88 ± 0.02^{bc}	**0.012**

$^{a\,b}$: Different letters in the same row indicate significant differences (P<0.05) n=4

groups were obtained with weekly weighing and are presented in Table 2. At the end of the study , no differences between the groups in terms of body weight gain (P>0.05), except for the first week, were found. The average body weight gain of the INGP group was found to be significantly lower than the other groups in thefirst week (P<0.05).

The feed consumption of the groups that had been determined on a weekly basis are shown in Table 3. The feed consumption of the animals in INGP group was significantly higher than in the CON and IN groups by the fifth week (P<0.05).The feed consumption of the chickens inthe INgroup was found to be significantly lower than the other groups by the sixth week (P<0.05). The feed consumption of the INGPgroup was significantly higher than that of the CON and INgroups(P<0.05). In addition, the feed consumption of the INGP group was higher than that of the CON and IN groups by the second half of the study and at the overall period(P<0.05).

The feed conversion ratios of the groups are shown in Table 4. It can be seen that the difference between the groups was significant both at the end of the fourth week and at the overall period. At the end

of the fourth week, it was seen that FCR in the CON groupwas significantly lower than the other groups (P<0.05).At the overall period, the feed conversion ratio of the GP and INGPgroups were significantly different from the CON group and GP group from IN group were significantly higher(P<0.05).

Carcass Yield and Intestinal Viscosity

Body weight, hot carcass weight, carcass yield and intestinal viscosity data from the slaughtered animals are shown as averages in Table 5. While there was a significant difference between the groups in terms of the pre-slaughter body weights and the hot carcass weights (P<0.05), no statistically significant difference between the groups in terms of the carcass yields was found (P>0.05). While the pre-slaughter body weights of INGP group were higher than the CON and GP groups, the hot carcass weights were also higher than in the other groups. No significant difference in intestinal viscosity values between the groups was detected (P>0.05).

Immunity and Antioxidant Status

The resultsfor the immunity and antioxidant parameters as measured from chicken plasma in the different groups are shown in Table 6. While the plasma IgG levels of IN and GP groups are similar to CON group (P>0.05), IgG level ofthe INGP group plasma was significantly lower than in these other groups(P<0.05). No differences in plasma IgM levels ofany group were found (P>0.05).Antioxidant enzyme activities except CAT did not show any significant changeresulting from the addition of inulin and grape pomace. While SOD and GPx enzyme activities were similar in all groups (P>0.05), CAT enzyme activity had significantly increased in groups that received grape pomace(P<0.05). No significant differences between the groups with respect to plasma MDA levels were found (P>0.05).The levels of plasma vitamins C, E and β carotene were significantly different between the groups (P<0.05), but vitamin A levels were similar between groups (P>0.05). Plasma β carotene level was significantly higher in the IN group than in the CON and INGP groups(P<0.05).Plasma vitamin C levelsof the CON and IN groups were lower than in the GP and INGP groups(P<0.05).Hence, it can be said that grape pomace increases the level of plasma vitamin C. Plasma vitamin E level was lower in the CON group compared to the other groups (P<0.05).

DISCUSSION

Growth Performance, Feed Consumption, and Feed Conversion Ratio

The bioavailability of grape pomace decreases due to high fiber and polyphenol levels depending on the tannin it contains. It has been reported that condensed

Table 5. Carcass yields and intestinal viscosity values of groups

	CON	IN	GP	INGP	P
Body weight before slaughter, g	1839.17 ± 74.06^{a}	1858.33 ± 67.96^{ab}	1725.00 ± 67.61^{a}	2040.83 ± 49.37^{b}	**0.013**
Hot carcass weight, g	1334.25 ± 58.62^{a}	1339.67 ± 51.43^{a}	1237.58 ± 50.47^{a}	1498.67 ± 44.31^{b}	**0.008**
Carcass yield, %	72.44 ± 0.48	72.04 ± 0.32	71.70 ± 0.42	73.34 ± 0.52	0.068
Intestinal viscosity, cP	1.44 ± 0.09	1.38 ± 0.06	1.39 ± 0.06	1.54 ± 0.09	0.479

$^{a\,b}$: Different letters in the same row indicate significant differences (P<0.05)

cP: centipoise n=12

Table 6. Plasma immunity and antioxidant status of groups

	CON	IN	GP	INGP	P
Ig G, ng/ml	18.93 ± 1.43^{b}	15.25 ± 1.33^{b}	18.85 ± 1.45^{b}	11.04 ± 1.11^{a}	**<0.001**
Ig M, ng/ml	2.02 ± 0.34	2.51 ± 0.69	1.51 ± 0.30	2.03 ± 0.21	0.476
SOD (U/ml)	5.25 ± 0.34	4.85 ± 0.17	5.47 ± 0.21	5.27 ± 0.17	0.261
CAT (nmol/dk/ml)	1.07 ± 0.29^{a}	2.47 ± 0.61^{a}	5.44 ± 0.59^{b}	4.64 ± 0.50^{b}	**<0.001**
GPX (nmol/dk/ml)	334.69 ± 8.87	339.90 ± 8.56	350.54 ± 12.45	336.31 ± 12.42	0.746
MDA (µmol/L)	1.18 ± 0.08	1.20 ± 0.11	1.14 ± 0.08	1.08 ± 0.03	0.760
β Caroten (µg/dl)	267.11 ± 10.50^{a}	333.89 ± 16.71^{b}	305.57 ± 14.73^{ab}	275.06 ± 24.94^{a}	**0.046**
Vitamin A (µg/dl)	29.42 ± 5.90	31.21 ± 3.56	26.48 ± 3.15	22.19 ± 1.58	0.239
Vitamin C (µg/dl)	14.47 ± 0.80^{a}	14.46 ± 0.62^{a}	16.78 ± 0.95^{b}	16.64 ± 0.44^{b}	**0.032**
Vitamin E(mg/dl)	0.35 ± 0.05^{a}	0.52 ± 0.04^{b}	0.54 ± 0.06^{b}	0.53 ± 0.02^{b}	**0.013**

$^{a\,b}$: Different letters in the same row indicate significant differences (P<0.05)

Ig: Immunoglobulin SOD: Superoxide dismutase CAT: Catalase GPX: Glutathione peroxidase MDA: Malondialdehyde n=12

tannin in the grape pomace structure can be as high as 104 g/kg dry matter (Kumanda, 2019; Güngör et al. 2021). Except for the first week, body weight gain did not change between the groups in our study data, whereas the FCR values decreased. Increased feed consumption in groups containing grape pomace also affected FCR values. Reyes et al. (2020) reported that the increase in feed consumption may be due to the color and odor of the grape pomace. In our study, ground grape pomace particles were viewed as black dots in diets containing grape pomace. In addition, grape pomace has beneficial effects on the liverdue to its phenolic compounds, but high levels can cause harmful effects. As a result of the interactions between the reactive hydroxyl groups of polyphenols and the carbonyl groups of proteins, polyphenol-protein complexes are formed. A reducetion in apparent protein digestion resulting from consumption of diets containing polyphenols is due to binding with polyphenol compounds of both dietary and endogenous proteins, such as proteins in the intestinal tract and digestive enzymes (Brenes et al. 2008). Viveros et al. (2011) determined that a decline in growth in the group consuming diets containing 7.2 g/kg of grape pomace extract occurred and interpreted this situation as the presence of polyphenols in pure form. While the FCR value was similar between the groups in the first and second three-week periods of our study, the negative effect developed for the overall period in GP group may be due to this. On the other hand, Brenes et al. (2008) found that low doses of GP (30 g/kg) did not produce any negative effects on protein and fat digestibility, high doses of GP (60 g/kg) caused a decrease in fat digestibility. This difference may be another reason for the reduction in FCR despite an increase in feed consumption in our study. Altough this result supports the results of our study, other studies that are not compatible with other results have been published. Chamorro et al. (2015) added 5% GP supplementsto broiler diets for 21 days and found no effects on feed consumption and FCR contrary toresults from our study. Ebrahimzadeh (2018b) also reported that 5% GPin broiler dietdproduced no effects on growth performance. These results, which are different from our study results, may be due to the different proportions of skin, stalk, seed, and/or species of the grape pomaces. In addition, poor FCR due to high feed consumption in the GP group through the entire experimental period (0-6 weeks) may not be upsetting because the resulting body weight gains are similar, suggesting that grape pomace could be used as raw material for feed instead of being considered a waste product. Inulin had no effects on growth performance, feed consumption, and FCR as found in other studies (Nabizadeh 2012; Wu et al. 2019). Although feed consumption increased in the INGP group in the growth and total experimental periods, the FCR was adversely affected compared to the control group only in the overall period. Even if feed consumption increased due to grape pomace, the body weight gain increased numerically both in growth and overall periods, and FCR increased in the INGP group. Wu et al. (2019) showed that inulin caused an increase in the concentration of *Lactobacillus* and *Bifidobacteria*, both of which are beneficial bacteria in the ileum and cecum, while leading to a reduction in the concentration of *Eacherichia coli*. In our study, inulin may have affected FCR by increasing nutrient availability by this beneficial bacteria during the growth period, but it was less effective in the overall period in the INGP group. A better FCR in the INGP versus the GP group may explain the positive effect of inulin on the FCR.

Carcass Yield and Intestinal Viscosity

No differences in carcass yields of the groups were noted, but hot carcass weights were higher in the INGP group as were pre-slaughter weights. These differencescould have been due to the fact that the selection of the INGP group was higher than the others with respect to random selection of animals in the pre-slaughter groups. Carcass yield did not change in similar studies, consistent with our results (Aditya et al. 2018; Ebrahimzadeh, 2018b). Water-soluble non starch polysaccharides (NSPs), such as β-glucan and arabinoxylan found in wheat and barley grains, were shown to adversely affect nutrient utilization and reduce growth performance in broilers. The undesirable effects of water-soluble NSPs on nutrition is actually due to an increase in intestinal viscosity (Rebole et al., 2010). Cell walls of grape shells contain 30% cellulose, xyloglucan, arabinan, galactan, xylan, mannan polysaccharides, and pectin polysaccharide, which are neutral polysaccharides (Pinelo et al., 2006). Due to the fact that the diets used in our study were based on corn and soybean meal and the ratio of grape pomace used was as low as 5%, intestinal viscosities were similar between the groups due to the low levels of NSPs. Additionally, inulin is classified as a prebiotic effective on bacteria (Knudsen et al. 2012). Prebiotics are also defined as “a non-digestible food ingredient, which beneficially affects the host by selectively stimulating the growth and/or activating the metabolism

of one or a limited number of health-promoting bacteria in the intestinal tract thus improving the host's intestinal physiology" (Gibson and Roberfroid, 1995). Accordingly, inulin did not adversely affect viscosity as it had the effect of improving intestinal health and therefore nutrient availability.

Immunity and Antioxidant Status

In many studies on humans and animals, inulin-type fructans have been shown to have immunomodulatory effects (Vogt et al. 2015). As a result of our study, it was seen that while inulin and grape pomace alone did not affect immunity, in the INGP group in their combination caused negativelyeffects on immunity and antioxidant staus. El-Kelawy et al. (2018) reported that 0.5 and 1 g/kg grape seed extract supplementation to the broiler basic ration caused an increase in plasma IgG and IgM levels contrary to our results. In other studies on broilers, these results were also observed (Nabizadeh, 2012; Iqbal, 2015). According to Nabizadeh (2012), this similarity is because probiotics indirectly benefit the host immune system by stimulating the growth of lactic acid-producing bacteria, while according to Iqbal (2015), this benefit was due to the resveratrol found in grape polyphenols. IgM level in our study did not change similar to results of Wu et al. (2019) and Ebrahimzadeh et al. (2018b). Even if the decrease in IgG levels in the INGP group is considered a negative effect, it was thought that more studies are needed on this subject.

Antioxidant enzymes form the first line of defense in protecting tissues and cells from the harmful effects of free radicals. SOD, CAT, and GPX undertake this defense by removing the free radicalprecursors. The activities of these enzymes in tissue and blood indicate the strength of the body's antioxidant defense system (Surai, 2016; Güngör, 2021). In our study, it was observed that plasma SOD and GPX activities were similar between the groups contrary to expectations. Shi et al. (2003) reported that due to the polyphenols in grape pomace, grape pomace has an antioxidant effect 20 times more than vitamin E and 50 times more than vitamin C. Ebrahimzadeh et al. (2018b) also reported that grape pomace at 5% and 7.5% levels caused a significant increase in plasma SOD and GPX levels compared to the control group in broilers. However, Tufarelli et al. (2021) determined that 5% grape pomace did not affect SOD and GPX in laying hens, similar to our study. In the present study, SOD and GPX activities as compared to CON, did not change in the IN and INGP groups. Shang et al. (2018) reported that dietary supplementation with inulin produced an increase in the activities of SOD, CAT, and GSH-Px in the serum. In our results, inulin supplementation did not cause an increase in antioxidant effect of inulin (Shang et al. 2018), whereas the plasma CAT activity increased dietary supplementation with grape pomace.On the other hand, it was seen that CAT activity increased in the INGP group by showing a synergetic effect with grape pomace. It can be said that inulin can act as an antioxidant for broilers by causing an increase in plasma β-carotene and vitamin E levels. Plasma β-carotene level increased in the IN group, but it was not changed in the GP and INGP group. Plasma vitamin E level also increased in other groups compare to CON group. This finding is compatible with Rho and Kim (2006) whose results demonstrated that fat-soluble vitamins are high in grape pomace. On the other hand, Jankowski et al. (2016) determined that the use of polyphenol-rich fruit pomaces in diets did not lead to an increase in the concentrations of fat-soluble vitamins in the blood. In our study,plasma vitamin A levels were also expected to increase after administration of grape pomace, similar to vitamin E; however, the vitamin A levels did not change. While plasma vitamin C levels increased in the GP group, it was similar to the control in the IN group. In addition, it also increased in the INGP group, similar to the GP group. Jankowski et al. (2016) observed that vitamin E levels decreased and vitamins A and C did not change in turkeys fed with polyphenol-rich fruit pomaces. They reported that the decrease in vitamin E levels may have been due to both oxidant and antioxidant effects of polyphenols according to species. In results of our study, the increase in vitamin C and E levels in GP and INGP groups may have been due to the fact that species of polyphenols in the pomace we used is acts as an antioxidant rather than an oxidant. Although the prebiotic effects of inulin have beenreported in many studies, its antioxidant effect has only been studied recently, and these results are consistent with our study. The result of our study confirm the antioxidant effects of inulin. When Liu et al. (2015) examined at the antioxidant activity of inulin at different inulinconcentrations (100, 200, 400 mg/kg) against liver damage induced by carbon tetrachloride in male mice, liver MDA levels were similar to the control group. Shang et al. (2020) determined that the use of different levels of inulin in laying hen diets'were reflected in their stored eggs and showed an linear increase in levels of antioxidant enzymes, such as SOD and CAT, and the MDA level decreased linearly with

the increasing inulin level. MDA, which has negative effects, such as changing the permeability by acting on the cell membrane, is the most important product of lipid peroxidation (Mercan 2004). However, in our study, MDA levels were found to be similar in the CON and other groups since no factor that would cause lipid peroxidation existed. A similar result was found by Tufarelli et al. (2021).

CONCLUSIONS

Based on the results of the current study, the use of grape pomace up to 5% alone in broiler rations is appropriate. Grape pomace may have a negative effect on feed efficiency due to the increase in feed consumption. However, this effect can be ignored due to its contribution to the evaluation of a waste product as feed material. It has been determined that inulin can be used up to 1% without causing any negative effects becasue of its capacity to improve antioxidant status. Since the antioxidant effects of prebiotics are a very new research topic, more research should be done. If grape pomace and inulin are to be used in combination, they should only be used after considering their negative effects on immunity. More studies with inulin and grape pomace mixtures at different doses in the presence of oxidant substances or with immune-enhancing additives are needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aditya S, Ohh SJ, Ahammed M, Lohakare J (2018) Supplementation of grape pomace (Vitis vinifera) in broiler diets and its effect on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality. Anim Nutr 4(2): 210-214.

Ahmed W, Rashid S (2019) Functional and therapeutic potential of inulin: A comprehensive review. Crit Rev Food Sci Nutr 59 (1): 1-13.

Ananymous (2004) Feed analysis methods, Ministry of Agriculture and Rural Affairs, Communique no: 2004/33.

AOAC (2005) Official methods of analysis, Association of Official Analytical Chemists, 18th ed, Chapter 4: 1-72.

Azizi M, Seidavi AR, Ragni M, Laudadio V, Tufarelli V (2018) Practical applications of agricultural wastes in poultry feeding in Mediterranean and Middle East regions. Part 1: Citrus, grape, pomegranate and apple wastes. World Poult. Sci. J. 74: 489-498.

Brenes A, Viveros A, Goni I, Centeno C, Sayago-Ayerdy S, Arija I, Saora-Calixto F (2008) Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens, Poult Sci 87: 307-316.

Brenes A, Viveros A, Chamorro S, Arija I (2016). Use of polyphenol-rich grape by-products in monogastric nutrition. A review. Anim. Feed Sci. Technol. 211, 1-17.

Buege JA, Aust SD (1978) Microsomal lipid peroxidation, In: Methods in enzymology (Elsevier), pp 302-310.

Chamorro S, Viveros A, Rebolé A, Rica A, Arija BD, Brenes AA (2015) Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. Food Res. Int. 73: 197-203.

Ebrahimzadeh SK, Navidshad B, Farhoomand P, AghjehgheshlaghFM (2018a) Effects of exogenous tannase enzyme on growth performance, antioxidant status, immune response, gut morphology and intestinal microflora of chicks fed grape pomace. S. Afr. J. Anim. Sci. 48(1): 2-18.

Ebrahimzadeh SK, Navidshad B, Farhoomand P, Aghjehgheshlagh FM (2018b) Effects of grape pomace and vitamin E on performance, antioxidant status, immune response, gut morphology and histopathological responses in broiler chickens. S. Afr. J. Anim. Sci. 48(2): 324-336.

El-Kelawy M, Elnaggar AS, Abdelkhalek E (2018) Productive performance, blood parameters and immune response of broiler chickens supplemented with grape seed and medicago sativa as natural sources of polyphenols. Egypt Poult Sci 38: 269-288.

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125: 1401-1412.

Guaragni A, Boiago MM, Bottari NB, Morsch VM, Lopes TF, da Silva AS (2020) Feed supplementation with inulin on broiler performance and meat quality challenged with Clostridium perfringens: Infection and prebiotic impacts. Microb.Pathog. 139: 103889.

Günal M, Yasar S, Forbes JM (2004) Performance and some digesta parameters of broiler chickens given low or high viscosity wheat-based diets with or without enzyme supplementation, Turk J Vet Anim Sci, 28: 323-327.

Güngör E, Altop A, Erener G (2021) Effect of raw and fermented grape pomace on the growth performance, antioxidant status, intestinal morphology, and selected bacterial species in broiler chicks, Animals, 11: 364.

Haag W (1985) Zur methodik und praktischen bedautung der vitamin-c-bestimmung beim rind in vergangenheit und gegenwart, Ph. D. Thesis, University of Justus Liebig, Gissen, Germany.

Iqbal Z, Kamran Z, Sultan JI, Ashraf S, Ali A, Ahmad S, Sohail MU (2015) Replacement effect of vitamin E with grape polyphenols on antioxidant status, immune, and organs histopathological responses in broilers from 1- to 35-d age. J. Appl. Poult. Res. 24:127-134.

Jankowski J, Juskiewicz J, Zdunczyk P, Kosmala M, Zielinski H, Antoszkiewicz Z, Zdunczyk Z (2016) Antioxidant status of blood and liver of turkeys fed diets enriched with polyunsaturated fatty acids and fruit pomaces as a source of polyphenols. Pol J Vet Sci 19: 89-98.

Knudsen KEB, Hedemann MS, Lærke HN (2012) The role of carbohydrates in intestinal health of pigs. Anim. Feed Sci. Technol. 173(1-2): 41-53.

Kumanda, C., Mlambo, V., & Mnisi, C. M. (2019). Valorization of red grape pomace waste using polyethylene glycol and fibrolytic enzymes: Physiological and meat quality responses in broilers. Animals, 9(10): 779.

Liu J, Lu J-f, Wen X-y, Kan J, Jin C-h (2015) Antioxidant and protective effect of inulin and catechin grafted inulin against CCl4-induced liver injury. Int J Biol Macromol 72, 1479-1484.

Martinek RG (1964) Method for the determination of vitamin E (total tocopherols) in serum, Clin Chem, 10: 1078-1086.

Mercan U (2004) Importance of Free Radicals in Toxicology.Van Vet J 15: 91-96.

Nabizadeh A (2012) Effect of inulin inclusion in low phosphorus di-ets on some hematological, immunological parameters and broilerchickens performance. Res. J. Anim. Sci. 6:60-66.

Pascariu SM, Pop IM, Simeanu D, Pavel G, Solcan C (2017) Effects of wine by-products on growth performance, complete blood count and total antioxidant status in broilers. Braz. J. Poult. Sci. 19: 191-202.

Pinelo M, Arnous A, Meyer AS (2006) Upgrading of grape skins: Signif-

icance of plant cell-wall structural components and extraction techniques for phenol release. Trends Food Sci Tech, 17: 579-590.

Rebolé A, Ortiz L, Rodriguez ML, Alzueta C, Trevino J, Velasco S (2010) Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat-and barley-based diet. Poult Sci 89: 276-286.

Reyes P, Urquiaga I, Echeverría G, Durán E, Morales MS, Valenzuela C (2020) Wine grape pomace flour in broiler diets effects growth and some meat characteristics. Anim. Prod. Sci. 60(9): 1210-1216.

Shang H-M, Zhou H-Z, Yang J-Y, Li R, Song H, Wu H-X (2018) In vitro and in vivo antioxidant activities of inulin. PLoS ONE 13(2): e0192273.

Shang H., Zhang H., Guo Y, Wu H, Zhang N (2020) Effects of inulin supplementation in laying hens diet on the antioxidant capacity of refrigerated stored eggs. Int.J.Biol.Macromol. 153: 1047-1057.

Shi J, Yu J, Pohorly JE, Kakuda Y, (2003) Polyphenolics in grape seeds-biochemistry and functionality. J. Medic. Food. 6: 291-299.

Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S (2016) Inulin: Properties, health benefits and food applications. Carbohydr.Polym 147: 444-454.

Surai P (2016) Antioxidant systems in poultry biology: Superoxide dismutase. J. Anim. Res. Nutr. 1: 8.

Suzuki J, Katoh N (1990) A simple and cheap methods for measuring serum vitamin a in cattle using only a spectrophotometer, Jpn J Vet Sci 52: 1281-1283.

Şengül AY, Şengül Ö, Daş A (2019) The Possibilities of Using Fruit Waste in Nutrition of Poultry. TURJAF 7(5): 724-730.

Tufarelli V, Baghban-Kanani P, Azimi-Youvalari S, Hosseintabar-Ghasemabad B, Slozhenkina M, Gorlov I, Viktoronova FM, Seidavi A, Laudadio V (2021) Effect of dietary flaxseed meal supplemented with dried tomato and grape pomace on performance traits and antioxidant status of laying hens. Anim. Biotechnol 1-8.

Viveros A, Chamorro S, Pizarro M, Arija I, Centeno C, Brenes A (2011) Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks, Poult Sci, 90: 566-578.

Vogt L, Meyer D, Pullens G, Faas M, Smelt M, Venema K, Ramasamy U, Schols HA, De Vos P (2015) Immunological properties of inulin-type fructans. Crit Rev Food Sci Nutr 55(3): 414-436.

Wu XZ, Wen ZG, Hua JL (2019) Effects of dietary inclusion of Lactobacillus and inulin on growth performance, gut microbiota, nutrient utilization, and immune parameters in broilers, Poult Sci, 98:4656-4663.