

ORIGINAL ARTICLE

The Effect of Red Grape pomace on Performance, Lipid Peroxidation (MDA) and some Serum Biochemical Parameters in Broiler

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ABSTRACT

Grape pomace (GP) is a source of polyphenols with powerful antioxidant. This study was conducted to evaluate the effect of different levels of GP (0, 2, 4 and 6%) on performance, lipid peroxidation (MDA) and some blood biochemical parameters in broiler chicken. A total of 240, male broiler chicks (Ross 308 strain) were designed in a completely randomized design with 4 treatment and 4 replication experimental diets. They were fed from 21 until 42 days old. Body weight, feed intake and feed conversion of broilers during the test period was measured and calculated weekly. Glucose levels, total antioxidant depletion and malondialdehyde were analyzed in the blood taken from 42 days old chicken after 6 hours of starvation from veins in wings. Result indicated that GP in diets had significant effect ($p < 0.05$) on performance traits, including body weight and feed intake of chicken during the test period. Feed conversion had no difference between treatments. GP also significant ($p < 0.01$) decreased blood glucose level, total antioxidant and MDA. Therefore, GP can be used in broiler chick's ration expecting to increase insolubility and decrease cholesterol and triglyceride in plasma. Also this is important in safety of meat costumers.

Key words: grape pomace, lipid peroxidation, broiler

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INTRODUCTION

In many countries, Lack of food source is a case the major constraints in the development of livestock and poultry farming systems. It should be mentioned that in these countries, the annual vast volume of waste products of agriculture industries produced that in animal feed and poultry can be used. Grape pomace (GP) is the residue left after juice extraction by pressing grapes in the wine industry. This product contains tolerable crud protein, high crud fat and fiber. Grape skins and seeds are rich source of flavonoids, including monomeric phenolic compounds such as (+)-catechins, (-)-epicatechin, and (-)-epicatechin-3-O-gallate and dimeric, trimeric, and tetrameric procyanidins. Studies have shown that flavonoids have the capacity to act as powerful antioxidant by scavenging free radicals and terminating oxidative reactions [1]. Flavonoids and flavones oligomers and polymers (proanthocyanidins) have been proven to possess powerful antioxidant properties [2]. The application of GP compounds in food technology has also demonstrated a potent edible oil antioxidant capacity and an inhibitor of the oxidation of fish lipids, frozen fish muscle, and cooked, cold stored turkey meat [3-4, 5]. Poultry meat is relatively rich in polyunsaturated fatty acids and is, therefore, readily susceptible to oxidative deterioration [6]. Increasing the unsaturation degree of the muscle membrane by dietary manipulation increases the susceptibility of chicken meat to oxidative deterioration during storage [7], and as a consequence, flavor and nutritional value are decreased. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have long been used to control lipid oxidation in stored

meat and meat products, but concern over their use [8, 9] has created a need and prompted research for alternative antioxidants, particularly from natural sources. Evidence is also available on the antioxidative effect of added tea catechins on susceptibility of chicken meat to lipid oxidation [10-11]. Thus, by increasing of fat oxidation, taste, smell and nutritional values of fat are reduced. Therefore, to prevent this problem, natural and syntactic antioxidant should be added to diets when the meat is stored. The objective of this study was to evaluate the effect of dietary GP on broiler chicken performance and serum.

MATERIAL AND METHODS

Test product

This study was conducted at the Agriculture and Natural Resources Research Center of West Azerbaijan, Iran. Sample of GP was analyzed for crude protein, moisture, crude fiber, Ca, p and NFE by procedures from the AOAC [12], and was analyzed for Polyphenols and tannins by procedure recommended from Makkar, H. P. [13]. Proximate, polyphenol and tannin contain in GP indicated that GP is an ingredient that it was used as a fiber and polyphenol source in chicken diets (Table1).

Table1. Proximate Composition of GP (g/kg dry matter)

Item	DM(g/kg)
Protein	107.10
Moisture	114.00
Fiber	347.10
Ca	5.60
P	2.90
NFE	377.00
Polyphenols	236.00
Tannins	186.00

Chickens and feeding and Management

Broilers were fed with starter diets from one to 20 days old, and then followed with the experimental diets (Table2). The diet was formulated to meet the nutritional requirements of broiler chicks recommended by Broiler Management Manual (Ross 308). About 240 broilers 21 day old male chicks were weight and distributed randomly into 4 treatments with 4 replicates (15 chicks in each replicate). Mash diet and water were provided ad libitum. At the end of the experiment, broilers were weighted, and feed consumption was recorded for feed efficiency computation.

Table2. Ingredients and nutrient composition of experimental diets (percent of diets)

Ingredient	Control	control+2% GP	control+4% GP	control+ 6% GP
Corn	64.73	63.56	62.39	61.21
Sunflower meal	7.28	4.85	2.43	0.00
GP ¹	0.00	2.00	4.00	6.00
Dicalcium phosphate	1.34	1.35	1.36	1.38
Salt	0.26	0.26	0.26	0.26
DL-methionine	0.07	0.09	0.10	0.12
L-lysine	0.25	0.23	0.21	0.19
Vitamin-mineral premix ²	0.50	0.50	0.50	0.50
Oyster sell	1.18	1.15	1.13	1.11
Analyzed composition				
ME (kcal/kg)				
protein	2943	2939	2934	2930
Ca	0.84	0.84	0.84	0.84
Available P	0.42	0.42	0.42	0.42
Na	0.15	0.15	0.15	0.15
Lysine	1.22	1.22	1.22	1.22
Methionine	0.44	0.44	0.44	0.44
fiber	4.49	4.49	4.49	4.49

¹GPC = grape pomace concentrate.

²Vitamin-mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B12, 12.5 g; riboflavin, 5.5 mg; Capanthothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg.

Collection of samples and measurement

At 42 d of age, 8 birds were randomly selected from each treatment, and blood samples were obtained by wing vein for subsequent determination of antioxidant activity (glucose, total antioxidant and MDA). The blood samples were allowed to clot in polypropylene tubes for 2 h at room temperature. Blood was taken into test tubes containing anticoagulant (EDTA) was poured in slowly. Then to separate the plasma from the blood was transferred to the laboratory of Agriculture. The tubes were centrifuged at 4000×g for 10 min and Blood plasma was separated and put in micro tubes. Plasma samples from each repetition 0.5 to 1 cc in 3 micro tubes was poured and frozen until tested.

Chemical Analysis

Dry matter (88.6), moisture (11.4), CP (10.71), crude fiber (34.71), Ca (0.56), p (0.29) and NFE (37.7) were analyzed according to the methods of the AOAC [12]. Estimation of Total Phenol and Tannins: It was estimated according to the procedure of Makkar et al., [13].

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Analytical procedure

Fifty microliter (μ l) of tannins extract for each sample was taken in test tube and volume was made to 1.0 ml with distilled water. Then, 0.5 ml Folin Ciocalteu reagent was added and mixed properly. Then 2.5 ml 20 per cent sodium carbonate solution was added and mixed it and kept for 40 minutes at room temperature. Optical density was taken at 725 nm in spectrophotometer and concentration was estimated from the standard curve. Total phenol was estimated as tannic acid equivalent and expressed on dry matter basis. Non-tannins phenol was estimated by precipitating tannins with polyvinyl polypyrrolidone (PVPP), which binds tannins. 200 mg PVPP was taken in test tube and then 2.0 ml distilled water and 2.0 ml tannins extract was added. Vortex it and kept in refrigerator for 15 minutes at 40 C. Then the mixture was again vortex and filtered through Whatman filter paper No. 1. Filtrate was taken for estimation of non-tannin phenol. 150 μ l of filtrate was taken in test tube and volume was made to 1.0 ml with distilled water and then processed like that of total phenol estimation. Concentration of non-tannin phenol was calculated from the standard curve and expressed on DM basis. Total tannins were calculated by subtracting non tannin phenol from total phenol. The standard was prepared (Fig 3.1) from the stock solution of tannic acid (0.5mg/ml) using 0, 10, 20, 30, 40 and 50 μ l in test tubes and volume was made to 1.0 ml. It gives a tannic acid concentration of 0, 5, 10, 15, 20 and 25 μ g respectively. Then 0.5 ml Folin reagent and 2.5 ml 20 per cent sodium carbonate were added. Whole content was mixed properly and after 40 minutes reading was taken at 725nm in spectrophotometer.

Estimation of Condensed Tannin (Proanthocyanidin)

Condensed tannin was estimated according to the method of Porter et al., [14].

Analytical procedure

0.5 ml of tannins extract was taken in test tube in triplicate and 3.0 ml butanol HCL and 0.1 ml of ferric reagent was added. Tube was vortex to ensure proper mixing. The mouth of the tube was covered with glass marble and then boiled it for 60 minutes. Similarly blank was prepared for each sample but without heating the reagent. The tube was cooled to room temperature and reading was taken at 550 nm using spectrophotometer. Glucose oxidase method was used to measure blood glucose [15]. The ferric antioxidant power (FRAP; FRAP assay) of the samples (Blood plasma) was estimated [16]. Briefly, FRAP reagent was mixed with distilled water and either the sample or appropriate reagent blank. Readings at 30 min were selected for calculation of FRAP values. Reduction power activities were as micromoles of Trolox equivalents per gram of DM. The concentrations of malondialdehyde (MDA) in plasma were determined by the method described by Ohkawa et al. [17]. A 500 micro liters of serum dissolved in 3 ml 1% phosphoric acid. After vortex 1 ml thiobarbituric acid solution was added to tube and within 45 minutes in boiling water bath is placed. After the test tubes to cool under cold water, 2 ml of normal Butanol was added. Vortex for 1 to 2 minutes and then was centrifuged for 10 minutes with rpm 3000 rpm. After separating the organic phase (supernatant) by measuring absorbance at 532 nm against Butanol Blanch was as normal. And then transfer the results to the standard curve; the concentration of MDA in serum samples was determined.

Measurement of blood triglyceride and clostrol

Plasma was separated from the blood in the EDTA bottles with a micropipette into a test tube for triglyceride and cholesterol analysis. The cholesterol and triglyceride assay of the blood plasma were done by enzymatic-colorimetric methods.

Statistical analysis:

The study design was Completely Randomized (CRD) With 4 treatments and 4 replicates in each treatment. Data of this study were subjected to analysis of variance using GLM procedures [18]. When significant differences were detected, means were compared by the Tukey range tests at 5% probability. Statistical model of this experiment was as follow:

$$X_{ij} = \mu + t_j + e_{ij}$$

X_{ij} = the value of each observation

μ = overall mean.

t_j = effects of GP source

e_{ij} = experimental error respectively

RESULTS

Growth performance

The effects of treatments on broiler performance (body weight, feed consumption and feed efficiency) are presented in (Table 3). Supplementation diets with GP significantly ($p < 0.05$) reduced body weight and feed efficiency in broilers during the 42 days rearing period. Also the addition of increasing concentration of GP in the chicken diets feed consumption increased but it isn't significant.

Table3. Performance of broiler chicks (21 to 42 d) fed diets containing grape pomace (GP)

treatment	Body weight g	Feed intake g	Feed efficiency g:g
Control	1609 ^{ab}	1220 ^{ns}	2.12 ^c
2% GP	1630 ^a	1237 ^{ns}	2.17 ^{bc}
4% GP	1574 ^{ab}	1254 ^{ns}	2.32 ^{ab}
6% GP	1559 ^b	1238 ^{ns}	2.37 ^a
Pooled SEM	15.1	8.7	0.04

^{a - b} Means in columns with no common superscript differ significantly ($P < 0.05$).

¹Data are means of 4 pens of 15 chicks.

²Probability value of contrast; NS = $P > 0.05$.

Blood characteristics

Comparison between treatments for blood glucose, total antioxidants, MDA at the end of growing period (42 days), was tested by Tukey test (Table4). Glucose levels of serum for GP treatments were higher in terms of control (Table 4). Increasing levels of GP in the diet also increased the amount of plasma antioxidants due to high level of flavonoids that have antioxidant properties in the blood.

The extent of lipid oxidation, as measured by MDA formation in plasma, was significantly lower than the control group (Table 4). Plasma triglyceride and cholesterol decrease ($p < 0.05$) when amount of GP increases.

Table 4 .Comparison means of different experimental groups on blood traits of broiler chickens at 42 days

Treatment	Glucose mm/l	Total antioxidant $\mu\text{m/g}$	MDA nm/mg, protein	TG g/dl	Chol. g/dl
Control	193.33 ^b	0.39 ^b	4.80 ^a	52.00 ^a	163.33 ^a
2% Gp	195.33 ^b	0.52 ^{ab}	4.46 ^b	42.33 ^b	151.67 ^b
4% Gp	204.00 ^{ab}	0.43 ^{ab}	3.33 ^{bc}	37.33 ^c	136.67 ^c
6% Gp	210.33 ^a	0.46 ^a	1.70 ^c	35.33 ^c	129.33 ^c
Pooled SEM	2.34	0.02	0.15	3.25	8.33

^{a - b} Means in columns with no common superscript differ significantly ($P < 0.05$).

¹Data are means of 8 chicks for each treatment.

DISCUSSION

The performance of chicks for each experimental group indicated that GP significantly ($p < 0.05$) reduced body weight and feed efficiency in broilers during the 42 days rearing period. High crude fiber in GP

(34.71 percent) is one of factors which it affected on bulk rations in feed processing. It caused decreasing feed intake and body weight. Another factor is high tannin content (1.86 percent) in GP also decreased broilers performance due to bond this material with different nutrients and enzymes affecting their digestibility and availability of these materials [19]. Thus, despite the high fiber and high tannin in GP, followed by increasing its level in the diet of a decreasing trend in the mean weight of chickens was observed. The effect of polyphenols has also been studied in chickens using ingredients like sorghum and faba bean. In general, relatively high dietary concentrations of polyphenols by the addition of these ingredients reduced performance in chickens as well as other livestock [20, 21]. Goni et al [22] reported that the inclusion of GPC up to 60g/kg did not influence performance. There are few references in the literature in relation to the use of grape by-products in chicken feed. Hughes et al [23] and Lau King [24] reported that reduced performance in chickens fed with feed containing grape without seeds. They also reported that their tannins are in grape pomace reduced the performance of the chicks. Because grape seed extract was a pure form containing 90.2% of total phenolics, expressed as gallic acid equivalent by the Folin method, and incorporated in the diet at 60 g/kg. In the current experiment, GP contained 2.36% of total polyphenols by the Folin method. Brenes et al [25] research showed that the use of grape pomace concentrate to 6 percent in the diet had no effect on broiler performance. Increasing levels of GP in the diet increased glucose level. The treatment with 6% GP had highest blood glucose levels due to high NFE (mainly glucose and fructose) is the grape pulp. Therefore, increasing levels of dietary GP in diet decreased amount of plasma MDA. These results were in contract to Goni et al [22] and Brenes et al [25] who's reported that the addition of GP in the diet up to 3 percent has no effect on the total amount of antioxidants blood. Results in this study also confirm that dietary GP can delay lipid oxidation in blood plasma chicken and reduce the potential risk induced by lipid oxidation. Grape skins and seeds are rich of flavonoids, including monomeric phenolic compounds such as (+)-catechins, (-)-epicatechin, and (-)-epicatechin-3-O-gallate and dimeric, trimeric, and tetrameric procyanidins. Studies have shown flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (1). Flavanols and flavanol oligomers and polymers (proanthocyanidins) have been proven to possess powerful antioxidant properties [2]. Plasma triglyceride and cholesterol decrease ($p < 0.05$) when amount of GP increases. As reported by Ikeda et al. [26], excessive amounts of catching in green tea may prevent the absorption of lipids in intestine resulting in prevention of the accumulation of the lipids in liver and other tissues. Moreover, decreasing of the cholesterol in tissues may be caused by negative effects of the catching on formation of micelle which are absorbed indirectly by bile acids [27]. Reduction of liver cholesterol and blood serum cholesterol of GTP may be caused by an increase in unabsorbable bile acids. The second factor may be the high amount of GP fiber. A lot of existing evidences shows that dietary fiber could result in reduction of the level of cholesterol in animals [28] by absorption of bile acids and various lipids. Moreover, the catabolism of liver cholesterol is affected significantly by phenolic compound in tannic acid [29]. Cholesterol is converted to bile acids exclusively in the liver that displays the prominent pathway for the elimination of the cholesterol from the body. This fact may also describe the reduction in cholesterol levels [30] suppose that there is a relationship between reduction of MDA caused by green tea and antioxidative effect of tea catechins. Excessive amounts of the catechins in GP may result in the same effect as reported by [31-32-33, 34].

CONCLUSION

In conclusion, supplementation red GP in broiler diets decreased broiler performance linearly after 2 percent. However its supplementation could increase blood glucose level and total antioxidant. The GP supplementation of 2% in broiler diets is suggested.

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