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Research article

Effect of supplementation of red grape pomace in ration on some blood traits of broiler

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Abstract

This study was conducted in the animal field of Veterinary Medicine College / University of Al-Qadisiyah., The trial lasted 42 days between 2016/10/19 - 2016/11/29 for the purpose of knowing the effect of adding grape pomace (GP) to the ration on some blood characteristics of broilers. In the study, 150 chicks (Ross-308) were randomized to three groups (50 chicks) for each group (25 replicates). The first group was the control treatment (G1) that was given no food additive. The second group was (G2) and was given 25 g of GP / kg of ration along experiment duration. The third treatment (G3) was given 50 g GP / kg of ration throughout the experiment period. The results showed decreases in the level of cholesterol both in G2 and G3 compared to G1 (P≤ 0.05). There was also a significant improvement in the level of triglycerides in G3 (P≤ 0.05) at age 21 days, while there was a significant improvement in these lipids in G2 and G3 (P≤ 0.05) at age 42 days. The results showed a significant decrease (P≤ 0.05) in the LDL of G2 and G3 at 21 days of age plus a significant decrease in G3 at 42 days of age. The results also showed a significant increase (P≤ 0.05) in the HDL of G2. G3 overran G1 and G1+ G2 at the age of 21 and 42 days respectively. The results revealed a significant decrease (P≤ 0.05) in glucose level of G3 at age 21 days and 42 days. G2 showed significant difference at age 21 days and no significant difference at age 42. In conclusion, the grape pomace in (2.5% or 5%) of broiler ration leads to improve the performance traits, health benefits and some of the blood parameters in broilers, the traits that we used are considered as health indicators, growth promoters, production and performance effectors.

Keywords: Broiler, Blood lipids, Glucose, Red Grape Pomace.

Introduction

The poultry industry is very important in most economies of the world because it provides essential food as a source of protein found in eggs and meat (1). There is increasing interest in food products that contain unsaturated fatty acids (2). Diet plays a key role in animal health and production, which is a factor that affects the quality of meat (3). The global demand for organic products of broilers that is "free of antibiotics" increases the search by the scientists to look for alternative of growth factors. Therefore, food additives of plant residues, such as fruit, vegetables, spices, etc., play a role in increasing health and production (4), disease protection and treatment, and increasing the metabolism performance of broilers (5). Most studies focus on medicinal plants that play a big role as natural antioxidants to prevent oxidative stress caused by free radicals reactions and protection of the vital functions of the cells, and one of these plants is Grape, which is a major fruit produced annually at a rate of 21

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million metric tons (6). GP is the waste resulting from squeezing grapes in factories that generated in large quantities that cause environmental and economic problems. Therefore, the use of these wastes represents a progress in overcoming these environmental issues and the importance of what it contains of polyphenols at large rates (7).according to (8) showed an improvement in the productivity and physiological characteristics of broilers that fed GP. Therefore, the study aimed to study the effect of the addition of GP to the ration on some blood traits in broilers as health promotion factor.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 395

Experimental design

This study was conducted in the animal farm of the College of Veterinary Medicine / University of Al-Qadisiyah., The trial lasted 42 days between 2016/10/19 - 2016/11/29. In the study, 150 chicks (Ross-308) were randomized to three groups (50 chick) for each group (25 replicates) with an average initial weight of 40g / chick obtained from a local hatchery. The first group was the control (G1) that was given no food additive. The second group was (G2) and was given 25g (2.5%) of GP / kg of ration for the duration of the experiment. The third treatment (G3) was given 50 g (5%) of GP / kg of ration throughout the experiment period. These percentages have been selected because of previous studies that had used different percentages of GP and some of them had not shown any effects on the above-mentioned traits, so we decided to study the percentage that we used in this study. The traits that were tested are the levels of cholesterol, LDL, HDL, glucose, and triglycerides.

Table (1) The materials used in this study

Materials	Origin
Glucose test Kit	Biochrom, England
Cholesterol measuring kits	Vaccines and Sera Institution, Baghdad, Iraq
Lipoprotein, triglycerides, EnzyChrom HDL and LDL/VLDL Kit	BioAssay Systems, USA

Chick management

The chicks raised in pens on wooden residual bedding (3-5 cm) in thickness on 6 m for each pen. When chicks arrived on the first day, the incubators were used to warm up the hall for the purpose of obtaining the desired thermal degree. The plastic dishes were used to provide the feed in the first week and then replaced with hung conical feeders. The water was freely accessed during the period of study. The room was equipped with the necessary number of lamps, air ventilators, and thermometers.

Table (2): Food contents of the ration

Ration ingredients	Ratio (%)
Yellow corn	51.4
Soybean (protein 45%)	31
Bran	1
Wheat	14
Premix	1
NaCl	0.3
Methionine	0.2
Lysine	0.1
CaO	1
Calculated chemical content	
Converted energy (KJ/Kg)	3051
Crude protein (%)	21.58

NRC,(8)

Ration

The chicks were fed ad-libitum as it was mentioned in table (2):

Red GP (RGP):

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GP was taken from the local retailers and was dried out under the sunshine. Then, it was added to the ration after grinding. No ground GP was added to the ration at 12 day of age.

The chemical analysis of the GP performed in the Studies and Researches Center, University of Baghdad as it is shown in the Table (3):

Table (3): Chemical analysis of GP

Contents	Ratios (%)
Dampness	3.16
Ash	2.56
Crude protein	13.22
Lipids	0.60
Carbohydrates	56.31
Total phenols	290

Vaccines:

Table (4) shows the vaccines that were used in the experiments. Vitamin C was used after each vaccine round to lower the effect stress generated by the vaccine administration for 3-5 days. Vaccines were administered via the drinking water after thirst for 3 hours. Then Newcastle (ND) and Infectious bronchitis (IB) vaccines were supplied using aerosol spraying before distributing the chicks to the assigned experimental groups. After that, the chicks were divided randomly to their groups. This was followed by sugary water 0.5 g/L was provided. The vaccine added to chlorine-free drinking water.

Table (4): Vaccine program

Age (day)	Vaccines
1	ND-B1+ IB Spray
10	ND-Lassota (drinking water)
14	Gumboro-IBD2 (drinking water)
20	ND-Lassota

NOTE: The blood samples collected by using 5 ml G×1" gauge syringe from wing vein, 2 ml of blood collected for each bird.

Biochemical blood test:

Glucose concentration (dl/mg):

It was measured using a kit and a protocol from (Biochrom, England).

Cholesterol concentration (mg/100 ml):

The cholesterol was measured using a kit from the Institution of Vaccines and Sera, Baghdad, Iraq. The test followed the manufacturer protocol.

Lipid concentration, Lipoprotein, and triglycerides concentrations:

The lipid profile including LDL, VLDL, and HDL were measured in the blood using a method adopted from (AOAC, 1980).

Statistical analysis:

One way, two-way ANOVA, and LSD (least significant differences) were used in the study. The significant differences were decided according to the level of probability, 0.05. The statistics were calculated using SPSS software (version 23).

Results

Cholesterol concentration

The result showed that there were significant differences between the experimental groups. Table (5) reveals these differences among groups throughout day 21 and day 42 of the study experiment.

Table (5): Cholesterol concentrations (Mean ± SE)

Group / Treatment	Age at 21 day	Age at 42 day
Control	175.66±0.66 Aa	185.66±0.88 Ab
GP (2.5%)	168.66±0.88 Ba	180.33±0.33 Bb
GP (5%)	164.33±1.85 Ca	178.2±1.2 Cb

*** Different letters refer to significant differences P≤0.05 and vice versa.**

***Small letters refer to vertical statistical reads between groups and vice versa to read between ages of the same group.**

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Triglycerides:

Table (6) shows the significant differences between the G2-G3 and G1 P≤0.05.

Table (6): Triglycerides concentrations (Mean ± SE)

Group / Treatment	Age at 21 day	Age at 42 day
Control	23.2± 0.75 Aa	26.66± 0.33 Ab
GP (2.5%)	20.33 ± 0.88 Ba	22.33± 0.33 Bb
GP (5%)	0.1±19 Ba	22.33± 0.33 Bb

*** Different letters refer to significant differences P≤0.05 and vice versa.**
*** Small letters refer to vertical statistical reads between groups and vice versa to read between ages of the same group.**

Low-density lipoproteins (LDL):

G2 and G3 showed significant differences compared to G1 P≤0.05. Table (7) reveals these significant differences.

Table (7): LDL concentrations (Mean ±SE)

Group / Treatment	Age at 21 day	Age at 42 day
Control	38.66 ± 0.66 Aa	48.1±0.57 Ab
GP (2.5%)	34.33 ± 1.2 Ba	43.33 ± 1.02 Bb
GP (5%)	34.31±1.76 Ba	39.33 ± 0.33 Cb

*** Different letters refer to significant differences P≤0.05 and vice versa.**
*** Small letters refer to vertical statistical reads between groups and vice versa to read between ages of the same group.**

High-density lipoproteins (HDL):

Table (8) shows significant differences between the study groups

Table (8): HDL concentrations (Mean ± SE)

Group / Treatment	Age at 21 day	Age at 42 day
Control	68.66 ± 0.66 Aa	88 ± 0.66 Ab
GP (2.5%)	85.3.± 0.1 Ba	89.66± 0.59 Ab
GP (5%)	82.66 ± 0.66 Cb	96.33± 0.669 Bb

***Different letters refer to significant differences P≤0.05 and vice versa.**
***Small letters refer to vertical statistical reads between groups and vice versa to read between ages of the same group.**

Glucose:

In table (9), the study groups show significant decreases in the concentration of glucose in G3. These changes revealed in table (9).

Table (9): Glucose concentrations (Mean ±SE)

Group / Treatment	Age at 21 day	Age at 42 day
Control	172.33± 1.45 Aa	176.33±0.88 Ab
GP (2.5%)	175.33± 0.33 Ba	177.11±0.3 Aa
GP (5%)	175.33± 1.88 Ca	175.33± 0.33 Ab

*** Different letters refer to significant differences P≤0.05 and vice versa.**
*** Small letters refer to vertical statistical reads between groups and vice versa to read between ages of the same group.**

Discussion

Adding grape pomace to the ration of broiler had placed significant overrunning via cholesterol levels of G3 and G2 when compared to G1. Moreover, G3 showed this incident when compared to G1 and G2 at 21 and 42 day of age table (5). These changes agree with (10) when he had used grape seed extract. The reason behind this effect linked to the polyphenol compounds (catechin, Gallic acid, and epicatechin). These compounds work on decreasing cholesterol via inhibiting the activity of pancreatic cholesterol esterase. This enzyme degrades cholesterol estate that found in food to release free cholesterol. Moreover, these phenolic compounds attach to the bile acids and decrease the cholesterol dissolving in micelles and leads to decreasing the absorption of cholesterol (11). The result also agrees with (12) who showed the decrease in total lipids and cholesterol via inhibiting the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase. The lignin and glucans compounds deactivate reactive oxygen species that increase the risk of getting atherosclerosis in humans (13). Our result did not match (14) result who had added 4% of GP to the ration of laying hens and did not decrease cholesterol and triglycerides. In case of triglycerides, our result indicates that GP adding has improved this lipid levels in G3

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over G2 and G1 at 21 day of age. In addition, G3 and G2 show significant enhancement of triglycerides over G1 at 42 day of age table (6). Our result agrees with (10) who revealed a decrease in triglycerides in the blood of broilers when added GP+ Vit C to the ration under thermal stress. (15) Showed that GP added to the food of rabbits that contained high ratio of cholesterol led to improve of antioxidant enzymes and lipid peroxides in the blood. Moreover, they showed that triglycerides and cholesterol were decreased thus will decrease the incidence of hypercholesterolemia. It was found that polyphenols were able to decrease triglycerides levels in the blood of rats that suffered from hypercholesterolemia (16) which agrees with our results. The red grape pomce and its components of polyphenols have reduced effects of lipids, triglycerides, total cholesterol, in addition to the proportion between LDL and HDL cholesterol in high-weight mice compared with low-weight mice Polyphenols act to reduce cholesterol uptake by inhibiting the action of cholesterol transients in the cell membranes (17). Our result did not match (14) where he pointed out that there were no changes in triglycerides and cholesterol in the serum when adding grape pomace with concentrations of 4% and 6% in rations of laying hens. For the LDL, our result reveals a significant decrease in their levels in the blood in G3 and G2 when compared to G1 at 21 day of age. In addition, a significant decrease was noticed in G3 when compared to G1 and G2 at 42 day of age table (7). These results come into match with (10) results that used GP as an additive to the ration of broilers which led to decrease the levels of LDL and VLDL in the blood. These decreases were linked to the effects of polyphenols that present in GP via decreasing the small molecules of LDL and increasing lipoprotein lipase (18). To come and study the effect of GP on HDL, we found that G2 has overrun G3, which overrun G1 at 21 day of age. Moreover, G3 overrun G2 and G1 at 42 day of age table (8). These results agree with (19) that had found a decrease in LDL and an increase in HDL when added flavonoid, a polyphenolic compound, to the ration of broiler. This effect was reasoned to the increase in HDL paraoxonase HDL PON that leads to increasing the cellular response via the increase in the receptors (20). HDL increasing is linked to the generation of is oflavone genistein by polyphenols that leads to activation of protein kinase mitogen (21). In the case of glucose, our study shows a significant decrease in G3 when compared to G2 and G1 at 21 and 42 day of age. In addition, G2 shows a significant difference when compared to G1 at 21 day of age table (9). These results come to agree with (10) who revealed that using grape seed extract in broiler ration led to a significant decrease in the level of glucose in the blood. This also matches (22, 14) who showed that using GP in ration of laying hens led to a significant decrease in the glucose levels of their blood. GP acts to protect β cells in the pancreas from the oxidative effects via antioxidant activity of proanthocyanidins compound that presents in GP (23). In this case polyphenols will inhibit the absorption of glucose from kidneys via the deactivation of Symporter the Sodium-glucose, a protein transporter that is found in the tubules of the kidneys (24), Polyphenols also frustrate carbohydrate digestion by inhibiting the action of (α-glucosidase, α-amylase) enzyme and inhibition of glucose transporters in intestine (25). The effects of grapes and their extracts which used in the studies on the production performance of chicken and blood characteristics can vary due to several factors, which include the difference in total polyphenols, which depends mainly on the type of grapes and the composition of the soil on which they grow. Techniques used to treat secondary waste from industrialization, for example, wine, vinegar and grape juice are factors affecting the effectiveness of polyphenols, the rate of addition of these products to the chicken diet and the extent to which it affects the rest of the components of the feed (14).

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References

1-Naji SA (2006). Guide of commercial, production of broiler, College of Agriculture/ University of Baghdad.

2-Narciso-Gaytán C, Shin D, Sams AR, Bailey CA, Miller RK, Smith SB, Leyva-Ovalle OR, Sánchez-Plata MX. Dietary lipid source and vitamin E effect on lipid oxidation stability of refrigerated fresh and cooked chicken meat. Poultry Science, (2010); 89, 726-2734.

3-Andersen HA, Oksbjerg N, Young JF, Therkildsen M. Feeding and meat quality-a future approach. Meat Science, (2005); 70, 543-554.

4-Windisch W, Schedle K, Plitzner C Kroismayr. A Use of phytogenic products as feed additives for swine and poultryJ. Anim. Sci., (2008)**;** 86 (E. Suppl.) pp. E140-E148.

5-Durmic Z, Blache D. Bioactive plants and plant products: Effects on animal function, health and welfare. Animal Feed Science and Technology, (2012); 176, 150-162.

6-Schieber A, Stintzing FC, Carle R. By-products of plant food processing as a source of functional compounds-recent developments.Trends Food Sci. Technol., (2002); 12:401-413.

7-Torres JL, Varela B, Garcı´a MT, Carilla J, Matito C, Centelles JJ, Cascante M, Sort X, Bobet R. Valorization of grape (Vitis,vinifera) by-products. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. J.Agric. Food Chem., (2002)**;** 50:7548-7555.

8-National Research Council (1994). Nutrient requirements of poultry, 9th revised edition. Washington, DC, National Academy Press.

9-Dorri SAS, Tabeidian M, Toghyani R, Jahanian F. Behnamnejad. Effect of different levels of GP on blood serum and biochemical parameters of broiler chicks at 29 and 49 days of age. Proc. 11th Int. and 4th Natl. Congress on Recycling of Organic Waste in Agriculture. (2012); 26- 27 April 2012. in Isfahan, Iran.

10-AOAC. (1980) Official Methods of Analysis. 13th Ed. Association of Official Analytical Chemists. Washington, D.C.

11-Hajati H, Hassanabadi A Golian A Nassiri-Moghaddam H Nassiri MR. The effect of grape seed extract and vitamin C feed supplementation on some blood parameters and HSP70 gene expression of broiler chickens suffering from chronic heat stress. Italian Journal of Animal Science volume.(2015); 14:3273.

12-Ngamukote S, Makynen K, Thilawech T, Adisakwattana S. Cholesterol lowering activity of the major polyphenols in grape seed. Molecules (2011); 16:5054-5061.

13-Ariana M, Samie A, Edris MA, Jahanian R. Effects of powder and extract form of green tea and marigold, and α-tocopheryl acetate on performance, egg quality and egg yolk cholesterol levels of laying hens in late phase of production. J.Med. Plants Res. (2011); 5:2710-2716.

14-Rimm EB, A Klatsky, D Grobbee, MJ Stampfer. Review of moderate alcohol consumption and reduced risk of coronary heart disease: Is the effect due to beer, wine, or spirits. BMJ.(1996); 312:731-736.

15-Kara K, Güçlüa BK, Baytok E, Şentürk M. Effects of GP supplementation to laying hen diet on performance, egg quality, egg lipid peroxidation and some biochemical parameters. Journal of Applied Animal Research, (2016); Vol. 44, No. 1.

16-Choi CS, Chung HK, Choi MK, Kang MH. Effects of GP on the antioxidant defense system in diet-induced hypercholesterolemic rabbits. Nutr Res Pract, (2010); 4: 114-120.

17-Wang L, Sun J, Yi Q, Wang X, Ju X. Protective effect of polyphenols extract of Adlay (Coix lachryma-jobi L. var. ma-yuen Stapf) on hypercholesterolemia-induced oxidative stress in rats. Molecules, (2012); 17: 8886-8897.

18-Agouni A, Lagrue-Lak-Hal AH, Mostefai HA, Tesse A, Mulder P, Rouet P, Desmoulin F, Heymes C, Martínez MC, Andriantsitohaina R. Red wine polyphenols prevent metabolic and cardiovascular alterations associated with obesity in Zucker fatty rats (Fa/Fa). PLoS ONE, (2009); 4(5): e5557.

19-Yang RY, Tsou SCS, Lee TC, Chang L-C, Kuo G, Lai P-Y. Moringa a novel plant rich in antioxidants, bioavailable iron, and nutrients. Am. Chem. Soc. Symp. Series, (2006). 925 (17): 224-239.

20-Kehui O, Mingsheng X, Yan J, Wenjun W. Effects of flavonoids on broiler performance, meat quality, and gene expression. Can. J. Anim. Sci. (2016); 96: 332–341.

21-Zagayko AL, Kravchenko GB, Krasilnikova OA, Ogai YO. Grape polyphenols increase the activity of HDL enzymes in old and obese rats, Oxid Med Cell Longev.: (2013); 593761.

22-Lamon-Fava S, Micherone D. Regulation of apoA-I gene expression: mechanism of action of estrogen and genistein. J Lipid Res.; (2004); 45:106-12.

23-El-Alfy AT, Ahmed AAE, Fatani AJ. Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats. Pharmacol Res. (2005); 52:264-270.

24-Ozgan A (2008). Use of grape seed oil in functional egg production [MSc Thesis]. Adana (Turkey): University of Cukurova.

25-Maghrani M, Michel JB. Eddouks, M., Hypoglycaemic activity of Retama raetam in rats. Phytother. Res. (2005); 19:125-128.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

26-Ding Y, Zhang Z, Dai X, Jiang Y, Bao L, Li Y, Li, Y. Grape seed proanthocyanidins ameliorate pancreatic beta-cell dysfunction and death in low-dose streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats partially by regulating endoplasmic reticulum stress. Nutr. Metab. (2013); 10:51.