



Effect of Adding Different Levels of Grapes (*Vitis vinifera* L.) Seeds and Leaf Powder or their Extracts on Some Bone Characteristics and Total Ash Content in Broiler Chickens

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ABSTRACT

Background: Some natural sources of polyphenols like grape seed, leaf or extracts, have many benefits in maintaining bone health in animals. This study aimed to investigate the efficacy of grape seeds, grape leaves powder, or their extracts on some bone characteristics and total ash content in broiler chickens.

Methods: Three hundred and twenty four one-day-old broilers were allocated to nine treatments with three replicates containing 12 chicks each. Chicks were fed nine experimental diets for 35 days; as a control diet without supplementation (T1), control diets supplemented with 15 and 30 g/kg grape seeds powder (GSP) (T2, T3), 15 and 30 g/kg grape leaf powder (GLP) (T4, T5), grape seed extract (GSE) at levels 2 and 4 ml/l added in drinking water (T6, T7) and with grape leaf extract (GLE) at levels 2 and 4 ml/l (T8 and T9), respectively.

Result: Significant increase ($p \leq 0.05$) in the bone length was recorded with GSP (30 g/kg), GLP (15 g/kg), GSE or GLE and calcium percent with GLE as compared with control. Furthermore, significant improvement was recorded in predictive skeletal weight of broilers fed GSE (T6 α T7) as compared to the other treatments. The study concluded that the best results were achieved at the 2 or 4 ml/l of grape seed extract in improving femur length, predicted skeletal weight and from grape leaf extract in improving calcium percentage in broiler bone ash.

Key words: Broiler, Calcium, Femoral bone, Grape, Phosphorus.

INTRODUCTION

Many factors influence bone development directly or indirectly in birds, among which nutritional factors, genetics, gender, and the absolute growth rate. On the other hand, bone tissue is complex and composed of inorganic substances such as calcium and phosphorus, which provide hardness and strength and organic substances, which give elasticity to bone (Adebisi *et al.*, 2009).

As is known, grapes represent an important source of bioavailable polyphenols such as flavonoids (Yamine *et al.*, 2018). Also, the compound proanthocyanidins extracted from the grape seed is one of the bioactive compounds that possesses a wide range of pharmacological and biochemical properties (Katiyar *et al.*, 2017). Phenolic compounds extracted from grapes have many benefits in maintaining bone health in animals. In this regard, Holman and Weaver (2014) showed that feeding rats on dried grapes increased bone calcium content as well as bone structure and strength. As noted by Shen *et al.* (2012), dried fruits and bioactive compounds, including phenolics, flavonoids and resveratrol extracted from fruits and grapes are important in maintaining the health and durability of bones and thus increasing their strength. These effects are attributed to the role of antioxidants in fruits in reducing the effects of oxidative stress. In the bones, resveratrol grape possesses important anti-inflammatory and antioxidant properties, which benefit bone health.

In addition, increased fruit intake has favorable effects on bone mineral concentration (Prynne *et al.*, 2006), as well

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as, a positive relationship between fruit consumption level and bone mineral density (Li *et al.*, 2013). According to Manach *et al.*, (2004), these phenolic compounds have attracted great interest as a nutritional intervention against degenerative diseases, including osteoporosis. On other hand, grape intake is thought to protect bone by blocking inflammatory molecules, that stimulate osteoclasts (Chu *et al.*, 2015).

As well as, grape seeds also contain many minerals, including calcium (0.56%), phosphorous (0.31%) and total ash (2.68%) (Hajati *et al.*, 2015), which are important in the formation of the skeleton of chickens. In the background of the above details, this research was conducted to determine the efficacy of grape seeds, grape leaves powder, or their

extracts on some broiler femur bone characteristics, the serum and bone content of calcium and phosphorous, and the total ash content at 35 days of age.

MATERIALS AND METHODS

This work was carried out at Poultry Field, College of Agriculture, University of Basra for a period of 5 weeks (35 days) from 8th October 2018 to 12th November 2018. Three hundred and twenty four day-old broiler chicks (Ross- 308) were randomly distributed among nine treatment groups containing three replicates of 12 birds each. Every group was fed on one of the following experimental diets. The first treatment was the control group, the birds in 2nd and 3rd treatments were given grape seed (GS) powder at 15 and 30 g/kg feed respectively, the 4th and 5th groups of birds were given grape leaves powder at 15 and 30 g/kg feed respectively, the 6th and 7th groups of birds were given grape seed extract (GSE) at 2 and 4 ml/l with drinking water, while the 8th and 9th groups of birds were given grape leaf extract (GLE) at 2 and 4 ml/l with drinking water, respectively. The basic diets were prepared according to the recommended nutritional needs of the broiler. The composition of the basal diet is presented in (Table 1). Chemical analysis of grape seeds and leaves were carried out according to AOAC (2016) (Table 2). The birds were fed a starter diet until 21 days of age, followed by a finisher diet from 22 to 35 days. All birds had *ad libitum* access to feed with the 35- day's period.

At the end of the 35 days experiment, three birds of similar body weight from each treatment were sacrificed to study thigh bone (Femur) characteristics. To preparing the bone, medical scissors were used to rid the thigh bone of flesh and connective tissues according to Hall *et al.*, (2003) method. Where the thigh bones were labeled and immersed in boiling water (100°C) for ten minutes and the remnants of soft tissues were removed by hand. They were then oven-dried for 6 h at 105 °C. The femur length and weight were determined. Bone Index (BI) was calculated according to the equation of Sedor *et al.*, (1991).

$$\text{Bone Index (BI)} = \frac{\text{Bone weight (g)}}{\text{Bone length (cm)}}$$

Femur ash content was determined by ashing the bone in a Muffle furnace for 6 h at 600°C according to the procedure of A.O.A.C. (2016), the percent of total ash was determined relative to the dry weight of the bone. Calcium percentage in the femur was calculated or analysed according to Cresser and Parsons (1979) and phosphorous according to Harner and Wilson, (1986). The Predicted Skeletal weight (g) was calculated according to the equation of Taylor *et al.* (1965):

Predicted Skeletal weight (g) =

$$2.997 + [6.601 \times \text{Tibia weight (g)}]$$

Serum calcium (Ca) and Phosphorous (P) were measured using commercial kits (Randox), then the Ca: P ratio in bone and serum was calculated.

Table 1: Ingredients and nutrient composition of broiler starter and grower diets.

Ingredient (%)	Starter diet	Grower diet
	1-21 days	22-35 days
Yellow corn	58.00	60.00
Wheat	04.00	04.00
Soybean meal (48%)	31.00	29.00
Vegetable oil	1.00	1.00
¹ Broiler protein concentrates (40%)	5.00	5.00
Limestone	0.70	0.70
Premix	0.15	0.15
Sodium chloride	0.15	0.15
Total	100	100
Calculated composition ²		
Metabolizable energy (Kcal /Kg)	3010	3035
Crude protein (%)	22.37	21.58
Crude fat (%)	2.76	2.82
Crude fiber (%)	3.72	3.62
Calcium (%)	0.63	0.61
Phosphorus available (%)	0.30	0.33
Lysine (%)	1.19	1.14
Methionine + Cysteine (%)	0.83	0.81
Calorie: Protein ratio	134.55	140.64

¹Broiler protein concentrate (Brocorn-5 special W), Exported by (Wafi B.V., Alblasserdam – Holland), supplied per kilogram of feed: Crude protein 40%, 2017 kcal/kg M.E, 5% fat, 2.20% crude fiber, 7.10% moisture, 28.30% ash, 4.20% calcium, 2.65% total phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine+ cysteine, 0.42% tryptophan, 1.70% threonine, 2.50% sodium, 4.20% chloride, 200 mg/kg copper, 1.600 mg/kg manganese, 2.000 mg/kg zinc, 2.000 mg/kg iron, 20.00 mg/kg iodine, 5.00 mg/kg selenium. ²Was calculated according to the chemical composition of feedstuff contained in NRC (1994).

Table 2: Proximate analysis (% on dry weight basis) of grape seeds and leave Powder.

Component (%)	Grape seeds	Grape leaves
Dry Matter	88.59	90.35
Crude protein	12.30	20.30
Crude fat	11.00	6.59
Ash content	3.15	15.76
Crude fiber	36.22	20.15
Available carbohydrate	37.33	37.20
Metabolized energy (Kcal/Kg) [*]	2421.62	2353.99

*ME was calculated according to Lodhi *et al.*, (1976).

Statistical analysis

The data were subjected to analysis of variance (One -way ANOVA) in accordance with Completely Randomized Design (CRD) using SPSS software (2015) to analyze the results. Duncan's new multiple range test (Duncan, 1955) was applied to the separated means at a significant level of 0.05 (SPSS, 2015).

RESULTS AND DISCUSSION

There were no effects of GSP, GLP, GSE and GLE supplementation on bone weight, bone index and total ash except for bone length and predicted skeletal weight (Table 3). A significant improvement ($p \leq 0.05$) was recorded in the bone length in the GSP (30 g/kg), GLP (15 g/kg), GSE, GLE groups as compared with control.

Also, the supplementation of grape seed extract (T6αT7) improved significantly ($P \leq 0.05$) predictive value for skeletal weight compared to the other treatments. While a significant decrease was observed when using the lower level of grape seed or leaf powder (15 g/Kg) and grape leaf extract (2ml/l) compared to the control.

The improvement in bone length and predicted skeletal weight (Table 3) may be attributed to the presence of the proanthocyanidin compound, which is extracted from grape seeds. A previous study concluded that feeding rats on proanthocyanidin led to an increase in bone calcium content (Yahara *et al.*, 2005). Also, rats fed on dried grapes increased bone calcium content, bone structure, and strength (Holman and Weaver, (2014). In this context, Shen *et al.*, (2012)

indicated the effect of dried fruits and bioactive compounds, including phenolic, flavonoids and resveratrol extracted from fruits, and grapes, indicating their importance in maintaining the health and durability of the bones and thus increasing their strength, attributing these effects to the role of antioxidants in fruits in reducing the effects of oxidative stress in the bones and that resveratrol possesses important anti-inflammatory and antioxidant properties, which benefit bone health. Additionally, a recent study, suggests that orally administered proanthocyanidin-rich grape seed extract improved implant osseointegration by alleviating the impaired bone health induced by ovariectomy as a model of estrogen hormone deficiency in mice (Tenkumo *et al.*, 2020).

The calcium and phosphorus in blood sera, bone ash, and the Ca to P ratio were not significantly affected ($P \geq 0.05$) by dietary GSP, GLP, GSE and GLE except for calcium percentage in bone ash (Table 4). Calcium percent increased ($P \leq 0.05$) in grape leaf extract groups (T8,T9) as compared to control, which reached 32.28 and 31.23%, respectively, while the remaining treatments did not differ in the level of calcium in bone ash.

Table 3: Effects of grape seed, leaf and extract on some bone measurements of the broilers.

Dietary treatments	Bone weight(g)	Bone length (cm)	Bone Index	Total ash (%)	Predicted Skeletal weight (g)
T1(Control)	7.27 ± 0.03	8.20 ^c ± 0.06	0.89 ± 0.01	43.01 ± 0.27	50.94 ^c ± 0.06
T2 (15 g/kg GSP)	7.20 ± 0.15	8.23 ^c ± 0.03	0.87 ± 0.02	41.74 ± 1.09	50.52 ^d ± 0.04
T3 (30 g/kg GSP)	7.33 ± 0.03	8.53 ^b ± 0.09	0.86 ± 0.01	40.54 ± 0.15	51.40 ^b ± 0.08
T4 (15 g/kg GLP)	7.23 ± 0.18	8.53 ^b ± 0.09	0.85 ± 0.03	42.56 ± 0.52	50.70 ^d ± 0.02
T5 (30 g/kg GLP)	7.27 ± 0.03	8.20 ^c ± 0.06	0.89 ± 0.01	41.62 ± 0.58	50.96 ^c ± 0.06
T6 (2 ml/l GSE)	7.36 ± 0.07	8.77 ^a ± 0.03	0.84 ± 0.01	40.47 ± 0.30	51.78 ^a ± 0.17
T7 (4 ml/l GSE)	7.43 ± 0.09	8.77 ^a ± 0.03	0.85 ± 0.01	45.99 ± 4.15	52.00 ^a ± 0.08
T8 (2 ml/l GLE)	7.17 ± 0.03	8.53 ^b ± 0.09	0.84 ± 0.01	43.83 ± 2.72	50.48 ^d ± 0.08
T9 (4 ml/l GLE)	7.33 ± 0.03	8.53 ^b ± 0.09	0.86 ± 0.01	40.89 ± 0.31	51.38 ^b ± 0.04
Significant	NS	*	NS	NS	*

Different letters (a-c) within a column denote significant differences between treatments ($p \leq .05$). NS: None significant; GSP: Grape seeds powder; GLP: Grape leaf powder; GSE: Grape seed extract; GLE: Grape leaf extract.

Table 4: Effect of grape seed, leaf and their extract on calcium and phosphorus level in serum and bone ash of broilers.

Dietary treatments	In blood serum			In bone ash		
	Calcium (mg/100ml)	Phosphorus (g/100 ml)	Ca : P ratio	Phosphorus (%)	Calcium (%)	Ca : P ratio
T1(Control)	2.46±0.06	1.25±0.04	1.94±0.15	28.73 ^b ±0.91	8.93±0.48	3.22±0.02
T2 (15 g/kg GSP)	2.42±0.06	1.26±0.13	1.92±0.09	29.17 ^b ±0.33	9.08±0.44	3.02±0.01
T3 (30 g/kg GSP)	2.47±0.05	1.31±0.08	1.99±0.03	28.87 ^b ±0.27	9.11±0.26	3.25±0.16
T4 (15 g/kg GLP)	2.37±0.04	1.19±0.03	1.96±0.14	29.01 ^b ±0.75	9.41±0.28	3.19±0.03
T5 (30 g/kg GLP)	2.46±0.02	1.33±0.05	2.06±0.11	28.51 ^b ±0.25	9.48±0.21	3.04±0.05
T6 (2 ml/l GSE)	2.39±0.04	1.17±0.05	2.02±0.05	28.87 ^b ±0.68	9.62±0.23	3.24±0.13
T7 (4 ml/l GSE)	2.37±0.02	1.23±0.06	1.79±0.1	28.34 ^b ±0.58	9.54±0.32	3.19±0.01
T8 (2 ml/l GLE)	2.40±0.03	1.16±0.02	1.98±0.14	31.23 ^a ±0.25	9.12±0.26	3.03±0.03
T9 (4 ml/l GLE)	2.38±0.02	1.20±0.03	2.06±0.02	32.28 ^a ±0.37	9.62±0.44	3.26±0.14
Significant	NS	NS	NS	*	NS	NS

Different letters (a-c) within a column denote significant differences between treatments ($p \leq .05$). NS: None significant; GSP: Grape seeds powder; GLP: Grape leaf powder; GSE: Grape seed extract; GLE: Grape leaf extract.

The improvement in calcium percent of the grape leaf extract treatments may be due to the presence of resveratrol compound in grape leaves, which has many properties in preserving bone health (Shen *et al.*, 2012). According to Holman and Weaver (2014), rats fed a diet enriched in grapes retained 44% more net calcium in the bones than rats fed a control diet. So, the researchers suggest that consuming grape products may improve calcium utilization and suppress bone turnover in ovariectomized rats, leading to improved bone quality. In contrast with our results, Kaya *et al.*, (2014) reported that serum calcium and phosphorus concentration were decreased in layer hens that were fed grape seed extract, at levels 20-25 mg/kg diets.

CONCLUSIONS

The present study demonstrated that supplementary grape seed extract at the levels of 2 or 4 ml/l of drinking water had a beneficial effect on the femur length, predicted skeletal weight and calcium percentage in bone ash. At the same time, the supplementation of grape seed, leaf and their extract had not affected the bone weight, bone index, total ash, serum calcium and phosphorus concentration, phosphorus percent in bone ash and Ca: P ratio among groups of the broiler.

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