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The Use of Grape Seed (*Vitis vinifera L.*) Extract as Feed Additive Against Negative Effects of Gentamycin in Broiler*

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Abstract: In this study, it was aimed to determine the effects of *V. vinifera L* seed extract against the negative effects of performance parameters, organ weights and blood parameters due to the administration of gentamycin in broilers. A total of 240 Ross 308 chickens were divided into 4 groups with 6 replications of 10 birds per cage. Treatment groups: Control group was fed a basal diet only; group V was fed a diet supplemented with grape seed extract (100 mg/kg); group G was fed a basal diet and administered gentamycin (50 mg/kg body weight intramuscularly); group V+G was fed a diet supplemented with grape seed extract, and gentamycin in levels as in group V and G. The highest weight gain and feed intake at the 3rd, 4th, 5th and 6th weeks was observed in group C, and the lowest, statistically significant, was in group G. The highest carcass weight was in group C. The highest aspartate aminotransferase and alanine aminotransferase levels were in group G. The lowest red blood cell value was in group C. Liver weight was increased significantly in group G. In this study, it was observed that the use of gentamycin harmed the health of broiler hens, but these effects could be reduced partially with the grape seed extract added to the feed. For this reason, grape seed extract may be used as a feed additive with healing effect.

Keywords: Feed additive, Gentamycin, Grape seed extract.

Broyler de Gentamisinin Olumsuz Etkilerine Karşı Üzüm Çekirdeği (*Vitis vinifera L.*) Ekstresinin Yem Katkısı Olarak Kullanımı

Öz: Bu araştırmada, broilere gentamisin uygulasından dolayı performans parametreleri, organ ağırlıkları ve kan parametreleri üzerine oluşan olumsuz etkilerini gidermede üzüm çekirdeği (*V. vinifera L*) ektsresinin etkinliğini belirlemek amaçlanmıştır. Toplam 240 adet Ross 308 dişi civciv, her bir kafeste 10'ar adet olacak şekilde 6 tekerrürlü 4 gruba ayrıldı. Uygulama grupları; C grubu (kontrol grubu) sadece basal diyet ile beslendi; V grubu üzüm çekirdeği eksratı katkılı diyetle beslendi (100 mg/kg); G grubu basal diyetle beslendi ve gentamisin uygulandı (50 mg/kg vücut ağırlığı kas içi); V+G grubu ise üzüm çekirdeği eksratı katkılı diyetle beslendi ve gentamisin uygulandı. İstatistiksel olarak anlamlı olan en yüksek ağırlık artışı ve yem tüketimi 3, 4, 5 ve 6. Haftalar da C grubunda, en düşük ise G grubunda görüldü ($P<0.05$). En yüksek karkas ağırlığı C grubunda oldu. Aspartat aminotransferaz ve alanin aminotransferaz seviyelerinin en yüksek olduğu grup G grubuydu. En düşük kırmızı kan hücre değeri C grubunda oldu ($P<0.05$). Karaciğer ağırlığı G grubunda önemli ölçüde arttı. Bu araştırmada, gentamisin kullanımının etlik piliç sağlığına zarar verdiği ancak üzüm çekirdeği ekstratı eklenen yem ile bu etkilerin kısmen azaltılabileceği görüldü. Bu nedenle üzüm çekirdeği ekstratının yem katkı maddesi olarak kullanılabileceği kanaatine varıldı.

Anahtar Kelimeler: Gentamisin, Üzüm çekirdeği eksratı, Yem katkısı.

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INTRODUCTION

Toxic effects may be observed in animals due to the use of gentamycin. Gentamycin decreases feed intake, reduces body weight and causes bleeding and swelling of the kidneys and liver (1). As a result of using gentamycin, damage to large tissues, cell death, detoxification and changes in inflammatory responses have also been observed (2). For all these negative reasons, and to reduce toxicity, it has become more preferable to use natural plants and plant extracts with anti-oxidative activities that have no adverse effects on health (3). Grape seed extract, a natural plant extract with abundant phenolic compounds (4), which is an unused end product of the fruit juice and wine industry, has recently attracted attention (5). Grape seed is one of the richest and most common sources of proanthocyanidins found in nature (6). The proanthocyanidins in grape seed consist of oligomeric and polymeric compounds composed of flavan-3-ol monomer units (7). Proanthocyanidins have many biological properties including antiviral, antimicrobial (8), anti-inflammatory (9) and antioxidant (10) properties. Proanthocyanidins have positive effects on health, such as in preventing skin diseases and damage from radiation (11).

This study investigated the healing effect of *V. vinifera L.cv. Merlot* seed extracts against the negative effects of gentamycin application on health and performance parameters in broilers.

MATERIALS and METHODS

Animals and Treatment

This study was performed in compliance with the guidelines approved by the Local Ethics Council of Animal Experiments at Ataturk University (Protocol Number 30.2.ATA.0.23.71-333). The study used 240 one-day-old Ross 308 female broilers. The animals were selected according to their weight and divided into four groups of 60 animals—six replicates with each pen containing 10 chickens. The animals' water and feed were provided *ad libitum*. The broilers chicken were maintained in standard housing conditions. The temperature started from 32-33 ◦C (the first day of age) and then gradually decreased to a constant temperature of 19 ◦C. The trial lasted seven weeks (42 days). The trial started after the 1-week adaptation period (0-7 days). The chickens were divided into groups in the second week (after the 8th day). The study had four groups: The Control group received a basal diet with no grape seed extract additive; group V received a basal diet plus grape seed extract (*V. vinifera L.*) in a dose of 100 mg/kg (from 8 days to 42 days), and group G received a basal diet plus gentamycin application (50 mg/kg live weight intramuscularly). Gentamycin was applied one time daily, for 8 consecutive days, starting on day 8th; group V+G received a basal diet plus grape (*V. vinifera L.*) seed extract by 100 mg/kg (from 8 days to 42 days) plus gentamycin application (50 mg/kg body weight intramuscularly).

Plant and Antibiotic Obtaining

As the plant material, *Vitis vinifera L. cv*. "*Merlot*" seeds were obtained from Merlot grapes from the "Doluca" Winery (Tekirdag, Turkey). The seeds were dried in the shadow for 4 days (temp. 25±1 °C). The dried seeds were ground to a fine powder in a mallet mill. Approximately 100 g of seed powder was extracted with 500 ml of ethanol for 2 days at room temperature. After the extract was filtered for two days, it was evaporated to dryness in a vacuum at 40 °C with a rotary evaporator. The supernatants were separated from the suspension with a filter (12). Table 1 shows the content analysis of the basal diet. The antibiotic gentamycin sulfate was supplied by the Ani-Med Center Drug Administration (Gentasol Forte, Ankara, Turkey).

Table 1. Composition of the experimental diets (%).
Tablo 1. Deneme rasyonlarının bileşimi (%).

Ingredients (%)	7–14 days	15–21 days	22–28 days	29 days <
Maize	37.0	37.2	36.5	34.0
Full fat soybean meal	27.0	21.0	19.5	20.0
Dry soybean	13.1	15.1	17.1	17.6
Wheat	7.30	10.5	12.6	14.84
Vegetable fat	0.71	3.07	5.20	5.58
Poultry meal	1.50	2.00	3.00	3.50
Maize gluten	8.41	6.55	1.50	–
Meat and bone meal	1.50	2.00	2.44	2.64
DCP (Dicalcium phosphate)	1.46	1.00	0.74	0.48
Methionine	0.24	0.23	0.23	0.24
Vitamin-mineral premix	0.50	0.50	0.50	0.50
Sodium chloride	0.21	–	0.18	0.17
Sodium bicarbonate	0.15	0.15	0.15	0.15
Marble dust	0.33	0.16	–	–
Antitoxin	0.10	0.10	0.10	0.10
Choline chloride	0.09	0.09	0.09	0.09
Threonin	0.08	0.08	0.07	0.07
Lysine	0.32	0.27	0.10	0.04
TOTAL	100	100	100	100
Calculated Values				
Dry matter	90.0	89.7	89.4	89.9
Crude protein	23.5	23.0	22.0	20.5
Metabolisable energy (MJ/kg)	12.7	13.4	13.8	13.9
Crude fat	5.56	5.35	7.40	7.45
Crude fibre	3.40	3.51	3.78	4.01
Ca	0.99	0.91	0.88	0.85
P	0.50	0.45	0.44	0.42

* Supplied per kg of diet: 10,000 IU vitamin A, 12 mg vitamin E, 2000 IU vitamin D, 36 mg niacin, 10 mg D-pantothenic acid, 3.61 mg riboflavin, 3.52 mg pyridoxine, 2.41 mg thiamine, 1.39 mg folic acid, 0.16 mg biotin and 0.03 mg vitamin B, 59 mg manganese, 41 mg zinc, 1281 mg iron, 7,9 mg copper, 0.31 mg iodine and 0.22 mg selenium.

Performance Parameters and Organ Weight Analysis

The data for performance parameters were calculated weekly. During the study, the feed and body weights of the birds in all cages were determined by weighing every week, and the average weight gain and feed consumption were calculated. Internal organ and carcass weights were calculated by comparison to the slaughter body weights.

Deaths during the experiment were recorded daily, and mortality was found by dividing the number of chickens by the number of dead chickens in the groups.

Blood Sampling Analysis

At the end of the research, broiler blood samples were taken after slaughtering all broilers, and then, the samples were taken into anticoagulant tubes. The samples were centrifuged at 4.000 rpm to separate the serum. The serum samples were stored at -20 °C until the time of biochemical and physiological analyses.

High-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in the blood samples. Biochemical parameters were measured with commercially available diagnostic kits with a Beckman Coulter AU5800 (USA) autoanalyzer. White blood cell (WBC), Red blood cell (RBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were determined in the blood samples. Hematological parameters were measured and counted with an automated hematology analyzer. All other chemicals were bought from the Sigma Chemical Co. (St. Louis, MO, USA).

Statistical Analysis

Statistical analysis was conducted by a general linear model (One-Way ANOVA) with the SPSS software package, version 20.00. Group effects were tested first, and then, post-hoc Tukey's tests were used to compare the group differences. The data are expressed as mean (M) ± standard deviation (SD) (13).

RESULTS

The highest live weight gain at the 2nd week was obtained in group G+V, whereas the lowest live weight gain was in group G (P<0.05). While the highest live weight gain at the 3rd, 4th, 5th and 6th weeks was obtained in group C, the lowest live weight gain was in group G (P<0.05). Feed intake during the 2nd week was the highest in group G+V and the lowest in group G. The highest feed intake at the 3rd, 4th, 5th and 6nd weeks were observed in group C, and the lowest was in group G (P<0.05). The best feed conversion rates at the 2nd and 4th weeks were seen in group C, and those at the 3rd, 5th and 6th weeks were in group V, whereas the worst of all weeks were in group G (P<0.05) (Table 2).

Table 2. Effect of feeding grape seed (*Vitis vinifera L.*) extract diet and gentamycin application on performance parameters in broilers (M±SD).

Tablo 2. Broilerde üzüm çekirdeği (*Vitis vinifera L.*) ekstratı katkılı diyetle beslemenin ve gentamisin uygulamasının performans parametrelerine etkisi (M±SD).

Parameters		C group	V group	G group	G+V group	P value
Initial body weight (g)		44.95±0.65	44.5±0.48	44.65±0.72	44.85±0.52	
Weight gain (g)	2 week	163.55±3.05^{a}	159.15±3.65^{a}	125.71±4.01^{b}	165.29±2.54^{a}	0.039
	3 week	175.29±6.24^{a}	167.89±5.61^{a}	79.24±7.42^{c}	150.74±5.54^{b}	0.023
	4 week	235.15±9.82^{a}	220.84±7.31^{a}	110.75±5.04^{c}	175.90±4.21^{b}	0.029
	5 week	282.41±8.56^{a}	266.47±7.41^{b}	162.19±5.06^{c}	240.44±5.38^{b}	0.034
	6 week	352.37±11.42^{a}	332.26±10.05^{ab}	235.05±7.24^{c}	290.34±10.27^{b}	0.043
Feed intake (g)	2 week	278.53±11.81^{a}	285.60±10.03^{a}	238.36±7.26^{b}	292.85±10.54^{a}	0.044
	3 week	334.03±17.14^{a}	306.85±12.51^{b}	157.95±6.76^{c}	298.60±10.36^{b}	0.038
	4 week	442.64±16.86^{a}	420.28±18.23^{a}	220.38±15.08^{c}	346.11±20.01^{b}	0.032
	5 week	542.15±22.40^{a}	496.50±18.42^{b}	323.21±15.47^{c}	464.27±18.14^{b}	0.026
	6 week	647.60±21.14^{a}	594.92±20.63^{b}	458.39±18.52^{c}	557.53±19.16^{b}	0.021
Feed conversion	2 week	1.71±0.04^{a}	1.79±0.06^{a}	1.90±0.04^{b}	1.77±0.03^{a}	0.032
	3 week	1.91±0.17^{ab}	1.83±0.16^{a}	1.99±0.15^{b}	1.98±0.16^{b}	0.043
	4 week	1.88±0.15^{a}	1.90±0.18^{a}	1.99±0.22^{b}	1.97±0.23^{b}	0.037
	5 week	1.92±0.18^{b}	1.86±0.16^{c}	1.99±0.23^{a}	1.93±0.16^{b}	0.026
	6 week	1.84±0.13^{a}	1.79±0.14^{a}	1.95±0.15^{b}	1.92±0.15^{b}	0.033
Mortality (%)		10.0	5.0	16.67	8.34	

Values are expressed as means±SEM. $^{a-c}$Mean values within rows with different letters are significantly different (P<0.05). C (Control group): The basal diet. Group V: The basal diet + grape seed extract (100 mg/kg) supplemented. Group G: The basal diet + gentamicin (50 mg/kg body weight intramuscularly) was made application. Group G+V: The basal diet + Grape seed extract (100 mg/kg) + gentamicin (50 mg/kg body weight intramuscularly) was applied.

The numbers of birds that died during the trial were 6 individuals in group C, 3 individuals in group V, 10 individuals in group G and 5 individuals in group G+V. The highest mortality rate was found in group G, and the lowest mortality rate was in group V (Table 2). While the highest carcass weight was in group C, the lowest was in group G (P<0.05). As the lowest percentage of the liver was in group C, the highest one was in group G (P<0.01). There were no significant differences among the groups in terms of the percentages of the heart and the gizzard (Table 3).

Table 3. Effect of feeding grape seed (*Vitis vinifera L.*) extract diet and gentamycin application carcass characteristics in broilers (M±SD).

Tablo 3. Broilerde üzüm çekirdeği (*Vitis vinifera L.*) ekstratı katkılı diyetle beslemenin ve gentamisin uygulamasının karkas özelliklerine etkisi (M±SD).

Parameters	C group	V group	G group	G+V group	P value
Carcass weight (g)	937.78±4.07^{c}	885.96±4.13^{bc}	536.04±3.51^{a}	780.15±3.96^{b}	0.017
Carcass ratio (%)	74.8±0.12	74.3±0.37	72.2±0.14	73.8±0.62	0.173
Liver (%)	2.49±0.05^{a}	2.52±0.04^{a}	2.95±0.03^{b}	2.60±0.02^{a}	0.005
Heart (%)	0.89±0.001	0.87±0.007	0.93±0.009	0.86±0.009	0.215
Gizzard (%)	2.88±0.02	2.84±0.03	2.87±0.02	2.92±0.03	0.614

Values are expressed as means±SEM. $^{a-c}$Mean values within rows with different letters are significantly different (P<0.05). C (Control group): The basal diet. Group V: The basal diet + grape seed extract (100 mg/kg) supplemented. Group G: The basal diet + gentamicin (50 mg/kg body weight intramuscularly) was made application. Group G+V: The basal diet + Grape seed extract (100 mg/kg) + gentamicin (50 mg/kg body weight intramuscularly) was applied.

While the AST (P<0.01) and ALT (P<0.05) values were increased in groups G and V+G, they were decreased in group V. As the lowest LDL level was in group V, the highest was in group C (P<0.05). There were no significant differences in the HDL levels among the groups (Table 4). While the RBC value was increased in groups V and G+V, it was decreased in group C (P<0.01). The highest MCH value was in group C, and the lowest was in group G+V (P<0.01). On the other hand, there was no significant difference among the groups in terms of the WBC and MCV values (Table 4).

Table 4. Effect of feeding grape seed (*Vitis vinifera L.*) extract diet and gentamycin application on blood values in broilers (M±SD).
Tablo 4. Broilerde üzüm çekirdeği (*Vitis vinifera L.*) ekstratı katkılı diyetle beslemenin ve gentamisin uygulamasının kan değerleri üzerine etkisi (M±SD).

Parameters	C group	V group	G group	G+V group	P value
HDL(mg/dl)	74.18±3.13	73.67±4.21	72.85±3.43	73.54±3.84	0.354
LDL(mg/dl)	126.36±2.21^{a}	117.45±2.35^{b}	125.58±3.06^{a}	124.61±2.24^{a}	0.023
AST(IU/L)	274.65±23.21^{b}	270.21±22.17^{b}	298.45±22.26^{a}	286.14±23.36^{ab}	0.005
ALT(IU/L)	11.78±0.12^{b}	10.77±0.13^{b}	18.60±0.12^{a}	15.62±0.11^{a}	0.033
RBC(x10^{6}/mm^{3})	2.46±0.46^{a}	3.14±0.48^{b}	3.02±0.32^{ab}	3.28±0.2^{b}	0.007
WBC(x10^{3}/mm^{3})	39.25±10.92	38.00±8.72	43.41±3.44	36.58±16.9	0.412
MCV(µ3)	124.37±26.2	121.9±26.99	120.21±21.06	123.05±14.54	0.245
MCH(pg)	39.01±8.64^{a}	26.15±3.21^{b}	26.02±6.77^{b}	24.39±3.11^{b}	0.004

Values are expressed as means±SEM. a-cMean values within rows with different letters are significantly different. HDL: High-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GM: Gentamicin, VV: Vitis vinifera, RBC: Red Blood Cell, WBC: White Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin.

DISCUSSION and CONCLUSION

In a study, chickens received *M. oleifera* extract and gentamycin. It was reported that the gentamycin group had decreased average feed intake, live weight gain, and FCR was affected negatively. However, the negative effects of gentamycin were found to be slightly improved in the groups that received the *M. oleifera* extract and gentamycin together. In the same study, the mortality rate was increased only in the groups treated with gentamycin and in the *M. oleifera* extract + gentamycin group (14). In another study on broilers, gentamycin administration increased lipid peroxidation levels and decreased antioxidant enzyme levels, but with the addition of Ocimum sanctum to diet, the enzyme levels returned to normal (15).

Other trials applying gentamycin to chickens have reported parallel results between increases in the dose of gentamycin administered and decreases in feed consumption and body weight (16-18). Another study added grape seed extract to the diets of poultry and reported that chickens infected with *Eimeria tenella* had improved performance (19). A study by Abu Hafsa and Ibrahim reported that adding grape seed by 20 g/kg in broiler rations had a positive effect on FCR and live weight gain; however, it did not affect feed intake (20). It was reported that addition of grape seed extract in chicken diets did not alter growth performance but improved the feed conversion rate. Grape seed added to a broiler diet had positive effects on the feed conversion rate and live weight gain, and it could result in better absorption of nutrients by slowing the bowel movements of tannins in the structure of grape seed (21). It is also believed to protect the intestines against oxidative damage and prevent digestive system disorders (22).

Previous studies found that the application of gentamycin increased mortality rates, and grape seed decreased mortality rates (18-20). In the study, only the mortality rate in group V was below acceptable limits. It was not found that the reason why the death rate in the control group was so high. The mortality rate with gentamycin administration can be attributed to toxic dose and depression (18, 20). The effect of grape seed is thought to be due to the anti-oxidant, anti-inflammatory and anti-bacterial properties of the proanthocyanidins it

contains. In another study conducted in pigs, it was reported that grape seed extract added to the diet reduced the toxic effect of aflatoxin B1 (23).

Khan et al. (16) reported that gentamycin application increased kidney, liver and heart weight. Due to the increase in the rate of gentamycin, the weight and size of the heart, liver, kidney and spleen increased (17,18). As a result of repeated administration of gentamycin at the safe dose level used in both the present study and other studies, changes in the structure and weight of internal organs emerged (1). It is thought that these changes were due to the toxic effects of gentamycin (14,16). Although grape seed extract in a broiler diet did not change pancreas and liver weights, it did change the length of the intestine (24). The group with grape seed extract addition by 3.6 g/kg had a decreased pancreatic weight but an increased intestinal length (24). Hajati et al. (25) studied broilers that were fed on a grape seed extract additive diet, which did not affect the broilers' liver, gizzard or cold carcass weights. In another study, 20 g/kg of grape seed added to chicken diets increased the chickens' gizzard weight. Moreover, the same process did not affect the weights of the heart, liver or spleen (20). In a study conducted on Japanese quails, the group that received grape seed additive exposed to normal temperature only had increased heart weight. However, the treatment did not have a significant effect on liver or spleen weight (26). The groups fed with a grape seed diet were similar.

In animals who were exposed to exogenous toxins, the ALT, AST and ALP enzymes were used to determine the damages and pathological conditions in the kidney and liver (27). The toxic effect of using gentamycin led to an increase in the AST and ALT ratios (18,28). Grape seed supplementation to broiler diets did not affect ALT and AST (20). Another experiment observed that broilers fed with grape seed proanthocyanidins extract diet had no significant differences in AST, ALT or total protein values (29). It is considered that the application of gentamycin in chickens led to various pathological conditions and consequently increased oxidative stress. However, grape seed extract has been determined to reduce oxidative stress (30,31). For this reason, ALT and AST ratios in the study decreased with the effect of grape seed extract. In a study conducted in rats, it was stated that grape seed extract significantly reduced LDL, VLDL, Chol levels (32). In another study, it was reported that while it increased HDL level, it did not affect LDL values (33). In another study, while there was no significant difference in HDL ratio as a result of gentamycin administration to rats, it was reported that there was a significant increase in LDL ratio (34). These reported results were similar to the results in the study. In achieving these results, it is thought that the effect of hyperlipemia is caused by oxidative stress and the hypolipidemic effects that proanthocyanidins suppressing intestinal lipid absorption and chylomicron secretion in the intestine (35).

In a study performed in rats, the grape seed did not affect the WBC, RBC or MCH values (9). In another study, it was reported that grape seed extract did not affect WBC, RBC or MCH values (33). In other studies that were conducted, the poultry hematological parameters have not been investigated. Hematological parameters, however, provide information about the health of animals. The results obtained in this study are thought to be related to the toxicity or side effects caused by gentamycin. The main reasons for the differences in the results obtained among studies are believed to be because of the rate of the grape seed supplement added to diets and the differences in the polyphenolic content of the grape seeds (19,32).

As a result, although the chickens experienced negative effects on performance parameters, blood values and organ weights as a result of the toxicity caused by the application of gentamycin, the grape seed extract (*V. vinifera L.*) could improve many of those negative conditions. However, to make a definitive conclusion, it is necessary to conduct more studies on the topic.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Islam NU., Khan MZ., Saleemi MK., Khan A., Bhatti SA., Yousaf M., Hassan ZU., 2011. Clinicopathological studies on gentamycin toxicity in White Leghorn commercial layers. Pak Vet J, 31, 305-308.
2. Akbarian A., Michiels J., Degroote J., Majdeddin M., Golian A., De Smet S., 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. J Anim Sci Biotechnol, 7, 37.
3. Lobo V., Patil A., Phatak A., Handra N., 2010. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev, 4, 118-126.
4. Stambuk P., Tomaskovic D., Tomaz I., Maslov L., Stupic D., Karoglan Kontic J., 2016. Application of pectinases for recovery of grape seeds phenolics. 3 Biotech, 6, 224.
5. Cosansu S., Juneja VK., 2018. Growth of Clostridium perfringens in sous vide cooked ground beef with added grape seed extract. Meat Sci, 143, 252-256.
6. Özcan MM., Al Juhaimi F., Gülcü M., Uslu N., Geçgel Ü., 2017. Determination of bioactive compounds and mineral contents of seedless parts and seeds of grapes. S Afr J Enol Vitic, 38, 212-220.
7. Zhang S., Li L., Cui Y., Luo L., Zhou P., Sun B., 2017. Preparative high-speed counter-current chromatography separation of grape seed proanthocyanidins according to degree of polymerization. Food Chem, 219, 399-407.
8. Kemperman RA., Gross G., Mondot S., Possemiers S., Marzorati M., Wiele TV., Vaughan EE., 2013. Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome. Food Res Int, 53, 659-669.
9. Charradi K., Mahmoudi M., Bedhiafi T., Jebari K., El May MV., Limam F., Aouani E., 2018. Safety evaluation, anti-oxidative and anti-inflammatory effects of subchronically dietary supplemented high dosing grape seed powder (GSP) to healthy rat. Biomed&Pharmacother, 107, 534-546.
10. Apaydin Yildirim B., Tunc MA., Yildirim S., Yildirim F., Kordali S., Ertekin A., Aktas Senocak E., 2017. The effect of Vitis vinifera L.cv. Merlot seed extract on gentamycin-induced nephrotoxicity in broilers. Europ Poult Sci, 81, 1-12.
11. Jing S., Zhang X., Yan LJ., 2015. Antioxidant activity, antitumor effect, and antiaging property of proanthocyanidins extracted from Kunlun Chrysanthemum flowers. Oxid Med Cell Longev, 2015, 1-10.
12. Kordalı Ş., Çakır A., Akcın TA., Mete E., Akcın A., Aydın T., Kılıç H., 2009. Antifungal and herbicidal properties of essential oils and n-hexane extracts of Achillea gypsicola Hub-Mor. and Achillea biebersteinii Afan. (Asteraceae). Ind Crops Prod, 29, 562-570.
13. SPSS (2004). SPSS for Windows Release 13.0. SPSS Inc.
14. Arafat N., Awadin WF., El-Shafei RA., Farag VME., Saleh RM., 2018. Protective Role of Moringa oleifera Leaves extract against gentamycin - induced nephro- and hepato-toxicity in chickens. AJVS, 58, 173-185.
15. Arivuchelvan A., Murugesan S., Mekala P., 2012. Antioxidant properties of Ocimum sanctum in broilers treated with high doses of gentamycin. IJDAD, 1, 143-146.
16. Khan I., Khan MZ., Saleemı MK., Javed I., Khan A., 2008. Pathological and Biochemical Effects of Intramuscular Gentamycin Administration in Chickens. Turk J Vet Anim Sci, 32, 345-351.
17. Javed U., Saleemi MK., Khan MZ., Khan A., Javed I., 2008. Pathological Effects of Gentamycin in Growing Broilers. Proceedings, The 15th Congress of FAVA. FAVA -OIE Joint Symposium on Emerging Diseases. 27-30 October 2008. Bangkok, Thailand, 17-18.
18. Javed U., Khan MZ., Saleemi MK., Khan A., Javed I., Rafique S., 2013. Toxico pathological effects of parenteral administration of gentamycin in growing Broilers. Int J Agric & Biol Eng, 15, 529-534.
19. Wang ML., Suo X., Gu JH., Zhang WW., Fang Q,

Wang X., 2008. Influence of grape seed proanthocyanidin extract in Broiler chickens: Effect on chicken coccidiosis and antioxidant status. Poult Sci, 87, 2273-2280.

20. Abu Hafsa SH., Ibrahim SA., 2018. Effect of dietary polyphenol- rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. J Anim Physiol Anim Nutr, 102, 268-275.
21. Ismail AM., Sedki AA., Abdallah AG., 2003. Influence of black seed, garlic and onion supplementation on reproductive performance in rabbits. Egypt J Agric Res, 81, 1193-1207.
22. Kermauner A., Laurencic A., 2008. Supplementation of rabbit diet with chestnut wood extract: Effect on in vitro gas production from two sources of protein. Proceedings of the 9thWorld Rabbit Congress, 10–13 Jun, Verona, Italy, 689-693.
23. Taranu I., Marin DE., Palade M., Pistol GC., Chedea VS., Gras MA., Rotar C., 2019. Assessment of the efficacy of a grape seed waste in counteracting the changes induced by aflatoxin B1 contaminated diet on performance, plasma, liver and intestinal tissues of pigs after weaning. Toxicon, 162, 24-31.
24. Brenes A., Viveros A., Goni I., Centeno C., Sa yago-Ayerdy SG., Arija I., Saura-Calixto F., 2007. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poult Sci, 87, 307-316.
25. Hajati H., Hassanabadi A., Golian A., Nassiri-Moghaddam H., Nassiri MR., Safari R., 2015. The effect of grape seed extract and vitamin C feed supplements carcass characteristics, gut morphology and ileal microflora in broiler chickens exposed to chronic heat stress. IJAS, 5, 155-165.
26. Erişir Z., Şimşek ÜG., Özçelik M., Baykalır Y., İflazoğlu Mutlu S., Çiftçi M., 2018. Effects of dietary grape seed on performance and some metabolic assessments in Japanese quail with different plumage colors exposed to heat stress. R Bras Zootec, 47, 1-10.
27. Center SA., 2007. Interpretation of liver enzymes. Vet Clin N Am-Small, 37, 297-333.
28. Saleemi MK., Khan MZ., Khan A., Javed I., 2009. Pathological effects of gentamycin administered intramuscularly to day old broiler chicks. Exp Toksicol Pathol, 61, 425-432.
29. Rajput SA., Sun L., Zhang N., Khalil MM., Gao X., Ling Z., Zhu L., Khan FA., Zhang J., Qi D., 2017. Ameliorative effects of grape seed proanthocyanidin extract on growth performance, immune function, antioxidant capacity, biochemical constituents, liver histopathology and aflatoxin residues in Broilers Exposed to Aflatoxin B1. Toxins, 9, 371-391.
30. Gines I., Gil-Cardoso K., Serrano J., Casanova-Marti A., Blay MT., Pinent M., Ardevol A., Terra X., 2018. Article effects of an intermittent grape-seed proanthocyanidin (GSPE) treatment on a cafeteria diet obesogenic challenge in Rats. Nutrients, 10, 315.
31. Wang EH., Yu ZL., Bu YJ., Xu PW., Xi JY., Liang HY., 2019. Grape seed proanthocyanidin extract alleviates high-fat diet induced testicular toxicity in rats. RSC Adv, 9, 11842.
32. Mansouri E., Khorsandi L., Moaiedi MZ., 2015. Grape Seed Proanthocyanidin Extract Improved some of Biochemical Parameters and Antioxidant Disturbances of Red Blood Cells in Diabetic Rats. IJPR, 14, 329-334.
33. Sano A., Uchida R., Saito M., Shioya N., Komori Y., Tho Y., Hashizume N., 2007. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. J Nutr Sci Vitaminol, 53, 174-182.
34. Ahmadvand H., Bagheris., Tamjidi-Poor A., Cheraghi M., Azadpour M., Ezatpour B., Moghadam S., Shahsavari G., Jalalvand M., 2016. Biochemical effects of oleuropein in gentamycin n-induced nephrotoxicity in rats. ARYA Atheroscler, 12, 87-93.
35. Blade C., Arola L., Salvado MJ., 2010. Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms. Mol Nutr Food Res, 54, 37-59.
36. Chamorro S., Viveros A., Centeno C., Romero C., Arija I., Brenes A., 2013. Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. Animal, 7, 555-561.