ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Araştırma Makalesi / Research Article
2015; 12(1) : 37-41

The Effects of Grape Seed on Some Biochemical Parameters in Ionophore Antibiotics Treated Broiler*

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Summary: Day-old Ross Mp3 male broiler chickens were used in this study. The animals were allocated to 8 groups, each comprising 10 broiler chickens. The first group was maintained for control purposes and was fed on normal feed throughout the trial period. To other groups it was given the grape seed (0.5%), lasalocid (100 ppm), salinomycin (60 ppm) and maduramycin (5 ppm), lasalocid (100 ppm) and grape seed (0.5%), salinomycin (60 ppm) and grape seed (0.5%), maduramycin (5 ppm) and grape seed (0.5%) into feed for a period of 6 weeks, respectively. At the end of the trial period serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), protein, albümin cholesterol, triglyceride, uric acid, glucose and creatinine levels were assayed. As a result, it was observed that grape seed has no negative effect on biochemical parameters. Findings obtained in the present study demonstrated that lasalocid has increased ALT activity, triglyceride and creatinine levels, while salinomycin increased ALT and ALP activities, triglyceride and creatinine levels, but decreased albumin levels. Maduramycin increased ALT and ALP activities along with triglyceride, uric acid and creatinine levels. In the group, which received a combination of grape seed and lasalocid, ALT and ALP activities and triglyceride levels had decreased; whilst in the group that was given a combination of grape seed and salinomycin, ALT activity and triglyceride levels were observed decrease. In the group fed on a combination of grape seed and maduramycin, it was ascertained that ALT and ALP activities, triglyceride, uric acid and creatinine levels had decreased ($P<0.05$). The results of the present study demonstrated that while lasalocid, salinomycin and maduramycin induced adverse effects on liver and kidney functions and fat metabolism in broiler chickens, the combined use of ionophore antibiotics and grape seed reduced the severity of the adverse effects of these feed additives.

Key Words: Biochemical toxicity, broiler, ionophore antibiotics

İyonofor Antibiyotikler Verilen Etçi Piliçlerde Üzüm Çekirdeğinin Bazı Biyokimyasal Parametreler Üzerine Etkileri

Özet: Çalışmada günlük Ross MP3 ırkı erkek etlik civciv kullanıldı ve her grupta 10'ar hayvan bulunan 8 grup oluşturuldu. Birinci grup kontrol olarak tutuldu ve çalışma süresi boyunca normal yemle beslendi. Diğer gruplara sırasıyla üzüm çekirdeği (%0.5), lasalosid (100 ppm), salinomisin (60 ppm), maduramisin (5 ppm), lasalosid (100 ppm) ve üzüm çekirdeği (%0.5), salinomisin (60 ppm) ve üzüm çekirdeği (%0.5), maduramisin (5 ppm) ve üzüm çekirdeği (%0.5) 6 hafta süreyle yemle birlikte verildi. Çalışma süresi sonunda serum aspartat aminotransferaz (AST), alanın aminotransferaz (ALT), alkalen fosfataz (ALP), protein, albumin kolesterol, trigliserit, ürik asit, glikoz ve kreatinin değerleri incelendi. Üzüm çekirdeğinin belirtilen biyokimyasal parametreler üzerinde herhangi olumsuz bir etkisinin olmadığı gözlendi. Lasalosid'in ALT, trigliserit ve kreatinin düzeylerinde yükselmeye; salinomisin'in ALT, ALP, trigliserit ve kreatinin düzeylerinde yükselme ve albumin düzeyinde azalmaya; maduramisin'in ALT, ALP, trigliserit, ürik asit ve kreatinin düzeylerinde yükselmeye yol açtığı belirlendi. Üzüm çekirdeği+lasalosid verilen grupta ALT, ALP ve trigliserit düzeylerinin düştüğü; üzüm çekirdeği+salinomisin verilen grupta ALT ve trigliserit düzeyinin düştüğü; üzüm çekirdeği+maduramisin verilen grupta ALT, ALP, trigliserit, ürik asit ve kreatinin düzeylerinin düştüğü belirlendi ($P<0.05$). Lasalosid, salinomisin ve maduramisin'in etlik piliçlerde karaciğer ve böbrek fonksiyonları ile yağ metabolizması üzerinde olumsuz etkilerinin bulunduğu; ancak iyonofor antibiyotiklerle birlikte üzüm çekirdeği verilmesinin bu antibiyotiklerin yol açtığı istenmeyen etkileri azaltıcı etkilerinin olduğu belirlendi.

Anahtar Kelimeler: Biyokimyasal toksisite, etçi piliç, iyonofor antibiyotikler

Introduction

Ionophore antibiotics have a common use in poultry production, particularly in poultry meat production. Compounds belonging to this group of antibiotics are capable of penetrating through biological membranes by forming fat-soluble

Geliş Tarihi/Submission Date : 02.04.2014
Kabul Tarihi/Accepted Date : 04.08.2014

* Bu çalışma 21. Ulusal Farmakoloji Kongresinde poster bildirisi olarak sunulmuştur.

compounds with various metals. When included in poultry feed at excessive levels, they cause disrupt of the active transport of various electrolytes and water, and therefore, metabolism, resulting in toxicity (4). Grape seed contains several biologically active polyphenols. Grape seed contains proanthocyanidines, quercetin and resveratrol (2, 9, 11, 12).

The present study was aimed at determining the effects of ionophore antibiotics given alone and in combination with grape seed on certain biochemical parameters.

Materials and Methods

All experimental procedures were approved by the Ethic Committee for Animal Experiments of Faculty of Veterinary Medicine, University of Erciyes (10/18, 22.05.2006). Day-old male broiler chicks of the Ross MP3 breed constituted the material of the study. The animals were allocated to 8 groups, each comprising 10 chicks. The first group was maintained for control purposes and was fed on normal feed throughout the trial period. The second group received grape seed alone (0.5%), while the third, fourth and fifth groups were administered with lasalocid (100 ppm), salinomycin (60 ppm) and maduramycin (5 ppm) alone, respectively. The sixth group was given a combination of lasalocid (100 ppm) and grape seed (0.5%), whilst the seventh group received a combination of salinomycin (60 ppm) and grape seed (0.5%). The eighth group was administered with a combination of maduramycin (5 ppm) and grape seed (0.5%). The described treatment regimens were used for a period of 6 weeks. At the end of the trial period, blood samples were collected from animals into dry tubes. Serum was extracted from the blood samples by centrifugation at 3000 rpm for 10 minutes. The measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, and total protein, albumin, cholesterol, triglyceride, uric acid, glucose and creatinine levels in serum samples were performed spectrophotometrically using commercial test kits. AST, ALT and ALP activities were expressed in U/L, while the other parameters were expressed in mg/dL. The SPSS 11.0 for Windows was used for statistical calculations. Data were given in the form of arithmetical mean values and ±standard deviations. Differences between groups were evaluated by one-way ANOVA according to $p < 0.05$ and post–hoc Duncan test.

Results

Findings obtained in the present study demonstrated that grape seed did not induce any adverse effect on the biochemical parameters investigated. It was ascertained that lasalocid increased ALT activity, triglyceride and creatinine levels; salinomycin increased ALT and ALP activities, triglyceride and creatinine levels, but decreased albumin levels; and maduramycin increased ALT and ALP activities, triglyceride, uric acid and creatinine levels. In the group that received a combination of grape seed and lasalocid, ALT and ALP activities and tryglyceride levels were observed to have decreased; while in the group that was given grape seed in combination with salinomycin, ALT activity and triglyceride levels were determined to have decreased. The combined administration of grape seed and maduramycin was observed to have resulted in decreased ALT and ALP activities, triglyceride, uric acid and creatinine levels ($P<0.05$).

Discussion

Grape seed is a non-toxic, non-mutagenic and non -carcinogenic substance (10). In the present study, grape seed extract included in broiler chicken rations at a rate of 0.5% for a period of 42 days did not cause any significant difference in the biochemical parameters investigated, when compared to the control group ($P>0.05$).

According to Hussein and El-Rahman (6) 60 ppm of salinomycin to broliers causes an increase in serum ALT and AST activities, while the administration of 300 ppm of salinomycin increases ALT and AST activities, total lipid, creatinine, urea and calcium levels, but decreases total protein, albumin and phosphorus levels. In this study, while lasalocid was increasing ALT activity, triglyceride and creatinine levels, salinomycin caused a rise ALT and ALP activities, triglyceride and creatinine levels and a decrease in albumin levels ($P<0.05$). These differences show that salinomycin causes a serious damage on the liver more than lasalocid like previous studies.

In a study conducted by Arun et al. (1), in which broiler chicks were administered with 5 and 8 ppm of maduramycin for a period of 6 weeks, it was determined that, in the group that received 8 ppm, serum AST, ALT, ALP and LDH activities, urea, creatinine and cholesterol levels had increased, while total protein, albumin and globulin levels had decreased. On the other hand, in the group, which was given 5 ppm of maduramycin, no significant differences were observed on investigated param

Table 1. Biochemical parameters in groups

	CONT	GS	LAS	SAL	MAD	GS+LAS	GS+SAL	GS+MAD	Statistical Significant (Anova)
AST (U/L)	227.17±40.92	208.50±39.98	265.01±60.88	252.14±56.37	246.12±48.67	220.11±56.83	232.59±57.96	233.90±38.20	P<0.05
ALT (U/L)	5.4±0.66^{a}	5.5±0.59^{ab}	7.79±0.96^{c}	10.67±1.33^{d}	11.9±1.94^{d}	6.19±1.17^{ab}	6.72±0.7^{abc}	6.96±1.14^{bc}	P<0.05
ALP (U/L)	1563.42±393.8^{a}	1698.33±355.6^{ab}	2030.44±579.6^{ab}	2061.86±353.1^{b}	2847±360.2^{c}	1792.94±247.7^{ab}	1740.88±254.8^{ab}	2021.78±355.1^{ab}	P<0.05
PRO (mg/dl)	3.51±0.52	3.68±1	3.24±0.94	3.45±0.55	3.41±1.08	3.46±0.74	3.38±0.47	3.45±0.79	P<0.05
ALB (mg/dl)	1.6±0.44^{b}	1.54±0.37^{ab}	1.4±0.43^{ab}	1.09±0.36^{a}	1.23±0.29^{ab}	1.43±0.44^{ab}	1.4±0.2^{ab}	1.5±0.32^{ab}	P<0.05
COL (mg/dl)	133.23±27.53	124.19±16.73	152.69±33.92	145.66±30.42	154.11±34.3	144.03±43.94	146.29±36.93	145.82±22.42	P<0.05
TRIG (mg/dl)	41.77±7.8^{a}	41.87±5.08^{a}	128.89±14.69^{c}	98.81±13.25^{b}	110.19±17.1^{b}	41.63±7.29^{a}	45.75±11.53^{a}	50.17±11.97^{a}	P<0.05
URIC (mg/dl)	4.99±2.07^{ab}	3.26±0.82^{a}	6.17±1.99^{b}	6.82±1.3^{b}	9.31±2.01^{c}	5±1.22^{ab}	4.82±1.89^{ab}	5.53±2.25^{ab}	P<0.05
GLU (mg/dl)	242.95±21.16	254.03±26.47	269.85±21.12	261.59±48.74	270.39±36.86	231.51±52.6	233.39±29.88	260.71±24.64	P<0.05
CRE (mg/dl)	0.08±0.03^{a}	0.11±0.03^{ab}	0.13±0.03^{b}	0.14±0.03^{bc}	0.19±0.06^{c}	0.12±0.03^{ab}	0.11±0.02^{ab}	0.12±0.02^{ab}	P<0.05

^{a,b,c}. Different superscripts in the same row indicate significant differences (P<0.05).

CONT: control, GS: grape seed, LAS: lasalosid, SAL: salinomycin, MAD:maduramycin, AST: aspartate aminotransferase, ALT:alanine aminotransferase, ALP: alkaline phosphatase, PRO: protein, ALB: albumin, COL: cholesterol, TRIG: triglyceride, URIC: uric acid, GLU: glucose, CRE: creatinine

eters. In the present study, it was ascertained that maduramycin increased ALT and ALP activities, and triglyceride, uric acid and creatinine levels ($P<0.05$). Contrary to the findings of the present study, no significant alteration having been observed in the biochemical parameters investigated in another study, in which maduramycin was administered at the same dose for the same time period (1).These findings can be attributed to the differences in the composition of the ration fed to the animals and the composition of the maduramycin premixes included in the feed. Increased serum ALP activity is generally observed in bone diseases, whilst increased creatinine levels are associated with damage to muscle tissue (8). It is reported that, serum uric acid levels are elevated in cardiovascular diseases and particularly in cases of chronic renal failure (13). In light of these data, it can be indicated that maduramycin causes damage to bone, muscle, kidney and liver.

In the present study, it was determined that lasalocid increased ALT activity, triglyceride and creatinine levels, while salinomycin increased ALT and ALP activities, triglyceride and creatinine levels but decreased albumin levels, meanwhile maduramycin increased ALT and ALP activities, triglyceride, uric acid and creatinine levels ($P<0.05$). The results of the present study demonstrated that, when administered to broiler chickens at the recommended doses, lasalocid caused less toxicity in comparison to maduramycin and salinomycin. Furthermore, the increase of ALT activity was observed to be smaller in the group, which was given lasalocid, in comparison to the groups that received salinomycin and maduramycin, while the increase of AST activity was observed to be greater. These findings suggest that lasalocid causes degeneration most particularly in tissues other than liver. In fact, in animals, an increase in ALT activity is attributed to liver damage, and an increase in AST activity is attributed to the widespread degeneration of liver, muscle and other soft tissues (8, 14). The results of the present study showed that triglyceride level was higher in the group given lasalocid, compared to the groups given salinomycin and maduramycin. The elevation of triglyceride levels is firstly associated with diet, but may also be observed in diseases of the liver, pancreas and kidneys (8). High triglyceride levels observed in the group given lasalocid may be attributed to degeneration of the liver, kidneys and pancreas.

When ionophores are administered at high doses, they cause the release of neuromediators, mainly noradrenalin, as a consequence of processes resulting in the accumulation of ions, such as sodium and calcium, in the cell, as well as the acceleration of intracellular oxidation processes, and thereby, degenerative disorders of tissues and organs, as a result of the generation of active oxygen groups, that may end in necrosis (4). Karabacak (7) reported that, the administration of salinomycin (60 ppm), lasalocid (100 ppm) and maduramycin (5 ppm) into broiler rations at treatment doses for a period of 42 days induced lipid peroxidation (in the groups given salinomycin, lasalocid and maduramycin MDA and NO levels were observed to increase, whilst CAT and GSH-Px activities were observed to decrease). The alterations determined in the biochemical parameters investigated in the present study can also be attributed to the degenerative disorders caused by the active oxygen groups generated by ionophores.

In a previous research conducted on the effects of grape seed in poultry, Wang et al. (15) ascertained that, when added to broiler rations at doses of 5, 10, 20, 40 and 80 mg/kg, a grape seed proanthocyanidine extract reduced the severity of coccidiosis-induced lipid peroxidation. Brenes et al. (3) reported that the addition of a grape seed extract to broiler rations at doses of 0.6, 1.8 and 3.6 g/kg for a period of 42 days increased antioxidant activity in both the feed ration and faeces. In the present study, in the group that received a combination of grape seed and lasalocid, ALT and ALP activities and triglyceride levels; in the group that received a combination of grape seed and salinomycin, ALT activity and triglyceride levels, and in the group, which was given grape seed in combination with maduramycin, ALT and ALP activities, triglyceride, uric acid and creatinine levels were determined to be decreased ($P<0.05$). The results obtained in the present study demonstrated that supplementation of the ration with grape seed resulted in amelioration on the biochemical parameters investigated, and thereby, suggest that grape seed may alleviate the toxic effects of ionophores. These effects of grape seed could be attributed to the radical scavenging activity and regulatory effect on the antioxidant/oxidant balance of the substances it contains, which have high antioxidant capacity (5, 16).

In conclusion, it was determined that, grape seed included in broiler rations at a rate of 0.5% for a period of 42 days did not cause any significant alteration in the biochemical parameters investigated, whilst the administration of lasalocid,

salinomycin and maduramycin at the recommended doses in feed induced adverse effects on liver and kidney functions and fat metabolism. However, the results obtained at the end of study demonstrated that the combined use of ionophore antibiotics and grape seed could reduce the adverse effects of ionophores.

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