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Physiological and histopathological effects of nettle seed (Urtica pilulifera), grape seed (Vitis vinifera), flaxseed (Linum usitatissimum) in broiler

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ABSTRACT

Objective: In this study, it was aimed to investigate the effects of nettle seed, grape seed and flaxseed added to the broiler diet on body weight, electrocardiogram, hematological and histopathological parameters.

Materials and Methods: In this study 40 daily Ross 308 female broilers were used. Chicks were divided into 4 groups of 10 chicks each. During 42 days, in addition to standard broiler feed, nettle seed, grape seed and flax seed with 30 gr/kg/day were added to groups II, III and, IV respectively. *The values of* erythrocyte (RBC), leukocyte (WBC), platelet (PLT), hematocrit (HCT), hemoglobin (Hb) and percentages of white blood cells were determined by conventional methods. The liver, kidney, spleen and lung tissues held in formaldehyde (10%), were passed through the alcohol and xylose serial in routine tissue tracking and were buried in paraffin blocks. They were prepared on the lam and stained with Hematoxylin-eosin (HE) and examined by light microscopy.

Results: It was determined that body weight was higher in the control group than those in the other groups in the second weighing and there was a decrease in the number of heart beat in group IV ($p \leq 0.05$). There was no significant difference with regard to hematological and histopathological findings.

Conclusion: Even if the additional nettle seed, grape seed and flaxseed (30 mg/kg) to broiler rations did not have any positive effect on body weight gain, it is also important that it has no negative effect on blood, heart, liver, kidney, lung and spleen.

Keywords: *Body weight, Electrocardiogram, Blood parameters, Tissue examination, Broiler, Nettle seeds, Grape seeds, Flax seed*

INTRODUCTION

Nowadays, it is very important to supply the increased food need, and animal foods are becoming a necessity to breed healthy generations and diets. Chicken meat, starting to get ranked first among animal foods, come at the beginning of food that people need for adequate and proper nutrition. There are some studies in which the effects of different plants were searched in order to maintain body weight gain healthily without changing the animal genetics and disordering its health (Brenes et al., 2010).

Many antibiotic alternatives with growth-promoting effects, such as herbs, are of interest to researchers (Omerovic et al., 2016). Stinging Nettle is one of the medicinal plants that has recently attracted attention as a phytogenic feed additive in poultry diets and has a long history of traditional medicinal use in many countries. (Ahmed and Parsuraman, 2016).

The cardiovascular effects of dietary flaxseed include an antihypertensive effect, an anti-atherogenic effect, a decrease in cholesterol, an anti-inflammatory effect, and suppression of arrhythmia. Although flaxseed is not very well known, it also consists of other potential biologically active compounds, such as proteins capable of exerting a biological effect, cyclinopeptides and cyanogenic glycosides. These compounds may also be responsible for the cardiovascular effects of flaxseed (Parikh et al., 2018).

Flaxseed and oil, nettle, grape seed and pomace have been used among plant-derived feed additives. Some researchers utilized flax seed and oil as feed additives to be able to maintain cardiovascular health by enriching poultry meat with omega-3 fatty acids (Chanmugam et al., 1992). Although poultry meat is preferable because of containing less fat, it can cause malnutrition in terms of polyunsaturated fatty acids (omega-3). Omega-3 fatty acids are effective in the prevention of coronary heart disease, cancer, arteriosclerosis and diabetes in people. Therefore, the number of studies, directed toward the enrichment of poultry meat with omega-3 fatty acids for adequate and proper nutrition of humans have increased.

Flaxseed is a rich source of α-linolenic acid (18:3 n-3). Feeding broilers flaxseed can increase n-3 fatty acids in meat tissues. However, non-starch polysaccharides in flax seed decrease nutrient digestibility and can have a negative impact on bird performance and muscle fatty acid content. The addition of carbohydrase enzymes to flax-based broiler diets can decrease the anti-nutritive effects of non-starch polysaccharides. (Apperson and Cherian, 2017).

Nettle contains formic acid, a high amount of chlorophyll, flavonoids, plant sterols, plant enzymes, phenylpropanoids, coumarins, terpenoids, potassium salts, vitamin C, polysaccharides, plant lignane, and lectin agglutinins with low molecular weight in their roots (Akbay et al., 2004). It was shown that there are many effects of polyphenols-rich grape pomace and seed, and phenolic compounds extracted from grape seed in some studies (Brenes et al., 2010).

The effects of active substances obtained from all medicinal and aromatic plants on poultry should be evaluated as a result of analyzes and commercials should be tested on animals alone or in combination, they should be added to the economy by mass production and contribute to the use of different feed additives (Kutlu ve Şahin, 2017).

Recently, the importance of some plant extracts in animal nutrition (Zajac et al., 2020; Saeed et al., 2021), hematological (Taş et al., 2011; Comba et al., 2014; Comba et al., 2016; Zajac et al., 2020; Comba et al., 2020; Abdul-Majeed, 2021; Çelik et al., 2022; Vadi and Comba, 2023) and histopathological (Comba et al., 2017; Yıldırım et al., 2017; Mis et al., 2018) studies on the effects of parameters are available in the literature. In addition, the effects of flaxseed 0%, 2.5%, 5.0%, 7.5% and 10% on growth performance in broiler chickens were investigated (Mridula et al., 2015). But, no studies were encountered in which the effects of giving the same amount (30 mg/kg) of nettle seeds, grape seed and flax seed in addition to broiler ration on body weight, electrocardiograms (ECG), some hematological and histopathological parameters were researched. In this study, it was aimed to investigate the effects of additional doses of nettle, grape and flax seed with 30 mg/kg which are used to improve *feed* efficiency and performance in poultry farming to the standard broiler ration on body weight, ECG, hematological and histopathological parameters.

MATERIALS and METHODS

Study Groups

This study was carried out with the decision of the Atatürk University Faculty of Veterinary Medicine Unit of Ethics Committee (2018/41). A total of 40 Ross 308 female broiler chicks were used in this study. Standard temperature (23±2 °C), humidity (50%±10) and light (23/1 h light/dark) were applied by providing appropriate conditions in the stell cage. Chicks were divided into 4 groups of 10 chicks each as control and experimental groups. The diets and drinking water were provided ad libitum. Additionally, nettle seed (30gr/kg/day), grape seed (30gr/kg/day) and flax seed (30gr/kg/day), were given to groups II, III and IV respectively, during 42 days, taken from local herbalists in Turkey. The samples were chopped into small parts with a blender. Compositions of all supplementary foods were determined according to AOAC (1994). The diets were isoenergetic and isonitrogenous. All the experimental diets were formulated to meet the minimum nutrient requirements of broilers (NRC, 1994). They were weighed every 10 days during the study period. After 42 days of work, Electrocardiograms (ECG) and blood, liver, kidney,

spleen and lung tissues were taken for all groups. The tissues were taken in formaldehyde (10%) and were examined histopathologically.

ECG

ECGs were taken by a device *(Cardio fax 6851, Nihon Kohen, Tokyo)* with an *electrode alligator Clip*. Electrodes were placed where *M. gastrocnemius* at the lower ends of the right and left legs and forepart of wing-body linkup of chickens are connected, after the gel was applied, in a quiet and dim environment. Animals were waited to sedate by wrapping lightweight cloth for 5 minutes. ECGs were taken at a deflection of 1mV = 10mm and a rate of 50 mm / sec. The derivations of Bipolar (I, II, III) and augmented unipolar (aVR, aVL, aVF) extremities were printed. The evaluation of duration and amplitudes of the waves in the trace was performed in the derivation of II, as calculation of the electrical axis was performed in the derivation of II and III (Figure 1).

Blood Collection

After ECGs were taken, blood samples from V. Subcutaneous of chickens were taken in an EDTA tube. The numbers of Erythrocyte (RBC), leukocyte (WBC) and platelet (PLT), hematocrit values (Hct) and the amount of hemoglobin (Hb) were estimated by hemocytometric method using Natt-Herrick solution, by micro hematocrit method and by Sahli's acid hematin method spectrophotometrically, respectively. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular *Hemoglobin Concentration* (MCHC) were calculated with Wintrobe's formulas red cell indice. Blood smears prepared from specimens were stained with the Grunewald-Giemsa staining method and percentages of white blood cells were determined.

Tissue collection

The liver, lung, kidney and spleen tissues of chickens were held in 10% formalin solution for histopathological examinations. After the tissues were fixed in 10% formalin solution for 48 hours, they were washed in running tap water for 10 minutes. Then they were passed through alcohol (70°, 80 °, 90 °, 96 ° and 100 °) and xylol series in routine tissue tracking and they were buried in paraffin blocks. A 4-*micrometer-thick cross-section* was received from each block and it was prepared on the lam for histopathological examinations. Preparations were stained with Hematoxylin-eosin (HE) and examined by light microscopy.

Statistical Analysis

Descriptive Statistics were expressed as Average, and Standard Deviation for the features studied. Kruskal Wallis test was used to compare the groups in terms of these characteristics. The Friedman test was used to compare the times within the groups. The statistical significance level in calculations was taken as 5% and SPSS statistical package program was used for calculations.

RESULTS

The intra-group means and standard deviations of the live weights (Table 1), electrocardiographic measurements (Table 2) and hematologic values (Table 3) were given for all groups. The liver and kidney (Figure 2), lung and spleen tissues (Figure 3) were examined histopathologically.

According to Table 1, there is no significance among the groups in the 1st and 4th weighing ($p \geq 0.05$) while the control group was found to have statistically high body weight compared to the other three groups in the 3rd weighing ($p \leq 0.05$). In addition, at the 2nd weighing the body weight in the control group was found higher than in the group IV ($p \leq 0.05$).

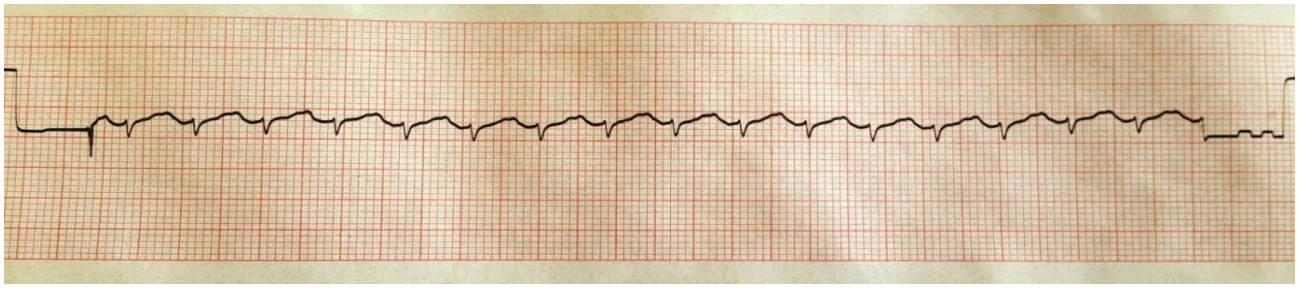

Figure 1. Electrocardiography (1mV and 50 msn) in chickens added flax seed to their feed.

Table 1. The average body weights with standard deviations for all groups (g).

Parameters	Group I (Control) X ± SD	Group II (Nettle seed) X ± SD	Group III (Grape seed) X ± SD	Group IV (Flax seed) X ± SD	p
1.weighing (1.day)	65±4.15	63±5.24	61±4.46	59±5.52	≥0.05
2.weighing (10. days)	132.10±10.24[a]	126.6±11.41[ab]	123.1±9.54[ab]	118.2±8.75[b]	≤0.05
3. weighing (20. days)	540.5±50.74[a]	448.3±46.68[b]	464.7±60.52[b]	445.3±55.46[b]	≤0.05
4. weighing (30. days)	1046.7±112.45	1011.7±128.51	1016.3±100.36	984.3±97.35	≥0.05
5.weighing (42. days)	2300.0±162.45	2240.0±155.68	2250.0±169.15	2283.5±175.23	≥0.05

a,b: The differences among groups carrying different letters in the same column are statistically significant ($p<0.05$).

Table 2. Mean and standard deviations of some ECG parameters in all groups.

	Group I (Control) X ± SD	Group II (Nettle seed) X ± SD	Group III (Grape seed) X ± SD	Group IV (Flax seed) X ± SD	p
No of heart beats per min	373±25[a]	355±25[a]	350±28[a]	310±23[b]	≤0.05
QRS wave (sec)	0.027±0.07	0.028±0.06	0.028±0.07	0.029±0.06	≥0.05
QRS wave (mV)	0.25±0.08	0.26±0.06	0.25±0.07	0.24±0.07	≥0.05
T (P+T) wave (sec)	0.048±0.014	0.049±0.013	0.049±0.013	0.051±0.013	≥0.05
T (P+T) wave (mV)	0.020±0.08	0.020±0.07	0.019±0.06	0.020±0.07	≥0.05
Q-T interval	0.13±0.018	0.13±0.017	0.12±0.016	0.14±0.017	≥0.05
Mean electrical axis of the heart	-82(-60- -120)	-90(-55- -120)	-85(-58- -115)	-84(-55- -118)	≥0.05

a,b: The differences among group averages carrying different letters in the same lines are statistically significant

Table 3. Mean and standard deviations of some hematological parameters in all groups.

Parameters	Group I (Control) X ± SD	Group II (Nettle seed) X ± SD	Group III (Grape seed) X ± SD	Group IV (Flax seed) X ± SD	p
WBC (10^3/mm^3)	14.42±2.45	15.59±2.34	13.24±1.84	15.78±1.96	≥0.05
Lymphocyte (%)	70.4±8.4	70.2±10.2	71.6±6.7	69.9±8.4	≥0.05
Monocyte (%)	5.5±1.4	5.8±1.2	5.1±2.4	6.4±2.1	≥0.05
Pseudo Eosinophil (%)	16.2±3.1	16.4±4.2	16.6±4.7	15.6±5.3	≥0.05
Eosinophil (%)	7.1±2.3	6.6±1.4	6.4±1.6	7.6±2.1	≥0.05
Basophils (%)	0.5±0.04	0.6±0.01	0.5±0.05	0.4±0.07	≥0.05
RBC (10^6/mm^3)	1.81±0.24	1.86±0.57	1.77±0.38	1.93±0.48	≥0.05
HCT (%)	19.75±1.72	19.94±2.61	18.6±1.82	20.84±2.26	≥0.05
Hb g/dl	8.42±0.53	8.06±0.74	8.12±0.92	8.66±0.71	≥0.05
MCV (fl)	108.42±2.74	107.64±3.14	109.22±2.36	110.2±3.52	≥0.05
MCH (pg)	45.64±2.45	43.72±3.64	45.04±3.52	44.76±3.26	≥0.05
MCHC (g/dl)	41.26±1.24	41.28±1.47	42.74±1.25	40.84±2.13	≥0.05
THR (10^3/mm^3)	40.25±5.61	41.12±7.35	41.3±6.53	40.02±7.42	≥0.05

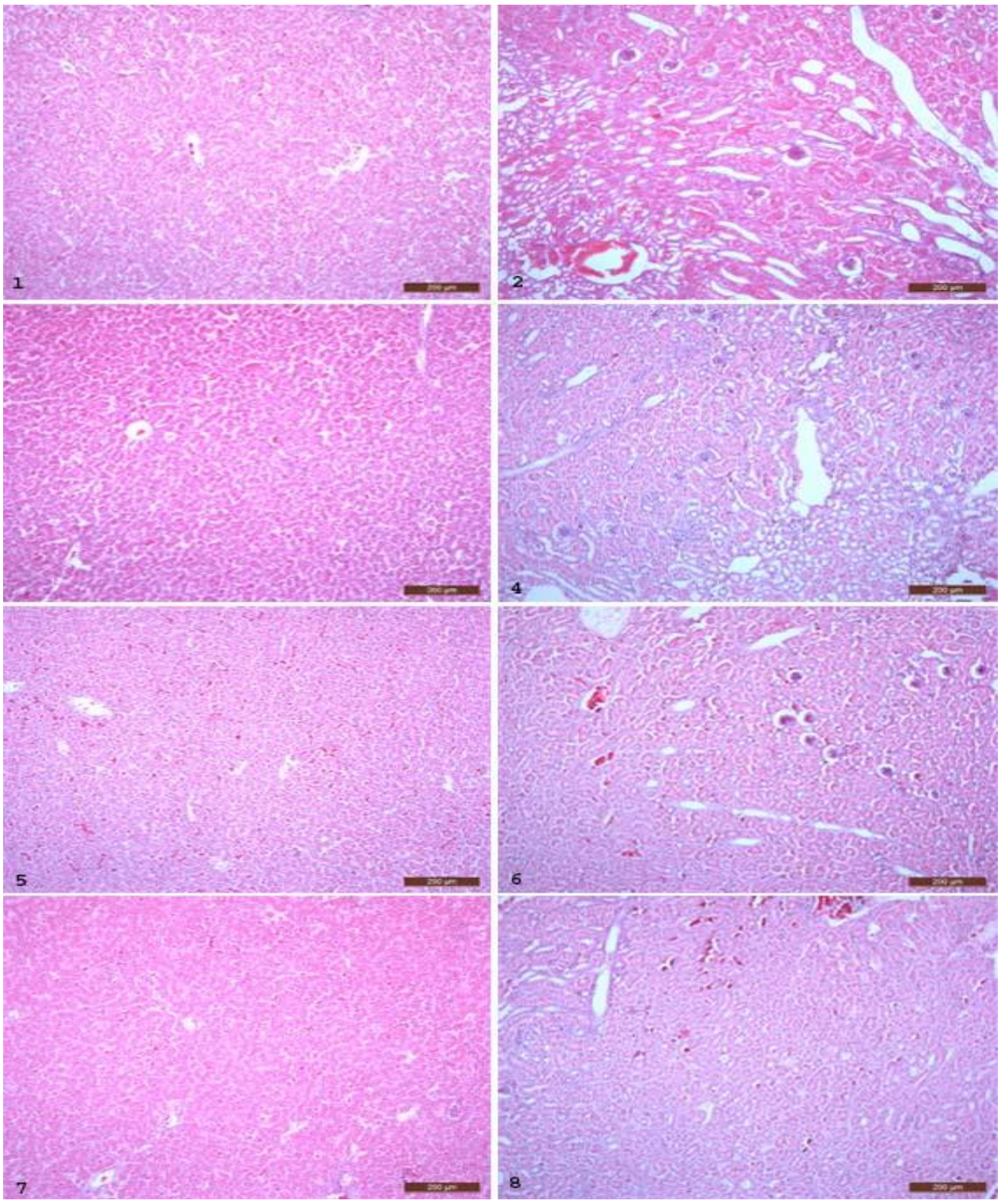

Figure 2. The normal histological structures of the liver and kidney tissues of broiler chickens in Group I (control group) (1-2), the normal histological structures of liver and kidney tissues of broiler chickens in Group II (nettle) (3-4), the normal histological structures of the liver and kidney tissues of the broiler chickens in Group III (grape seed) (5-6), the normal histological structures of the liver and kidney tissues of the broiler chickens in Group IV (flax seed) (7-8). Bar: 200 μm HxE

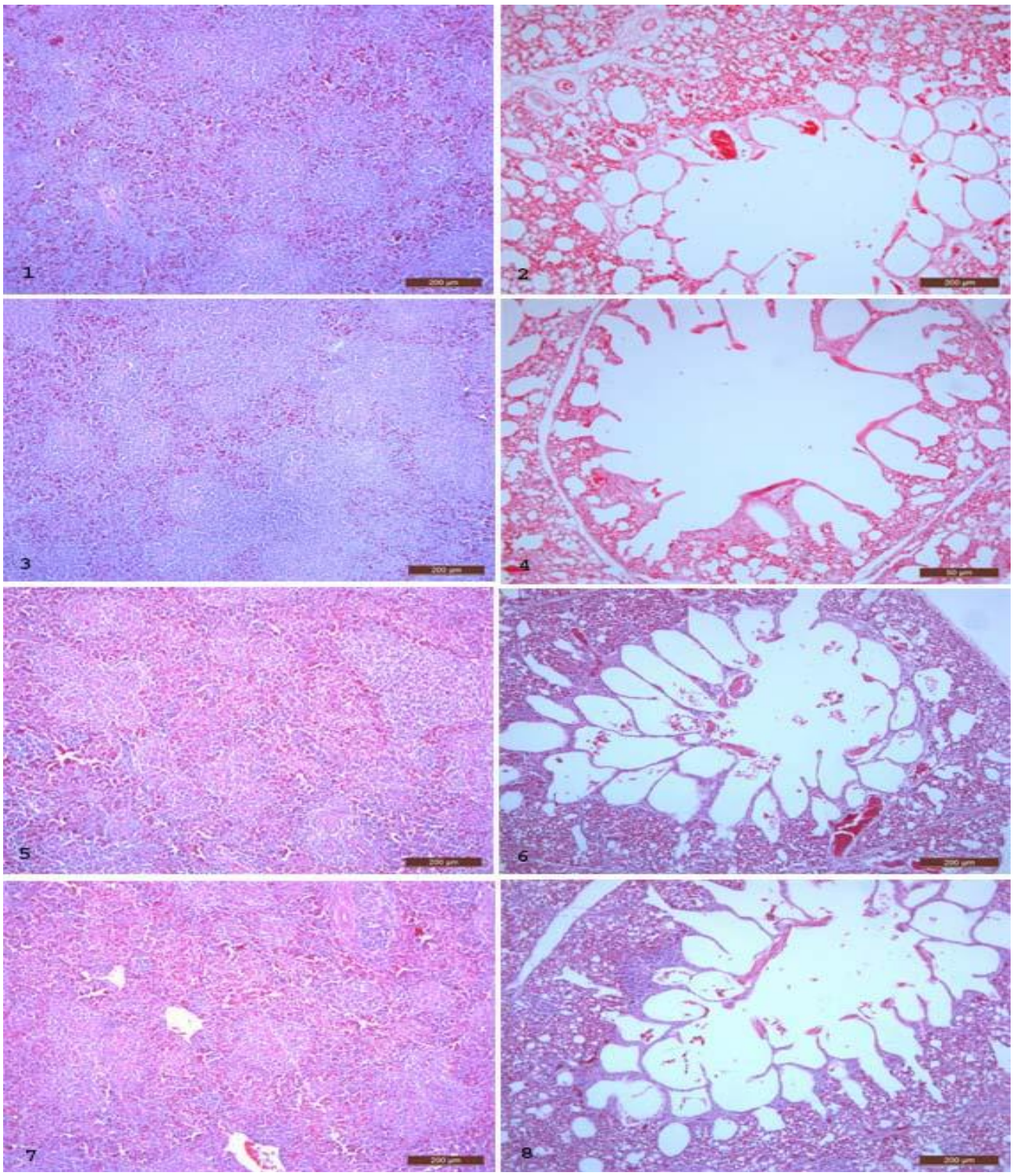

Figure 3. The normal histological structures of the spleen and lung tissues of broiler chickens in Group I (control group) (1-2), the normal histological structures of spleen and lung tissues of broiler chickens in Group II (nettle) (3-4), the normal histological structures of the spleen and lung tissues of the broiler chickens in Group III (grape seed) (5-6), the normal histological structures of the spleen and lung tissues of the broiler chickens in Group IV (flax seed) (7-8). Bar: 200 μm HxE

The number of heartbeats in group IV was lower than those in other groups according to Table 2 ($p \leq 0.05$). A significant difference cannot be found in the other ECG parameters among the groups ($p \geq 0.05$).

According to Table 3, there was no significant difference between the control and experimental groups in terms of hematological parameters.

Histopathological findings

It was not encountered with any toxicological findings as the results of necropsy and histopathological examination of liver, lung, kidney and spleen tissues. In all groups, remark cords were in normal sequence, the sinusoidal spaces were regular no pathological conditions were found in the portal and central regions in the livers (Figures 2-1, 3, 5, 7). Bronchus, bronchial, and alveoli had a normal histological appearance in the lungs in all groups (Figures 3-2, 4, 6, 8). There were not any pathological conditions in the kidney, tubules or glomerulus in all groups (Figures 2-2, 4, 6, 8). It was seen that follicular structures were protected in the spleen in all groups (Figures 3-1, 3, 5, 7). There were not any histopathologic changes among the groups with regard to histopathological evaluation.

DISCUSSION

The inclusion of nettle in the diet significantly reduced liver, heart, bursa and abdominal fat compared to the control. Ahmadipour and Khajali (2019) reported that when 1% and 1.5% nettle were included in broiler rations, body weight gain was significantly improved, but feed intake did not change significantly. In another study (Hashemi et al., 2018), in which stinging nettle leaf extract powder was added to broiler rations at 0.15%, 0.20 and 0.25% levels, it was stated that it resulted in significantly better growth. Flaxseed supplementation did not affect the weekly body weight of broiler chicks during the first three weeks, but thereafter it reduced significantly with increasing levels of flaxseed in the diets. Birds fed on 10% flaxseed showed a reduction of 10.08% in body weight as compared to the control group. Diets containing 5.0–7.5% flaxseed resulted in significantly lower weight gain, higher feed conversion ratio, energy efficiency ratio and lower protein efficiency ratio as compared to control and 2.5% flaxseed diets. (Mridula et al., 2015).

In the present study, it was determined that nettle seed, grape seed, and flax seed extracts added to the standard ration affected the body weight averages between the groups in the second and third weighing, but did not affect the first and fourth weighing. These weighing values with 10-days intervals were determined in accordance with the literature (Eser et al., 2012).

Nettle supplemented in broiler diets exerts positive effects regarding production performance. Nettle provides nutrients and bioactive components, which stimulate growth and feed utilization, modulate metabolic processes and support immune system in broilers (Milosevic et al.,2021). In a study conducted by Al-Salihi et al., (2018) with the extract of nettle leaves added to drinking water at a concentration of 10, 15 and 20 ml/l, the immunostimulatory effect of stinging nettle in broilers was reported. Hashemi et al. (2018) reported a positive effect of nettle on hemoglobin, hematocrit, and blood content in broiler chickens fed increasing amounts of nettle. Furthermore, nettle extract can stimulate the innate cell mediated immune reaction by lymphocyte propagation in laying hens (Sandru et al., 2007). In another study flaxseed (15%) treatments did not induce changes in the levels of such indices as RBC, MCHC, MCH, MCV, and PCV. In contrast, the dietary inclusion of flaxseeds to the diet decreased the hemoglobin level in comparison with the control (by 9%) and the other treatments (Zajac et al., 2020). Furthermore, adding nettles (0.25 or 0.50 %/kg diet) showed that crushed nettle plant led to an increase in the values of packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, and it's shortened clotting time, as well as a significant increase ($p \leq 0.05$) in the number of basophils compared to the control group (Abdul-Majeed at al., 2021). In our study, the change in blood parameters was not statistically significant. We think that this situation may be due to the dose difference as well as related to the way the substance is administered.

It is known that factors such as race, age, gender, pregnancy, lactation, muscle activities, region, season, ambient temperature, care and nutrition have an effect on hematologic parameters, ECG values, growth and development in animals (Belge et al., 2003; Çınar et al. 2006). The taste, flavor and consumption amount of the feed additives are among the significant factors affecting the quality of feed utilization in poultry feeding (Çınar and Dönmez, 2001). It has also been reported that polyphenols (flavonoid, anthocyanin, elagi-tannin, proanthocyanidin) can adversely affect the performance of animals by reducing the absorption of fat and proteins by composing complexes with macromolecules (such as fat and protein) and enzymes (protease and lipase) in the digestive tract (Manach et al., 2004).

It was declared that the addition of 0.6, 1.8 and 3.6 % of grape pomace extracts (Brenes et al., 2010), and 0.5, 1 and 2 % of milled grape seeds or 200, 400 and 800 ppm grape seed extracts (Turan ve Öztürk,,

2010) to broiler feed did not affect body weight, feed consumption or the rate of feed utilization. Hughes et al. (2005) reported that the addition of 0.2, 0.5 and 1.0% of grape seeds to broiler feed did not have a negative effect on feed intake and body weight but the addition of 3.0% of that reduced these parameters cumulatively. Brenes et al., (2010) stated that high levels of consumption of polyphenols in the grape seed can cause adverse effects on animal health and performance. Our results have supported the literature in terms of there were no differences for in ECG, blood and histopathological parameters between the control and added grape seed group (Hughes et al., 2005; Turan and Özturk, 2010; Brenes et al., 2010).

Cardiovascular disease remains the leading cause of mortality and morbidity worldwide. The inclusion of functional foods and natural health products in the diet are gaining increasing recognition as integral components of lifestyle changes in the fight against cardiovascular disease. Several preclinical and clinical studies have shown the beneficial cardiovascular effects of dietary supplementation with flaxseed (Parikh et al., 2018).

Dietary flaxseed protects against ventricular fibrillation induced by ischemia-reperfusion in normal and hypercholesterolemic Rabbits. (Ander BP et al., 2004). It was reported that the addition flaxseed oil at different levels to broiler ration did not affect feed consumption, body weight gain, and the feed utilization ratio (Manilla et al., 1999; Çebi, 2010). However, Kralik et al. (2003) detected that the addition of 13.5% of flax seed to broiler increased the levels of mono-unsaturated fatty acids and α-linoleic fatty acids in breast meat and abdominal fat while it decreased the level of saturated fatty acids significantly. Furthermore, they expressed that the ratio of omega-6/omega-3 in breast meat and abdominal fat decreased significantly. Similarly, Lopez-Ferrer et al. (2001) detected that the addition of flax seed oil reduced the amount of saturated fatty acids. Reducing blood LDL significantly, makes using of flax seed oil important in poultry.

Clinical trials in humans have not found any changes in platelet aggregation with flaxseed. Edel et al. (2016) also reported no change rate and degree of increased platelet aggregation by increasing the dose of flaxseed from 10 to 40 g/day in food. Another study also demonstrated flax seed prevents leukocyte and platelet adhesion to endothelial cells in rats (Haliga et al., 2013). In another clinical study, it was reported that a flaxseed diet for 12 months had no effect on heart function, heart rate and heart diastolic duration. (Caligiuri et al., 2014).

In this study, no differences were observed between the control group and groups that *flaxseed* was added to their *feed* in terms of blood and tissue values. Only the number of heartbeats was lower than the control group (Table 2) but this value of 310±23 is similar to values reported for poultry in literature (Emre et al., 1994; Çınar et al., 1996; Çınar et al., 2006) and it was among the reference values.

In this study, the averages of the mean electrical axis of the heart were found as -82 (-60-120), -90 (-55- -120), -85 (-58- -115) and -84 in groups (I, II III and IV), respectively. These values show parallelism those were reported by Çınar et al., (1996), Çınar and Dönmez (2001) and Çınar et al., (2006). It can be interpreted as the heart proceeds forward and right in the broilers studied. It has been shown that the incidence of subclinical heart diseases is high in fast-growing broilers and that the deaths occurring in relation to these diseases lead to significant economic losses (Baghbanzadeh and Decuypere, 2008). Blood parameters are related to the health status of the animals (Çınar et al., 2006), and have a diagnostic importance in assessing the general state of the animal.

CONCLUSION

In conclusion, even if the additional nettle seed, grape seed and flaxseed (30 mg/kg) to broiler rations did not have any positive effect on body weight gain, it is also important that it has no negative effect on blood, heart, liver, kidney, lung and spleen. This state indicates that these substances do not cause any pathological circumstances in the case of adding to broiler ration and these can be used physiologically. The results suggest that the presence of these substances at certain levels is necessary for blood production and normal metabolic activities in the animals and these have positive effects on carcass quality and substantiality.

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