The effects of humic acid addition to ration on the fattening performance and some oxidative stress parameters in Anatolian Merino lambs

Research Article

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Article info
Submission: 15-06-2021
Accepted: 31-01-2022
Publication: 30-04-2022

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
- http://dergipark.org.tr/vetbio

ABSTRACT

In this study, it is aimed to determine the effects of adding humic acid to ration of Anatolian Merino lambs on the fattening performance and some oxidative stress level by using thiol / disulfide balance measurement method. In the study, singleton 32 male Anatolian Merino lambs with an average age of 3 months were used. The experiment was carried out by forming a control group without additives and totally four trial groups, three of which were added with humic acid (2, 4, 6 g/kg), with 8 replications in each group for a total of 70 days. The lambs housed in the group partitions were fed with 400 g/day/head of alfalfa grass as roughage and lamb grower feed ad-libitum containing 2750 kcal/kg DM ME, 16% CP until the end of the trial. At the end of the research; intra-group native thiol (NTL, µmol/l) values increased on the 30th day ($P<0.05$) in all groups except the control group, while total antioxidant status (TAS, mmol/l), total thiol (TTL, µmol/l) and disulfide values increased on the 60th day in all groups including the control group ($P\leq0.001$) was observed. In terms of lambs' feed consumption, live weights, body weight gains, feed conversion ratios, TAS, total oxidant status (TOS, µmol/l), oxidative stress index (OSI), TTL, NTL and disulfide was found that there was no significant difference between groups ($P>0.05$). It was concluded that the humic acid additive was not effective on the fattening performance, but 4 or 6 g/kg could be added to the lamb rations due to the increase in thiol groups, which have an important role in the antioxidant defense system.

Keywords: Humic acid, lamb, oxidative stress, ruminant, thiol/disulfide balance

INTRODUCTION

Various organic acids are used in livestock rations to increase the acidity of the feeds and prevent the deterioration of the feed, to maintain the balance between pathogens and beneficial microorganisms in the digestive system, to improve the digestion and absorption of ingested nutrients and to promote growth. Humic acid is one of these organic acids (Islam et al., 2005; Váradyová et al., 2009). These compounds originate from humus, which is formed by some substances such as carbohydrates, amino acids and phenols, which are released by the decay and decomposition of organic materials in the soil over time (Gau et al., 2001; Ying et al., 2001). They are defined as complex organic substances that are composed of humic, fulvic acid and some micro minerals, which can transfer electrons due to their chemical properties and can be chelate with many metal ions thanks to these properties (Ritchie and Perdue, 2003). In their natural state, they are insoluble in water and are not biologically active. The salts they form with the elements sodium, potassium and nitrogen are called "humate". Humates are soluble in water and biologically active (Eren et al., 2000; Küçükersan et al., 2005).

How to cite this article
Budak, D., Daş, BD., Özkurt, G., Halıcı İ. (2022). The effects of humic acid addition to ration on the fattening performance and some oxidative stress parameters in Anatolian Merino lambs. *Journal of Advances in VetBio Science and Techniques, 7*(1), 89-89. https://doi.org/10.31797/vetbio.1056653

The use of humic acids in animal feeds started with some humic acid preparations developed for the treatment of diarrhea and digestive disorders in calves, pigs, cats and dogs between 1980-1990. However, it is seen that humic acid products, which are known to be an important and effective substance in plant nutrition, are used to increase the efficiency of feed and drinking water of various animals (Demirulus, 2011).

The growth rate and yield potential of lambs are directly proportional to the feed efficiency level. Poor care and feeding conditions are effective in the deterioration of the oxidant-antioxidant balance of the organism and the formation of oxidative stress. This causes a decrease in the growth performance of the developing young ruminants (Altınçekiç, 2016; Serin, 2015). Oxidative stress can be defined as the deterioration of molecular and cellular functions as a result of the loss of the balance between the body's antioxidant defense and the production of free radicals that cause peroxidation of the lipid layer of the cells. Under oxidative stress, damage occurs to biomolecules such as lipids, proteins, and DNA. Free radicals; oxidized bases cause a variety of tissue damage, including DNA chain breaks and DNA-protein crosslink formation (Yokuş and Çakır, 2002). Oxidative damage caused by the rise of reactive oxygen species above physiological levels and the increase in the production of free radicals leads to damage to cell membrane lipids and weakening of cellular protein functions (Devasagayam et al., 2014; Pratic`o, 2005; Valko et al., 2004). Under normal conditions, there is a balance between free oxygen radicals and radical toxicity and the production of a protective antioxidant system. Oxidative stress, which occurs as a result of the disruption of this balance between antioxidants and oxidants in favor of oxidants, is a part of the mechanisms of cellular and molecular tissue damage in diseases (Celi, 2011). In the antioxidant defense system of the organism against free radicals, first of all, enzymatic or non-enzymatic antioxidant mechanisms in the cells come into play. Damage caused by radicals is prevented in the body by the enzyme systems of superoxide dismutase, catalase and glutathione S-transferase, as well as important biological thiols such as glutathione, cysteine, homocysteine, N-acetylcysteine, ɣ-glutamylcysteine. Thiols, also known as mercaptans, are organic chemical compounds containing hydrogen and sulfur atoms and sulfhydryl (-SH) groups attached to the carbon atom, which show antioxidant properties in preventing the formation of any oxidative stress state (Erel and Neşelioğlu, 2014; Sen and Packer, 2000; Turell et al., 2013). Thiols are involved in oxidation reactions via oxidants. They form covalent S-S bonds between the sulfhydryl groups of two cysteine amino acids in the structures of proteins. These are called disulfide bonds (Cremers and Jakob, 2013). Native thiols are molecules that contain an unreduced functional thiol group. They are responsible for the antioxidant defense mechanism. When oxidative stress increases, their amount decreases. Total thiol represents the thiol/disulfide level in equilibrium and total of oxidized-unoxidized thiols. Under oxidative stress conditions, lead to the formation of reversible disulfides bonds between oxidative residues of cysteine, low molecular thiol and protein thiol groups. The disulfide bonds formed can separate into thiol groups again. Thus, dynamic thiol/disulfide equilibrium can be achieved. It has a critical role in many cellular activities such as dynamic thiol/disulfide balance, antioxidant protection mechanism, enzymatic activity and cell growth. Today, it is a marker associated with many diseases in the medical field (Erel and Neşelioğlu, 2014). Thiol groups of sulfur-containing amino acids such as cysteine and methionine in proteins are the primary target point of reactive oxygen species. Oxidation of reactive oxygen species and thiol groups into

reversible disulfide bonds is the first manifestation of protein oxidation. Biological importance of thiols and disulfides; It can be explained by the preservation of the structure of proteins, the regulation of protein and enzyme functions, their roles in receptors, transporters and transcription (Ateş et al., 2015).

In this study, it was aimed to determine the effects of humic acid addition to the ration on fattening performance and some oxidative stress parameters by thiol/disulfide balance measurement method in Anatolian Merino lambs (TUIK, 2021), an important breed whose breeding is quite common in Turkey.

MATERIAL and METHOD

In the study average age of 3 months, singleton Anatolian Merino lambs were used as animal material. The research was carried out in May-June, when the average daily air temperature was 20-25°C. 32 Male lambs were selected from the Anatolian Merino herd in the farm, from male lambs with the same birth time and body weight as possible. The roughage material of the study consisted of dry alfalfa hay and the concentrate feed material consisted of lamb grower feed containing 2750 kcal/kg DM ME and 16% CP. A.O.A.C. (1984) was used as the method of determining the values of dry matter (DM) crude protein (CP), crude cellulose (CC), ether extract (EE) and crude ash (CA); Van Soest (1994) procedure was followed for determining the amounts neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Metabolic energy (ME) (Anonymous, 2016), non-nitrogen extract (NNE=DM- (CP+CA+EE+CC)), cellulose (CL=ADF-ADL) and hemicellulose (HCL=NDF-ADF), values were derived from the analysis results on feed materials through calculation. The analysis results of the chemical compositions of the roughage and lamb grower feed used in the ration are given in Table 1.

Table 1. Chemical composition of feeds (%)

Nutritions	Lamb grower feed	Alfalfa hay
ME, kcal/kg DM	2750	1.38
DM	88.73	93.29
CP	16.00	9.17
CC	7.14	37.42
EE	2.89	0.99
CA	7.65	11.33
NNE	54.55	41.09
NDF	29.24	53.61
ADF	9.70	42.43
ADL	0.90	9.76
CL	8.8	32.67
HCL	19.54	11.18

The trial was carried out for a total of 70 days to reach the intended statistical data, the first 10 days of which was the adaptation to feed period, and the 60 days of the fattening period. At the beginning of the trial, the lambs were classified according to their first weighing body weights and divided into 4 groups homogeneous with 8 animals in each group randomly distributed. Group feeding was applied to the lambs. Health control of the lambs were also performed in the adaptation period. Trial groups were fed st group (no additive control group), 2^{nd} group (2 g/kg humic acid added), 3^{rd} group (4 g/kg humic acid added), 4^{th} group (6 g/kg humic acid added) with concentrated feed. While concentrated feeds were given ad-libitum, the excess feeds in the feeders were collected and weighed every

two weeks. Alfalfa hay used as roughage was given by weighing 400 g per animal per day, and at the end of two-week periods, the increased amounts in the manger were collected, weighed and recorded. Fresh and clean drinking water was always available in front of the lambs.

Body weights were determined individually by weighing every two weeks until the end of the trial, the first of which was at the beginning of the trial period. Weighings were made at the same hours (08:00) before the morning feeding. In each weighing period, the excess feed in front of the animals was collected and weighed, and the amount of feed consumed by removing from the amount of feed offered to the animals was recorded.

At the beginning (day 0), middle (day 30), and end (day 60) of the trial, blood samples were taken from the jugular veins into tubes with anticoagulants, from all lambs before the morning feeding. These blood samples were collected in flat gel tubes (Becton Dickinson and Company, New Jersey, USA), all samples were centrifuged at 3000 rpm for 10 minutes and stored at -80°C until analysis. Then, enzymatic and non-enzymatic measurements of all antioxidant and oxidant molecules were made in these blood samples with native thiol (--SH), total thiol (--SH+-- S--S--), Total antioxidant status (Total Antioxidant Level - TAS), Total oxidant status (Total Oxidant Level -TOS) kits (RelAssay Diagnostic, Turkey) (Erel and Neşelioğlu, 2014). The disulfide level was calculated with the formula (serum total thiol - serum native thiol)/2. All results are reported as micromoles per liter (µmol/l), TAS millimoles (mmol/l) (George and Hero, 1979).

For total thiol measurement, 10 µl of reagent 1 (R1) (10µl of R1' is used for free thiol measurement) and 10 µl of sample were mixed. Afterwards, R2 and R3 were added and the first absorbance (A1) reading was made spectrophotometrically at 415 nm wavelength (Schimadzu UV-1201 spectrofotometer, Kyoto, Japan). The second absorbance (A2) reading was taken at the same wavelength at the 10^{th} minute when the reaction peaked, and the measurement was completed by obtaining the A2-A1 absorbance difference. It was used 14.100 mol/l-1 cm-1 which is the molar extinction coefficient of 5-thiol-2-nitrobenzoic acid (TNB) for the calculation of total and free thiol levels.

Antioxidants in the sample convert the dark blue-green ABTS (3-ethyl-benzothiazoline 6 sulfonate) radical solution to the colorless ABTS form. The change in absorbance at 660 nm is related to the total amount of antioxidants. The kit has been calibrated with a stable antioxidant standard called Trolox Equivalent, similar to vitamin E. Oxidants in the sample oxidize the ferrous ion-clamp integrated with the ferric ion. The oxidation reaction is prolonged by the amplifying molecules present in the reaction medium. Ferric ion forms a colored compound with chromogen in acidic medium.

The total amount of oxidant molecules in the sample was determined in relation to the darkness of the color measured in the spectrophotometer. The kit was calibrated with hydrogen peroxide, the results were given as micromoles of hydrogen peroxide per liter (µmol H_2O_2 Equi v./l) (Erel and Neşelioğlu 2014). By taking the percentage of the ratio of TOS level to TAS level; OSI was calculated according to TOS (µmol H_2O_2 equiv/l) / TAS (mmol Trolox equiv/l) formula (Erel, 2005).

One-way analysis of variance (ANOVA) was used to test the significance of the difference between the independent group means in terms of each parameter studied, and the Duncan test was used to control the significance of the differences (Duncan, 1955; Düzgüneş et al., 1983). Data are given as arithmetic mean±standard deviation (X±SX).

RESULTS

The values of roughage, concentrate feed and total feed consumption averages obtained from lambs in two-week periods from the beginning of the study until the end of the trial are given in Table 2. The roughage consumption decreased in the 4th group, which consumed 6 g/kg humic acid added concentrate, in a 2-4 week period compared to the other groups (P<0.05). No statistically significant difference was observed between the 1st group, which is the control group without humic acid, and the other 3 groups with additives, in terms of roughage and concentrate feed and total feed consumption averages (P>0.05).

Table 2. Average values the consumption of roughage, concentrate and total feed of lambs

Properties	Trial Groups				P values *
	1	2	3	4	
Roughage, kg					
0-2 week	44.7±4.81	38.0±4.40	43.2±4.65	33.2±5.18	0.736
2-4 week	38.0±4.09[a]	24.8±3.87[ab]	34.8±3.75[a]	18.5±4.88[b]	<0.05
4-6 week	43.2±4.65	25.6±3.75	39.6±4.26	35.4±3.81	0.091
6-8 week	33.2±3.57	42.0±4.52	45.2±4.87	37.8±4.38	0.656
Concentrate, kg					
0-2 week	236±57.05	238±53.09	232±43.02	235±33.58	0.779
2-4 week	240±58.01	243±58.29	245±38.56	250±41.95	0.071
4-6 week	223±53.90	225±54.54	235±51.85	233±57.21	0.534
6-8 week	201±48.59	204±48.88	210±47.10	207±49.02	0.912
Total, kg					
0-2 week	280.7±32.36	276±30.78	275.2±26.39	268.2±22.16	0.753
2-4 week	271.8±29.56	267.8±23.42	279.8±24.43	268.5±25.14	0.193
4-6 week	261.4±51.75	250.6±22.61	274.6±23.28	268.4±19.94	0.261
6-8 week	247.0±23.07	246±22.20	255.2±19.93	244.8±19.35	0.853

*Difference between the averages shown with different lower case on the same line are significant.

In Table 3, the average values of the live weight (kg) and daily live weight gains (g) obtained in 2-week periods until the end of the trial, including the fattening initial body weights, are given. Accordingly, it was observed that 0, 2, 4 and 6 g/kg humic acid additives, respectively, did not differ statistically between the groups in terms of the specified parameters (P>0.05), and it was revealed that the humic acid additive did not affect the fattening performance at each dose.

Table 3. Average values of lambs live weight (kg), live weight gains (g/day)

Properties	Trial Groups				P values
	1	2	3	4	
Live weight, kg					
Beginning (0. day)	38.79±4.18	38.62±4.55	38.76±3.67	38.76±3.65	1.000
0-2 week	43.38±5.03	43.98±5.11	44.27±3.8	43.97±3.81	0.976
2-4 week	46.4±4.95	47.88±5.09	48.41±3.41	46.98±4.56	0.761
4-6 week	48.27±4.82	50.3±4.62	50.49±3.74	49.28±4.79	0.680
6-8 week	51.13±4.74	53.88±4.32	53.03±4.17	52.09±4.8	0.583
Live weight gain, kg/day					
0-2 week	4.59±2.31	5.36±1.01	5.51±0.6	5.21±3.0	0.752
2-4 week	3.02±0.77	3.9±0.79	4.14±0.8	3.01±4.34	0.593
4-6 week	1.87±0.92	2.42±1.58	2.08±0.58	2.3±0.72	0.639
6-8 week	2.86±0.47	3.58±0.95	2.54±0.88	2.81±0.89	0.057

As seen in Table 4, no statistically significant difference was observed between the group means in terms of TAS (mmol/l) values (P>0.05). On the other hand, when the in-group values of the 1st, 2nd and 3rd groups were examined, it was observed that the mean TAS (mmol/l) at the beginning of the experiment was found to be lower than the TAS (mmol/l) obtained on the 30th and 60th days (P<0.05). However, the mean of intergroup and intragroup differences in TOS (µmol/l) and OSI values were found to be insignificant (P>0.05).

Table 4. Averages of intergroup and intragroup TAS (mmol/l), TOS (µmol/l) and OSI values obtained during the trial

Properties	Trial Groups				P values
	1	**2**	**3**	**4**	
TAS					
0. day	1.17^{a}±0.21	1.09^{a}±0.10	1.13^{a}±0.11	1.65±1.04	0.113
30. day	1.54^{b}±0.11	1.40^{b}±0.16	1.46^{b}±0.22	1.49±0.15	0.363
60. day	1.59^{b}±0.19	1.59^{b}±0.12	1.53^{b}±0.14	1.61±0.16	0.636
*P values	**0.013**	**<0.001**	**0.004**	0.062	
TOS					
0. day	8.74±4.02	6.93±3.09	6.06±1.80	8.56±4.31	0.300
30. day	10.05±4.68	7.86±2.95	6.40±2.37	8.63±4.11	0.215
60. day	6.92±2.12	5.65±2.33	6.60±2.64	7.87±4.64	0.463
P values	0.459	0.121	0.368	0.895	
OSI					
0. day	0.74±0.31	0.65±0.33	0.55±0.18	0.65±0.40	0.647
30. day	0.65±0.29	0.55±0.17	0.43±0.15	0.57±0.24	0.227
60. day	0.44±0.13	0.36±0.15	0.43±0.15	0.47±0.25	0.532
P değerleri	0.264	0.059	0.236	0.717	

*Difference between the averages shown with different lower case on the same column are significant

The averages of the intragroup and intergroup differences of TTL (µmol/l), NTL (µmol/l) and disulfide values obtained from the groups during the trial are given in Table 5. It was observed that all three parameters did not differ in statistical significance between the groups (P>0.05). On the other hand, it was determined that the values of the mean TTL (µmol/l) values in each group were lower than the mean TTL (µmol/l) values obtained at the beginning of the trial (day 0) and at the 30th day at the end of the trial (day 60) (P≤0.001). In terms of NTL (µmol/l), the unadded control group was not affected, while the other groups were affected by the humic acid additive, and the values obtained on the 30th day showed a significant increase compared to the 0th day (P<0.05). In other words, it was observed that the native thiol level in the plasma increased on the 30th day with the addition of 2, 4 or 6 g/kg humic acid. However, at the end of the trial, although this value increased at the same level for each group, the differences observed in the groups on the 60th day were found to be statistically insignificant (P>0.05). When the disulfide values were examined, it was determined that the disulfide values increased significantly at the end of the trial in each group on the 0th, 30th and 60th days (P≤0.001).

Table 5. Intragroup and intergroup averages of TTL (µmol/l), NTL (µmol/l) and disulfide values obtained during the trial

Properties	Trial Groups				P values
	1	**2**	**3**	**4**	
TTL					
0. day	396.37[a]±95.82	361.66[a]±73.65	390.44[a]±91.50	333.76[a]±127.44	0.523
30. day	483.31[a]±98.46	487.08[a]±85.28	474.91[a]±70.22	495.12[a]±123.64	0.976
60. day	1545.60[b]±97.03	1519.20[b]±85.96	1513.80[b]±56.58	1587.50[b]±76.20	0.222
*P values	**0.001**	**0.001**	**0.001**	**<0.001**	
NTL					
0. day	292.50±115.60	229.37[a]±71.46	205.80[a]±60.04	216.06[a]±115.11	0.864
30. day	382.17±152.74	386.06[b]±117.81	370.39[b]±104.07	420.77[b]±144.20	0.173
60. day	331.17±61.41	307.23[ab]±92.32	308.50[ab]±81.96	346.75[ab]±104.87	0.696
*P values	0.097	**0.008**	**0.002**	**0.013**	
Disulfide					
0. day	51.93[a]±42.92	66.14[a]±43.55	92.32[a]±49.86	58.85[a]±37.01	0.239
30. day	50.57[a]±55.50	50.51[a]±27.67	52.26[a]±36.50	37.18[a]±29.43	0.830
60. day	607.22[b]±37.41	605.99[b]±63.01	602.65[b]±55.92	620.38[b]±55.49	0.889
*P values	**0.005**	**0.005**	**<0.001**	**<0.001**	

*Difference between the averages shown with different lower case on the same column are significant

DISCUSSION

In general, no research has been found in the literature on the evaluation of the oxidative stress level with the addition of humic acid to the ration by measuring the thiol/disulfide balance in ruminants. Therefore, the comparison of the results obtained is limited.

Studies on humic substances are mostly concentrated in poultry, and studies on the use of humic acids as productivity enhancers in ruminants are limited. Kara et al. (2012), humic acid added to quail feeds at a rate of 0.5%; reported that they significantly increased the live weight, live weight gain and feed conversion ratio. It has been reported that humic acid compounds provide optimum pH formation in the digestive tract, suppress harmful bacterial species, reduce mycotoxin levels and contribute to the development of intestinal health (Islam et al., 2005). It has also been emphasized that they significantly reduce digestive disorders and have antiviral and antibacterial effects (Taklimi et al. 2012). Studies on the mechanism of action of humic acid on fattening performance may give conflicting results on the subject. In this study, humic acid was not effective on fattening performance parameters of lambs. Although there are studies reporting that the fattening performance improves with the increase in feed efficiency and growth rate in lambs fed with humic acid supplementation (Covington, 2012; Wang et al., 2020), there are also studies reporting that humic acid compounds have no effect on performance, in support with the results obtained from this study (McMurphy et al., 2009; Silva et al., 2011). Fattening performance is under the influence of many factors such as breed, gender, age, care and feeding style, amount and quality of feed, and feed consumption increases in parallel with the age and live weight of lambs (Esen and Yıldız, 2000). In a study conducted by Şahin and Boztepe (2010) to determine the effects of live weight per fattening on fattening performance in Anatolian Merino male lambs, the difference between the groups in terms of live weight, daily average live weight gain and feed conversion ratio was found to be statistically insignificant. Consistent with the results of this study, the fact that the fattening performance parameters did not change may be related to the race. This result is also consistent with the results of a study showing that the humic acid contribution was not effective on the growth performance of kids (El-Zaiat et al., 2018).

Also, it was reported that humate administration at 10 ml/day and 15 ml/day for 8 weeks improved growth performance in the newborn kids and also had an effect at 21 d on skin reaction to phytohemagglutinin suggesting a possible effect on cell-mediated immune response (Agazzi et al., 2007). It has been emphasized that the addition of 1% and 2% humic acid in lambs diets increases the daily average weight gain without affecting the feed conversion rate, but 5% humic acid can reduce the growth performance of the lambs (Covington, 2012). On the other hand, there are studies reporting that the addition of humic acid does not affect blood biochemical parameters in sheep (Tunç and Yörük 2012), rams (Galip et al., 2010), beef cattles (McMurphy et al., 2009) and calves (Silva et al., 2011). In this study, it was observed that roughage consumption decreased in the 3rd group fed with 4 g/kg humic acid added ration compared to the 4th group fed with 6 g/kg additive in the 2-4 week period. This may be related to the feed preference of the lambs as other fattening performance parameters did not change during the 2-4 week period and the total trial period. It has been stated that the lambs consume by choosing the feed they need for nutrients, and their feed preferences vary according to many factors such as species, age, environmental conditions and physiological condition of the animal (Çavuşoğlu and Akyürek, 2018). On the other hand, the increase in plasma NTL level in group 4 in the same period can be explained as the activation of antioxidant mechanisms when feed consumption is directed towards concentrate feed.

In a study conducted with quails, it was reported that the use of humic acid in the ration at high doses such as 600 mg/kg caused a decrease in antioxidant levels, and 360-480 mg/kg doses had no effect on TAS (İpek et al., 2008). The increase in TAS in the control, 1st and 2nd groups on the 60th day is an indication that antioxidant molecules in the plasma increase and resistance against diseases develops. TAS reveals the total activity of all substances with antioxidant properties in the serum. It is expected that feeding conditions that require high energy for many body functions increase the level of oxidative stress (Koch and Hill, 2017). The fact that TOS and OSI did not differ in all other groups may be due to be healthy of the animals and the adequate energy content of the feeds. While TOS increases significantly in disease states, TAS and OSI tend to decrease (Mert et al., 2019). Although it is not statistically affected by humic acid supplementation, it can be said that TOS was higher in the control group on the 30th day compared to the supplemented groups, and especially 4 g/kg humic acid supplementation may have an effect on reducing the oxidant level.

TTL and disulfide levels increased in all groups, while NTL increased in groups with humic acid supplementation. This increase is more pronounced in lambs fed with 4 g/kg and 6 g/kg humic acid additives. It is emphasized that humic acid prevents the formation of free radicals and reduces stress factors by supporting the immune system (Huber and Parzefal, 2007; Paciolla et al., 1998). In a study that carried out to investigate the effect of dystocia that the type of birth does not occur within physiological limits and requires interventions from the outside, on total thiol and native thiol, significant reducing were found in total thiol and native thiol levels in kids in the dystocia group compared to the normal birth group (Akkuş, 2021). Therefore, this result can also be associated with the fact that lambs are healthy born and healthy animals.

According to the results of this study, the fact that there was no statistical difference between the groups in terms of some oxidative stress parameters is similar to the results of the research conducted by Avcı et al. (2013) on Merino sheep. By measuring the dynamic thiol-

disulfide balance, which plays a role in the development of many diseases, a lot of information can be obtained related to the health and nutritional status of the animal (Erel and Neşelioğlu, 2014). Since the increase in disulfide values in each group at the end of the trial was found to be significant in the control group and the humic acid additive did not make any difference between the groups, it can be said that the additive did not have an effect on the thiol/disulfide balance.

CONCLUSION

It was determined that there was no statistically significant difference between the groups in terms of feed consumption, live weight and live weight gain of the lambs, and it was concluded that the humic acid additive did not have any effect on the fattening performance.

At the end of the research; It was observed that the mean NTL values within the group increased on the 30th day in all groups except the control group, and the TAS, TTL and disulfide mean values on the 60th day in all groups including the control group. At the beginning (day 0), middle (day 30), and end (day 60) of the trial; intragroup differences were found to be significant in terms of TAS, NTL and disulfide. It has been concluded that 4 or 6 g/kg humic acid can be added to lamb rations due to the increase in thiol groups, which have an important role in the antioxidant defense system.

Today, no research has been found in the literature on the evaluation of oxidative stress level with thiol/disulfide balance measurement regarding the nutrition of ruminants. The number of studies conducted in ruminants with the addition of humic acid is also very limited. There is a need for new research on the subject.

ACKNOWLEDGMENT

Authors thanks to Aksaray University Scientific Research Projects Coordination Unit for their support. (This work was supported by Aksaray University Scientific Research Projects Coordination, grant number: 2019/031)

Ethical approval: The research was approved by the Animal Experiments Local Ethics Committee of Konya Bahri Dağdaş International Agricultural Research Institute with the date and number of 31.01.2019/91/294997.

Conflict of interest: The authors declared that there are no actual, potential or perceived conflicts of interest for this article.

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