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RESEARCH ARTICLE

The Effects of Nutritional Periods on Oxidative Stress Levels in Lambs During Birth-Weaning Period

Duygu BUDAK[1]*, Besime DOĞAN DAŞ[2], Güzin ÖZKURT[3], Yavuz KAL[4]

[1]Aksaray University, Faculty of Veterinary Medicine, Zootechnical and Animal Nutrition Department, 68100, Aksaray, Turkey
[2]Harran University, Faculty of Veterinary Medicine, Zootechnical and Animal Nutrition Department, 63200, Şanlıurfa, Turkey
[3]Aksaray University, Faculty of Veterinary Medicine, Department of Veterinary Biochemistry, 68100, Aksaray, Turkey
[4]Bahri Dagdas International Agricultural Research Institute, 42070, Konya, Turkey

ABSTRACT

In this study were used 40 newborn singleton Akkaraman and Merino lambs with 4 trial groups consisting of selected 10 lambs homogeneous according to race and gender. Trial was carried out 100 days, covering 5 feeding periods with 21-day periods the first application is on the 14th day from the birth of lambs. The lambs were fed with lamb starter feed for the first two months, then with lamb grower feed *ad-libitum* and 250 g/day/head dry alfalfa hay. At the end of the study; it was determined that the lowest total antioxidant status (TAS) was in Akkaraman male lambs, the highest was in Merino's female, and it was found higher in Merino females than males ($P<0.05$). There was no difference between the groups in terms of total oxidant status (TOS) and native thiol (NTL), during the trial ($P>0.05$). Oxidative stress index (OSI) obtained from Akkaraman lambs was found to be higher than Merino, lower OSI was reached in female Merino's, and total thiol (TTL) were increased in Merino male lambs compared to Akkaraman ($P<0.05$). It was concluded that the Merino had better adaptation to feeding after weaning than the Akkaraman.

Keywords: Lamb, oxidative stress, ruminant, thiol/disulfide balance

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Doğum-Sütten Kesim Dönemi Aralığında Besiye Alınan Kuzularda Beslenme Dönemlerinin Oksidatif Stres Düzeyleri Üzerine Etkileri

ÖZ

Bu çalışmada, ırk ve cinsiyete göre homojen seçilmiş 10 kuzudan oluşan 4 deneme grubu ile 40 adet yeni doğan tekiz Akkaraman ve Merinos kuzular kullanılmıştır. Deneme, kuzuların doğumundan itibaren, ilk uygulama 14. günde olmak üzere 21 günlük periyotlar ile 5 besleme periyodunu kapsayan 100 gün sürede gerçekleştirilmiştir. Kuzular ilk iki ay kuzu başlangıç yemi, sonrasında deneme süresince kuzu büyütme yemi ile *ad-libitum* ve 250g/gün/baş kuru yonca otu ile beslenmişlerdir. Araştırmanın sonunda; en düşük total antioksidan seviye (TAS)'nin Akkaraman erkek kuzularında, en yüksek değerinin ise Merinos ırkı dişi kuzularda olduğu, Merinos ırkı dişilerde de erkeklerine göre daha yüksek bulunduğu tespit edilmiştir ($P<0.05$). Deneme süresince total oksidan seviye (TOS) ve nativ tiyol (NTL) değerleri bakımından gruplar arasında farklılık olmadığı gözlenmiştir ($P>0.05$). Akkaraman ırkı erkek ve dişi kuzulardan elde edilen oksidatif stres indeksi (OSI), Merinos ırkı erkek ve dişi kuzulara ait OSI'nden yüksek bulunmuş, dişi Merinoslarda daha düşük OSI'ne ulaşıldığı ($P<0.05$), total tiyol (TTL) düzeylerinin Merinos ırkı erkek kuzularda Akkaraman ırkı erkek kuzulara göre arttığı tespit edilmiştir ($P<0.05$). Merinos ırkının Akkaraman ırkına göre sütten kesim ardından yeme adaptasyonunun daha iyi olduğu sonucuna varılmıştır.

Anahtar kelimeler: Kuzu, oksidatif stres, ruminant, tiyol/disülfit dengesi

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ORCID ID; *DB: 0000-0001-9327-3830, BDD: 0000-0003-2163-2632, GÖ; 0000-0003-2732-9490, YK; 0000-0002-3672-0048.*
*Corresponding author e-mail: budakduygu@aksaray.edu.tr

INTRODUCTION

The health, growth rate and performance of the lambs from the moment they born, are closely related to their nutritional status and are an important criterion for increasing the quality and productivity, which is the main purpose of animal production. The growth rate and yield power of lambs are directly proportional to the level of feed efficiency. Poor care and feeding conditions are the obvious cause of stress factors (Serin 2015, Altınçekiç 2016).

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 (Yokuş and Çakır 2002, Celi 2011). Hydroxyl, superoxide, nitric oxide, lipid peroxide, reactive oxygen and nitrogen species (ROS and RNS) from free radicals produced by normal cell metabolisms as a result of oxidative stress cause tissue damage by lipid peroxidation (Mercan 2004, Tabakoğlu and Durgut 2013, Karabulut and Gülay 2016). The resulting oxidative damage; it is seen as the main cause and indicator of diseases that occur with the deterioration of tissue functions such as cancer, cardiovascular diseases, immune system diseases, degenerative diseases (Pratic`o 2005). Reactive oxygen species (ROS) are primary molecules that cause oxidative damage when they rise above physiological levels. Increased production of free radicals leads to damage to cell membrane lipids and weakening of cellular protein functions (Valko et al. 2004, Devasagayam et al. 2014). In the antioxidant defense system of the organism against free radicals, primarily enzymatic or non-enzymatic antioxidant mechanisms in the cells come into play. The damage caused by radicals is prevented by the enzyme systems of superoxide dismutase, catalase and glutathione S-transferase in the body, as well as important biological thiols such as glutathione, cysteine, homocysteine, N-acetylcysteine. 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 (Sen and Packer 2000, Erel and Neşelioğlu 2014).

The plasma thiol level consists mostly of albumin and protein thiols, with small amounts of low molecular weight cysteine, homocysteine, glutathione, N-acetylcysteine and ɣ-glutamylcysteine biothiols (Turell et al. 2013). Thiols participate in oxidation reactions via oxidants and form covalent disulfide bonds. These are defined as S-S bonds formed between the sulfhydryl groups of two cysteine amino acids in proteins (Huber and Parzefall 2007, Cremers and Jakob 2013). Under oxidative stress conditions, lead to the formation of reversible disulfides between oxidative residues of cysteine, low molecular thiol stacks and protein thiol groups. The disulfide bonds formed can be reduced back to thiol groups. Thus, dynamic thiol/disulfide balance can be achieved. Dynamic thiol/disulfide balance has a critical role in many cellular activities such as, antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and cell growth. Today, it is a very topical indicator in the field of medicine and is associated with many diseases (Erel and Neşelioğlu 2014). The thiol groups of sulfur-containing amino acids (cysteine, methionine..) in proteins are the primary target point of ROS. The oxidation of thiol groups in the environment with ROS to reversible disulfide bonds is the earliest manifestation of radical-mediated protein oxidation. Biological importance of thiols and disulfides; can be explained by the stabilization of the structures of proteins, the regulation of the functions of proteins, the regulation of enzyme functions, their roles in receptors, transporters, Na-K channel and transcription (Ateş et al. 2015).

The primary immune response to pathogens, stable oxidant/antioxidant balance and vitality ability of lambs born and growing with insufficient immune system capacity depend on the nutritional level during growth and development stages from birth. On the other hand, it is expected that the transition from intrauterine to extrauterine environment with birth, that is, the increase in the oxygen requirement during the adaptation at birth and then the feeding conditions that require high energy for many body functions, increase the level of oxidative stress. Disruption of dynamic thiol-disulfide balance plays a role in the development of many diseases. By measuring the dynamic thiol-disulfide balance, can be obtained a lot of information related to the animal's health and nutritional status.

In this study, it was aimed to determine the effects of feeding on the oxidative stress level of female and male lambs of Anatolian Merino and Akkaraman offspring, which are the most preferred growing in our country (TUIK 2020), by using the thiol/disulfide balance measurement method at the developmental stages from birth.

MATERIAL and METHOD

This study was carried out with the decision of the local ethics committee of animal experiments in Bahri Dağdaş International Agricultural Research Institute with the code 293286/90.

In the study, a total of 40 newborn singleton heads; Akkaraman (20 head; 10 male and 10 female) and Anatolian Merino (20 head; 10 male and 10 female) lambs were used. Healthy and homogeneous animals

with the same birth weight were selected by taking birth weights and necessary records (single birth type, ear number, gender, breed) from birth. The experiment was continued for a total of 100 days from the birth of the lambs, covering the 3-week period in 21-day periods after the first two weeks (0-14. days) when they were fed only with milk. The experiment was continued for a total of 100 days from the birth of the lambs, covering the first two weeks (0-14. days) when they were fed only with milk, and the 4 week period in 21-day periods. In the study, 4 experimental groups were formed, each of which consisted of 10 lambs and the lambs with the same weight average of each group were randomly distributed. Lambs housed in group compartment, each group is arranged so that there are 10 animals in each group; Akkaraman female lambs (AFL) were kept in the 1st group compartment, Akkaraman male lambs (AML) were kept in the 2nd group compartment, Anatolian Merino female lambs (MFL) were kept in the 3rd group compartment, and Anatolian Merino male lambs (MML) were kept in the 4th group compartment. Fattening lambs, according to the feeding period; the period when they are fed only with milk from birth (0-14 days), the period of adaptation to solid feed in addition to milk feeding (15-36 days), the period when they are fed with milk and solid feed (36-57 days), the weaning period (58-78 days) and only solid feeding period (79-100 days) by considering 5 periods (5 feeding periods).

Group feeding was applied in the study, and lambs were fed with only milk until the 14th day from birth, and with milk from the 15th day to the 36th day, as well as with ad-libitum lamb starter feed (including 2.80 Mcal/kg dry matter, 17% crude protein). At the same time, while starting solid feed, dry alfalfa hay was started to be given to the lambs as roughage. After lambs had been get used to consuming 250 g of dried alfalfa hay per animal daily, it was kept constant by giving 2.500 g daily to each group throughout the trial. Starting from the 58th day of the trial, it was pass to lamb grower feed (including 2.75 Mcal/kg dry matter, 16% crude protein). By adding daily increasing amounts to the reduced lamb starter feed, the transition was ensured with practice. Up to the 78th day, they were fed with milk as well as ad-libitum lamb grower feed. Thus, the lambs which were provided with sufficient solid feed consumption for weaning and reached 4 times their birth weight, were weaned on the 78th day and after the 79th day fed only with feed until the 100th day which corresponds to the end of the trial. Fresh and clean drinking water was always available in front of the lambs.

AOAC 1984 was used as the method of determining the amounts 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), organic matter (OM), non-nitrogen extract (NNE), cellulose (CL) and hemicellulose (HCL) values were derived from the analysis results on feed materials through calculation (Table 1).

During the trial, the live weights of the lambs were started to be determined together with their birth weights and they were recorded on the first 15th day, then once every three weeks at the same time (08:00) in the morning. Lambs were hungry for 12 hours before weighing in order to prevent variation (hungry-full) in live weight.

Table 1. The chemical composition of feed materials used in the study

Nutrients	Lamb started feed	Lamb grower feed	Alfalfa hay
ME, Mcal/kg DM	2.80	2.75	1.38
DM,%	90.44	88.73	93.44
*OM,%	82.74	81.08	82.86
CP,%	17.00	16.00	9.57
CC,%	7.14	8.10	34.96
EE,%	3.70	2.89	0.92
CA,%	7.70	7.65	10.58
*NNE,%	53.94	54.55	37.41
NDF,%	-	29.24	50.09
ADF,%	-	9.70	42.43
ADL,%	-	0.90	9.11
*CL,%	-	8.8	33.32
*HCL,%	-	19.54	7.66

*OM=DM-CA, NNE=DM-(CP+CA+EE+CC), CL=ADF-ADL, HCL=NDF-ADF

Blood samples were taken from animals 4 hours after feeding at the beginning of each feeding period (5 periods). 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 10th 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 H2O2 Equi v./L) (Erel and Neşelioğlu 2014). By taking the percentage of the ratio of TOS level to TAS level; Oxidative Stress Index (OSI) was calculated according to TOS (μmol H2O2 equiv./L) / TAS (mmol Trolox eqiv./L) formula (Erel 2005).

Analysis of the data; The data of variance was used to determine the differences between the groups, and Duncan test was used to control the significance of the differences. T-Test was used to analysis the significance of the difference between the means of two independent groups for each parameter studied (Duncan 1955, Düzgüneş et al. 1983). Variance analyzes were performed using Duncan Tests SPSS statistical package programs.

RESULTS

The average live weights (kg) of the lambs obtained during the feeding periods (with 21-day periods) from their birth are given in Table 2. Accordingly, in terms of live weight characteristics was observed no statistically significant difference between the group averages in the 1st, 2nd and 5th periods during the fattening period from the birth of the lambs ($P>0.05$). However, in the 3rd and 4th periods, the body weight differences of AML and MML were found to be significant ($P<0.05$) and the weights of MML were lower.

Table 2. Average live weight (kg) of the lambs obtained during the feeding periods (in 21-day periods) from their birth

Feeding periods	Groups				SEM	P
	AFL	AML	MFL	MML		
1	5.335	5.241	5.335	5.320	0.092	
2	14.830	15.718	14.517	14.415	0.267	
3	22.525	24.336[a]	22.685	20.745[b]	0.457	<0.05*
4	29.790	31.891[a]	29.470	28.509[b]	0.585	<0.05*
5	35.450	37.291	34.140	37.291	0.607	

SEM: Standart error of the mean

*Difference among the averages shown with different letters on the same line are significant

Intra-group TAS (mmol/L), TOS (μmol./L) and OSI values obtained during the feeding periods of the lambs from the beginning of the study are given in Table 3. Accordingly, no statistical difference was observed between the 1st, 2nd, 3rd and 4th feeding periods of AFL in terms of TAS (P>0.05). However, TAS values obtained in the 1st and 4th periods were found to be lower compared to the 5th period (P<0.05). In AML, while no statistical difference was observed in the 1st, 2nd, 3rd and 5th periods (P>0.05), the decrease detected in the 3rd period compared to the 1st and 5th periods was significant (P<0.05). Intra-group mean of TAS values of MFL did not change (P>0.05). TAS values obtained from MML during feeding periods were found to be lower in the 1st period compared to the 3rd and 5th periods (P≤0.001). Similarly, it was determined that there was a significant decrease in the 1st period compared to the other periods (P<0.05) and in the 4th period compared to the 1st, 3rd and 5th periods (P<0.05). As in AFL, it was observed that TOS values were highest in the 1st and 2nd periods and the lowest in the 5th period (P<0.05) in MML, but the difference in the 3rd and 4th periods was insignificant when compared to the other periods (P>0.05). In AML and MFL, the decrease observed in TOS values in the 5th periods compared to the 3rd and 4th periods was found to be statistically significant (P<0.05), a significant decrease was detected in the 5th period compared to the 1st and 2nd periods (P≤0.001). OSI was found to be higher in the 1st period compared to the 3rd and 4th periods in AFL (P<0.05), and it showed a significant decrease in the 5th period compared to the 1st and 2nd periods (P≤0.001). It was determined that the OSI value, which was highest in the 2nd period from AML, was the lowest in the 5th period (P≤0.001). This decrease observed in the 5th period was also detected in merino female and male lambs. The decrease in MFL in the 5th period compared to the 1st (P≤0.001), 2nd and 3rd (P<0.05) periods was found to be statistically significant. OSI values obtained from MML were significantly higher in the 1st period than in the 2nd period (P<0.05) and the 3rd 4th and 5th periods (P≤0.001). It was determined that this value was quite low in the 5th period compared to the 1st and 2nd periods (P≤0.001).

Intra-group TTL (μmol/L), NTL (μmol/L) and disulfide values obtained during the feeding periods of the lambs from the beginning of the study are given in Table 4. A decrease was observed in the TTL level obtained from AFL in the 5th period when the lambs were fed only with solid feed, compared to the other periods, and in AML compared to the 1st and 2nd periods (P<0.05). TTL values obtained in the 2nd and 3rd periods of MFL were found to be higher compared to the 5th period (P<0.05), and it was determined that the TTL values obtained in the 1st and 5th periods in MML compared to the other periods decreased significantly (P≤0.001).

Table 3. Intra-group TAS (mmol/L), TOS (μmol./L) and OSI values obtained during feeding periods of lambs

		Properties							
Groups	Period	**TAS**	**SEM**	**TOS**	**SEM**	**OSI**	**SEM**	**P**	
	1	1.348^{a}	0.218	13.308^{a}	1.054	1.110^{aA}	0.110	*	**
	2	1.640^{ab}	0.168	14.946^{a}	1.832	0.920^{A}	0.083	*	**
AFL	3	1.627^{ab}	0.127	11.669^{ab}	1.221	0.765^{b}	0.074	*	
	4	1.396^{a}	0.179	10.694^{ab}	0.889	0.785^{b}	0.087	*	
	5	1.918^{b}	0.119	10.029^{b}	0.825	0.545^{aB}	0.049	*	**
	1	1.766^{a}	0.207	13.331^{A}	1.000	0.800^{a}	0.104	*	**
	2	1.287^{ab}	0.159	14.208^{A}	1.738	1.136^{bA}	0.079	*	**
AML	3	1.112^{b}	0.120	11.706^{a}	1.159	1.048^{bA}	0.070	*	**
	4	1.281^{ab}	0.170	11.124^{a}	0.843	0.951^{A}	0.083	*	**
	5	1.472^{a}	0.113	7.827^{bB}	0.783	0.528^{bB}	0.047	*	**
	1	2.043	0.218	13.393^{A}	1.118	0.717^{aA}	0.110		**
	2	2.486	0.168	13.930^{A}	1.943	0.571^{a}	0.083	*	**
MFL	3	2.532	0.127	11.754^{a}	1.296	0.468^{a}	0.074	*	
	4	2.406	0.179	10.380^{a}	0.943	0.463	0.087	*	
	5	2.508	0.119	6.761^{bB}	0.875	0.282^{bB}	0.049	*	**
	1	1.370^{aA}	0.197	12.287^{a}	0.953	1.080^{aA}	0.099	*	**
	2	2.005^{bc}	0.152	13.598^{a}	1.657	0.702^{bAB}	0.076	*	**
MML	3	2.243^{bB}	0.115	11.119^{ab}	1.105	0.504^{BC}	0.067		**
	4	1.897^{c}	0.162	10.513^{ab}	0.804	0.613^{aBC}	0.079	*	**
	5	2.335^{bB}	0.108	8.939^{b}	0.747	0.398^{bC}	0.045	*	**

SEM: Standart error of the mean

*Difference among the averages shown with different lower case in the same group column are significant (P<0.05).

**Difference among the averages shown with different upper case in the same group column are significant (P≤0.001).

Table 4. Intra-group TTL (µmol/L), NTL (µmol/L) and disulfide values obtained during feeding periods of lambs

		Properties							
Groups	**Period**	**TTL**	**SEM**	**NTL**	**SEM**	**Disülfide**	**SEM**	**P**	
AFL	1	476.444^{a}	23.138	233.66^{aA}	15.683	121.38^{A}	10.186	*	**
	2	531.889^{a}	26.594	282.33^{b}	16.128	124.77^{aA}	12.652	*	**
	3	485.556^{a}	24.038	302.22^{B}	17.237	91.66^{bc}	9.198	*	
	4	482.444^{a}	29.031	272.1^{b}	12.470	105.16^{ab}	13.845	*	
	5	431.556^{b}	19.702	292.333^{b}	12.123	69.61^{cB}	9.056	*	**
AML	1	420.400^{a}	21.951	201.00^{A}	14.878	109.70^{aA}	9.663	*	**
	2	490.900^{b}	25.230	271.60^{Ba}	15.300	109.65^{aA}	12.003	*	**
	3	463.200	22.804	316.20^{Bbc}	16.352	73.50^{bc}	8.726	*	**
	4	453.300	27.542	286.20^{Bab}	11.830	83.55^{ab}	13.135	*	**
	5	417.800^{a}	18.691	319.50^{Bc}	11.501	49.15^{cB}	8.591	*	**
MFL	1	442.222	23.138	187.55^{A}	15.683	127.33^{aA}	10.186	*	**
	2	490.556^{a}	26.594	301.33^{B}	16.128	94.61^{ab}	12.652	*	**
	3	494.000^{a}	24.038	312.55^{B}	17.237	90.72^{b}	9.198	*	
	4	448.778	29.031	292.66^{B}	12.470	78.05^{bc}	13.845	*	
	5	403.222^{b}	19.702	286.11^{B}	12.123	58.55^{cB}	9.056	*	**
MML	1	413.364^{A}	20.929	200.72^{A}	14.186	106.31^{abc}	9.214	*	**
	2	530.273^{B}	24.056	277.54^{caBC}	14.588	126.36^{a}	11.445	*	**
	3	525.000^{B}	21.743	334.90^{bB}	15.591	95.04^{bc}	8.320	*	**
	4	522.182^{B}	26.260	286.90^{aBC}	11.279	117.63^{ab}	12.523	*	**
	5	448.727^{A}	17.821	263.81^{cC}	10.965	92.45^{c}	8.191	*	**

SEM: Standart error of the mean

*Difference among the averages shown with different lower case in the same group column are significant (P<0.05).

**Difference among the averages shown with different upper case in the same group column are significant (P≤0.001).

The highest NTL level of AFL was observed in the 3rd period, this level increased statistically significantly compared to the 1st period when they were fed only with milk (P≤0.001). It was determined that the NTL detected in the 1st period in AML and MFL decreased significantly compared to the other periods. In MML, the NTL value was found to be the lowest in this term (P≤0.001), and it decreased significantly in the 2nd period compared to the 3rd period (P<0.05). In terms of disulfide values, a significant decrease was observed in AFL and AML compared to the 1st and 2nd periods, and in the 5th periods compared to the 1st period in MFL (P≤0.001). In MML, the decrease observed in the 3rd period compared to the 2nd period and in the 5th period compared to the 2nd and 4th periods were found to be statistically significant (P<0.05).

In terms of mean values between groups, TAS level obtained from lambs in all feeding periods and during the experiment was found to be at the highest level in MFL and later in MML (Table 5). While no difference was observed between Akkaraman female and male lambs in terms of this value (P>0.05), the increase observed in MFL can be attributed to the decrease in TOS in the 5th period. However, there was no significant difference between the groups in terms of TOS during other feeding periods and during the trial (P>0.05). Depending on these changes, it was determined that OSI decreased significantly in merino female and male lambs.

The intergroup TTL (µmol/L), NTL (µmol/L) and disulfide values obtained during the feeding periods of the lambs are given in Table 6. According to the results obtained regarding the TTL levels in the blood, there was no statistically significant difference between the groups in each feeding period of the lambs (P>0.05). However, it was determined that TTL levels did not differ in females of both offspring during the trial (P>0.05), but increased in MML compared to AML (P<0.05). On the other hand, the mean NTL value, which did not differ between the groups during the experiment, was found to be higher in AFL than MFL in the first feeding period (P<0.05). The mean NTL (µmol/L) value of AML was found to be higher in the 5th period compared to MML (P≤0.001). It was observed that disulfide values of MML were higher than females and higher than AMLs (P<0.05).

Table 5. Intergroup TAS (mmol/L), TOS (µmol./L) and OSI obtained during feeding periods of lambs

Period	Groups				SEM	P	
	AFL	AML	MFL	MML			
TAS(mmol/L)							
1	1.348^{a}	1.766^{ab}	2.043^{b}	1.370^{a}	0.105	*	
2	1.640^{acA}	1.287^{aA}	2.486^{bB}	2.005^{c}	0.081	*	**
3	1.627^{aA}	1.112^{bA}	2.532^{B}	2.243^{B}	0.061	*	**
4	1.396^{aA}	1.281^{aA}	2.406^{aB}	1.897^{b}	0.086	*	**
5	1.918^{aA}	1.472^{bA}	2.508^{B}	2.335^{cB}	0.058	*	**
1-5	1.586^{a}	1.384^{a}	2.395^{b}	1.970^{c}	0.045	*	
TOS(µmol/L)							
1	13.308	13.331	13.393	12.287	0.516		
2	14.946	14.208	13.930	13.598	0.898		
3	11.669	11.706	11.754	11.119	0.599		
4	10.694	11.124	10.380	10.513	0.436		
5	10.029^{a}	7.827	6.761^{b}	8.939	0.405	*	
1-5	12.129	11.639	11.244	11.291	0.214		
OSI							
1	1.110^{a}	0.800^{bc}	0.717^{b}	1.080^{ac}	0.053	*	
2	0.920^{a}	1.136^{aA}	0.571^{bB}	0.702^{abB}	0.040	*	**
3	0.765^{a}	1.048^{bA}	0.468^{bB}	0.504^{bB}	0.036	*	**
4	0.785^{ac}	0.951^{aA}	0.463^{bB}	0.613^{cb}	0.042	*	**
5	0.545^{bcA}	0.528^{acA}	0.282^{aB}	0.398^{a}	0.024	*	**
1-5	0.825^{a}	0.892^{a}	0.500^{b}	0.659^{c}	0.018	*	

SEM: Standart error of the mean

*Difference among the averages shown with different lower case on the same line are significant ($P < 0.05$).

**Difference among the averages shown with different upper case on the same line are significant ($P \leq 0.001$).

Table 6. Intergroup TTL (µmol/L), NTL (µmol/L) and disulfide values obtained during feeding periods of lambs

Period	Groups				SEM	P	
	AFL	AML	MFL	MML			
TTL(µmol/L)							
1	476.444	420.400	442.222	413.364	11.154		
2	531.889	490.900	490.556	530.273	12.820		
3	485.556	463.200	494.000	525.000	11.588		
4	482.444	453.300	448.778	522.182	13.995		
5	431.556	417.800	403.222	448.727	9.498		
1-5	481.578	449.120^{a}	455.756	487.909^{b}	5.713	*	
NTL(µmol/L)							
1	233.66^{a}	201.00	187.55^{b}	200.72	7.560	*	
2	282.330	271.60	301.33	277.54	7.775		
3	302.220	316.20	312.55	334.90	8.309		
4	272.100	286.20	292.66	286.90	6.011		
5	292.333	319.50^{A}	286.11	263.81^{B}	5.844		**
1-5	276.533	278.900	276.044	272.782	4.458		
Disulfide							
1	121.38	109.70	127.33	106.31	4.910		
2	124.77	109.65	94.61	126.36	6.099		
3	91.66	73.50	90.72	95.04	4.434		
4	105.16	83.55	78.05^{a}	117.63^{b}	6.674	*	
5	69.61	49.15^{A}	58.55^{a}	92.45^{bB}	4.365	*	**
1-5	102.52^{ac}	85.11^{b}	89.85^{ab}	107.56^{c}	2,516	*	

SEM: Standart error of the mean

*Difference among the averages shown with different lower case on the same line are significant ($P < 0.05$).

**Difference among the averages shown with different upper case on the same line are significant ($P \leq 0.001$).

DISCUSSION

In general, no research has been found in ruminants on the evaluation of oxidative stress level in the field of animal nutrition by thiol/disulfide balance measurement in the literature. Therefore, the comparison of the results obtained is limited.

Growing performance is under the effect of 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 this study expected was a situation that the live weights obtained from the groups by periods will increase in male lambs compared to female lambs. However, it can be said that the decrease observed in MML in the lambs feding solid feed together milk and reduced milk periods compared to females and Akkaraman offspring is related to the need for higher protein in MML's fattening, as can be understood from the results of some studies (Esen and Yıldız, 2000, Sawal et al. 2011).

Insufficient, excessive or unbalanced nutrition is a factor 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 newborn and developing young ruminant (Serin 2015, Altınçekiç 2016).

Serum or plasma concentrations of different types of oxidant (malondialdehyde, nitric oxide) and antioxidant concentration (superoxide dismutase, glutathione peroxidase, catalase, vitamin E and selenium) can be measured separately by direct or indirect methods. However, measuring each parameter individually has advantages and disadvantages. These measurements do not provide an overall cumulative measure of oxidative and antioxidant status. Individual measurements require time consuming, costly and complex techniques. Therefore, the measurement of TAS, TOS and OSI reflects this situation and is more economical (Harma et al. 2005). There are studies reporting that the TAS level, which shows the total activity of all substances with antioxidant properties in the serum, is high in healthy sheep or lambs (Heidarpour et al. 2013, Mert et al. 2019, Garret et al. 2021). The decrease in the in-group TAS values of AML in the 3rd period was found to be significant compared to the 1st and 5th periods. This situation is related to the decrease of antioxidant molecules in the blood of Akkaraman male lambs during this period. The increase observed until the 5th period is related to the antioxidant defense mechanism that develops in response to the high oxidant level, especially in the period until the adaptation to solid feeds. Similar situations were encountered in the males of the Merino. Although merino male lambs had higher compliance with the feed, it showed a decrease in the 4th period, and the antioxidant level increased significantly during the weaning period. This finding, which is valid for TAS, is also similar to the results of research showing that Roman lambs develop high antioxidant capacity during the suckling period (Mialon et al. 2021). In related to mean TAS values between groups obtained from lambs in all feeding periods and during the experiment at the highest level in MFL and later in MML can be explained by the fact that Merino lambs are better adapted to feed and reach higher antioxidant capacity than Akkaraman lambs during their developmental stages. There was no significant difference between the groups in terms of TOS during other feeding periods and during the trial. Depending on these changes, it was determined that OSI decreased significantly in merino female and male lambs. Accordingly, it can be said that the immunity of the groups develops better especially when the lambs consume milk and solid feed together. When the results obtained from this study are considered, similar to the results of the research, which stated that Norduz sheep developed better resistance to diseases due to the higher TAS level and lower OSI than Morkaraman offspring, the same can be said for Merino compared to Akkaraman in this study (Mis et al. 2018).

Thiol or sulfhydryl groups (SH), which form the most active and functional form of the sulfur atom, as well as antioxidant defense are of great importance in enzyme function, protein functionality, detoxification, regulation of transcription factors, signal transduction, apoptosis and cellular stimulation mechanisms (Akkuş 2021). It can be said that the highest TTL values obtained from lambs increase due to rising of thiol compounds, which show antioxidant properties in the blood, especially during the periods when they consume solid feed with milk in MML. It is understood that the antioxidant defense mechanism develops depending on the total thiol level in lambs in this process where solid feed is consumed with milk. As at the TTL level, intra-group differences in NTL have been observed to a significant size. It was concluded that the disulfide value, which increased more significantly in the Merino race, developed in parallel with the high level of TTL and NTL in all groups. In other words, the thiol/disulfide balance was found to be weaker in Akkaraman offspring, but it was observed that the balance achieved by the high blood total thiol level in male lambs shifted towards thiol groups. In oxidative stress situations, while the native thiol and total thiol values are expected to decrease, the disulfide value is expected to increase (Erel and Neşelioğlu 2014). Accordingly, it can be said that the disulfide level tends to decrease in parallel with the periods when the antioxidant defense mechanism of all trial groups is activated.

CONCLUSION

When the results obtained from the study were evaluated in general, it was determined that the lowest antioxidant capacity was found in Akkaraman male lambs, the highest in Merino female lambs, and it was found higher in Merino females than males. It has been observed that the total antioxidant capacity can increase in lambs consuming solid feed together milk. The highest TAS value was observed in the 5th period, which is the weaning period, in Merino male lambs as well as in Merino females. On the other hand, it was determined that oxidant parameters TOS, OSI and disulfide decreased gradually with adaptation to solid food in all 4 groups. The fact that the oxidative stress index is higher in Merino lambs, especially in females, is largely a result of higher blood antioxidan molecules and total thiol levels in Merino lambs than in Akkaraman lambs. This situation explained that the lambs of both breed were able to compensate for oxidative stress with the adaptation developed in the transition from milk to solid feed. According to the results, it can be said that the oxidative stress caused by the transition from milk to solid food and weaning is sufficiently tolerated by the antioxidant systems of the lambs. When all oxidant antioxidant parameters were evaluated, it was concluded that the adaptation of Merino lambs after weaning to feed was better than Akkaraman lambs.

By measuring the dynamic thiol-disulfide balance, which plays a role in the development of many diseases, a lot of information related to the health and nutritional status of the animal can be obtained. However, there is a need for research on the determination of oxidative stress by thiol/disulfide balance measurement method in ruminants.

Conflict of interest: The authors declared that there is no conflict of interest.

Ethical Approval: This study was approved by the Animal Experiments Local Ethics Committee of Konya Bahri Dağdaş International Agricultural Research Institute with the date and number of 31.01.2019/90.

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