# Effects of Defaunation and Urea on Glutathione and Malondialdehyde Levels in Blood and Ruminal Fluid of Ramlıç Lambs\*

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Received: 03.04.2002

**Abstract:** The objective of this study was to investigate ruminal fluid and whole blood oxidant-antioxidant status in normal and fauna-free small ruminants. Forty male Ramlıç lambs, a local breed in the Afyon area, with an average body weight of 32-36 kg and approximately 210 days old were used. The lambs were divided equally into 4 groups (faunated (F), defaunated (D), faunated + urea (FU) and defaunated + urea (DU)). Malondialdehyde (MDA) and glutathione (GSH) concentrations in the ruminal fluid and blood were measured. In comparison with the controls, all parameters were significantly higher in the fauna-free animals. The ruminal fluid MDA concentration decreased and the GSH level increased when 2.9% urea was added to rations. The defaunation procedure causes MDA formation to increase in the blood and ruminal fluid. The presence of 2.9% urea in rations may lead to lower oxidative stress and an augmented GSH level in rumen of fauna-free animals. Therefore, urea can be an important local antioxidant for the rumen of ruminants. As a result, defaunation may make the animals more susceptible to oxidative stress. Urea supplementation appears to reduce oxidative stress status and to support the antioxidant defense system in the rumen. Urea supplementation may be of major importance for fauna-free ruminants, whose ruminal antioxidant status may not be sufficiently well prepared to enhance performance.

Key Words: Defaunation, urea, oxidative stress, glutathione, malondialdehyde, antioxidants

## Defaunasyon ve Ürenin Ramlıç Kuzularda Kan ve Rumen Sıvısı Glutatyon ve Malondialdehit Düzeylerine Etkileri

**Özet:** Bu çalışma, defaunasyon işlemi uygulanan ve rasyonlarına üre katılan hayvanlarda kan ve rumen sıvısının oksidan-antioksidan statüsünü araştırmak amacıyla, 32-36 kg ağırlıklarında ortalama 210 günlük 40 erkek Ramlıç kuzuda gerçekleştirildi. Ramlıç ülkemizde geliştirilmiş önemli bir koyun ırkıdır. Oksidan-antioksidan göstergeler ise bir canlı için önemli yaşamsal parametreler olarak kabul edilmektedir. Araştırma, yemlerine 2,9 oranında üre katılmış kuzular (FU), defaunasyon uygulanan kuzular (D), hem defaunasyon uygulanmış hem de yemlerine 2,9 oranında üre katılmış kuzular (D) ve faunalı normal beslenen kuzular (F) olmak üzere 4 grup oluşturularak gerçekleştirildi. Rumen sıvısı ve kan örneklerinde lipid peroksidasyonu ürünlerinden malondialdehit (MDA) ve glutatyon (GSH) düzeyleri araştırıldı. Defaunasyon işleminin hem rumen hem de kan MDA ve GSH düzeylerini yükselttiği izlendi (P < 0,01). Öte yandan rasyona üre ilave edilen defaunalı hayvanlarda rumen sıvısında MDA düzeyi azalmış; GSH konsantrasyonu ise, hem F hem de FU grubundan daha yüksek düzeye ulaşmıştır. DU grubunda rumen MDA düzeyi kan MDA düzeyi nanla düşüktür. Bu veri, ürenin rumende oksidatif strese karşı antioksidan yapıyı güçlendirerek peroksidasyonu azaltabilecek potansiyel bir antioksidan olabileceğini göstermektedir. Sonuç olarak; defaunasyon işleminin ruminantlarda oksidatif strese yol açabileceği, ancak rasyona katılan 2,9 oranındaki ürenin hayvanın verimlilik ve direncini azaltan oksidatif stresten korunmada etkili olabileceği kanaatine varılmıştır.

Anahtar Sözcükler: Defaunasyon, üre, oksidatif stres, glutatyon, malondialdehit, antioksidan

<sup>\*</sup> This study was carried out with the experimental animals from project VHAG-1579, supported by the Scientific and Technical Research Council of Turkey (TÜBİTAK).

### Introduction

Since 1970, several reports have been issued on the positive effects of defaunation on the performance of ruminants (1-3). To our knowledge, there is no evidence to explain the effects of defaunation on oxidant-antioxidant status in ruminants. On the other hand, in recent years much attention has been paid to the issue of the oxidant-antioxidant balance and its effects in many medical disciplines (4,5).

The main aim of this study was to investigate the effects of the defaunation procedure and of urea on oxidant-antioxidant status in ruminants. This article is probably the first research into the results of defaunation and urea supplementation in fauna-free animals with regard to the oxidant-antioxidant balance. The effects on MDA and GSH levels of defaunation and of adding urea to rations are presented below. MDA is commonly measured by its reaction with thiobarbituric acid, and it is accepted as a general marker of oxidative stress and lipid peroxidation (6). GSH is a substrate for glutathione peroxidase, which serves to remove radical metabolites (4,7).

#### Materials and Methods

Animals and diet: Forty male, healthy, Ramlic lambs, approximately 180 days old, and weighing an average of 32-36 kg were used. The lambs were equally divided into 4 groups (faunated (F), defaunated (D), faunated + urea (FU) and defaunated + urea (DU)) as similar as possible with regard to live weight at the beginning of the experiment. The lambs in groups F and D were fed control rations, and the lambs in the groups FU and DU were fed the experimental ration show in Table 1. In addition, 350 g of dry alfalfa was given daily to each lamb. All diets were pelleted except for the dry alfalfa. All lambs in each group were housed in a pen isolated from the others. The lambs in each group were fed twice a day, their rations being divided into 2 equal parts as 3% of live weight. Water was available ad libitum during the experimental period. The lambs in groups FU and DU were adjusted to the ration with urea 2 weeks after the defaunation procedure. The lambs in the experimental groups were adjusted to their diet 2 weeks after defaunation.

	F and D groups	FU and DU groups
Barley	48.0	61.8
Oats	21.5	24
Molasses	2.0	6.5
Cottonseed meal	17.4	-
Soybean meal	5.0	-
Sunflower meal	3.1	-
Urea	-	2.9
Sodium sulfate	-	1.8
Granite powder	1.8	1.8
NaCl	0.5	0.5
Dicalcium phosphate	0.5	0.5
Mineral mix (Remineral 2)	0.1	0.1
Vitamin mix (Rovimix 301-F)	0.1	0.1

Table	1.	Composition	of the	rations	(%).
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Mineral mixture (Remineral 2, Roche, İstanbul, Turkey), contained 50 g manganase, 50 g iron, 50 g zinc, 10 g copper, 0.15 g selenium, 0,15 g cobalt and 0.8 g iodine per kg.

Vitamin supplement (Rovimix 301-F, Roche, İstanbul, Turkey), composed of 15, 000, 000 IU Vitamin A; 4, 000, 000 IU Vitamin D3; 20 g Vitamin E; 4 g Vitamin B<sub>1</sub>; 10 g Vitamin B<sub>2</sub>; 5 g Vitamin B<sub>6</sub>; 15 g calcium-D-pantptothenate; 20 g niacin; 20 mg Vitamin B<sub>12</sub>; 50 mg D-Biotin and 200 g choline chloride per kg.

Defaunation procedure: In this study, the method of Ankrah et al. (8) was employed for defaunation. One day prior to starting the defaunation procedure, all the lambs in groups D and DU were fed half of their ration. In these groups, feeding was stopped in the first 3 days of the defaunation period. The lambs in groups D and DU were defaunated with a solution (100 ml daily for each animal) of dioctyle sodiumsulphosuccinate (DSS: Sigma Co, Cat no: 4422) (2% W/V) delivered directly into the rumen through a polyethylene tube inserted down the esophagus. This was given for 3 days without any interval. On days 2 and 3, 200 ml of a substrate solution (20 g starch, 40 g sucrose, 20 g casein and 20 g electrolyte mix NaCl, 37; KH<sub>2</sub>PO<sub>4</sub>, 37; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 18.5; MgSO<sub>4</sub>, 3.7; CaCl<sub>2</sub>, 3.7% of total mix) was infused into the rumen of each lamb in groups D and DU via the esophagus 2 h post-dosing with DSS. The substrate solution was used to sustain the bacterial population in the rumen when the animals were off feed (8). All lambs were fed their assigned diets starting from the last day of DSS dosage. Since omasal protozoa are extremely difficult to remove and are responsible for reinoculating transiently defaunated rumens. Two weeks from the beginning of the defaunation period, a 100 ml solution of DSS (5% W/V) was again infused into the rumen of each lamb in groups D and DU without fasting the lambs. This procedure has been found to be very effective in completely removing protozoa from the rumens of lambs and allowing them to return to a normal appetite in a short period (8).

Blood samples were collected from the jugular vein, and rumen contents were sampled from each lamb in all groups 4 h after the morning feeding on day 90 of the experimental period. All ruminal fluids collected via stomach tube were immediately measured for pH using a glass electrode and then strained through 4 layers of cheesecloth to yield rumen fluid for  $NH_3$ -N (Sigma-Cat no: 640B). Samples of the rumen contents from defaunated lambs were examined for protozoa via a microscope every other week.

MDA and GSH measurements: Blood and ruminal fluid MDA and GSH assays were performed within 2 h of sample collection. MDA was estimated by the double heating method of Draper and Hadley (6). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid solution was added to 0.5 ml blood and to ruminal fluid samples and placed in a 90 °C water bath for 15 min. After cooling, the mixture was centrifuged at 1000 g for 10 min, and 1 ml of the supernatant was added to 0.5 ml of 6.7 g/l TBA solution in a test tube and placed back in a 90 °C water bath for 15 min. The solution was cooled in water and its absorbance was measured by using a Shimadzu UV 1601 spectrophotometer at 532 nm. GSH determination was performed by the classic method of Beutler et al. (7) in fresh blood and ruminal fluid specimens.

Statistical analysis: ANOVA was used for statistical calculations using SPSS 11.0. The least significant difference test was used to compare the differences among the groups, and within the same group by 2-way Dunnett test. Differences were considered at P < 0.05.

## Results

Mean total concentrations and statistical results of MDA and GSH in blood and ruminal fluid are summarized in Tables 2 and 3. The defaunation procedure used in the study is seen to be very effective. All defaunated lambs remained protozoa-free until the end of the 90-day study period. The ruminal fluid GSH level in group F was the lowest among the groups. There were significant effects of defaunation and urea on the blood and ruminal fluid MDA (P < 0.001), and on blood and rumen GSH concentrations (P < 0.01). All these markers increased

Table 2. MDA and GSH values (Mean ± SEM).

Groups	Blood MDA (nmol /ml)	Rumen MDA (nmol /ml)	Blood GSH (mg/dl)	Rumen GSH (mg/dl)
Faunate (F)	1.395 ± 0.121	3.723 ± 0.254	7.275 ± 0.206	6.605 ± 0.282
Controls + urea (FU)	1.406 ± 0.118	$4.005 \pm 0.267$	7.255 ± 0.217	7.101 ± 0.228
Defaunate (D)	2.004 ± 0.128	5.534 ± 0.253	8.608 ± 0.231	7.538 ± 0.237
Defaunate + urea (DU)	2.069 ± 0.121	3.784 ± 0.254	7.530 ± 0.213	7.983 ± 0.263

Table 3. Effects of defaunation and urea on MDA and GSH levels in lambs.

	Faunate		Defaunate				Probability		
	F <sup>a</sup>	FU <sup>b</sup>	D <sup>a</sup>	DU <sup>b</sup>	SEM	FD <sup>c</sup>	F*FU <sup>d</sup>	F*FU*D*DU	
Number of samples	10	9	9	10					
Blood MDA (nmol/ml)	139	1.41	2.00	2.01	0.19	< 0.01	0.96	< 0.01	
Rumen MDA (nmol/ml)	3.72	4.01	5.53	3.78	0.26	< 0.01	0.44	< 0.01	
Blood GSH (mg/dl)	7.28	7.25	8.61	7.53	0.06	< 0.01	0.22	< 0.01	
Rumen GSH (mg/dl)	6.61	7.10	7.54	7.98	0.07	<0.01	0.05	<0.01	

<sup>a</sup> Plant protein

<sup>b</sup> Non protein nitrogen (urea)

<sup>c</sup> Faunate vs. defaunate

<sup>d</sup> Group F vs. Group FU

<sup>e</sup> Faunate and defaunate vs. plant protein and non protein nitrogen

after the defaunation procedure. Urea supplementation returned the ruminal fluid MDA level in the DU group animals to the control values. On the other hand, the increase in blood GSH levels in group D is an interesting result (P < 0.001). Urea may augmente the GSH concentrations of ruminal fluid in faunate and defaunate lambs (P < 0.001).

### Discussion

Defaunation is a supporting factor in the energy metabolism of ruminants (9). It also has an increasing effect on energy production (1). In animals, energy is largely produced by cellular oxidation. Oxidation reactions are an essential biological procedure for the formation of high energy compounds used to fuel metabolic processes, but these reactions can be injurious to cells if they are overproduced. All aerobic tissues are susceptible to damage mediated by oxidative changes (10). Oxidative stress essentially effects polyunsaturated fatty acid in cell membranes, producing lipid peroxidation reactions and free radical metabolites (10,11). Polyunsaturated lipids, proteins, DNA and carbohydrates are susceptible to oxidative stress (4,11).

In this study, the fauna-free lambs supplemented with urea were more resistant to oxidative stress than the defaunated group. Urea supplementation resulted in an augmented GSH status and reduced MDA in ruminal fluid. Supplementation with urea appears to reduce oxidative stress and to support antioxidant enzyme activity in lambs' ruminal fluid. However, it is unclear why only ruminal fluid GSH and MDA concentrations are affected by the addition of urea to the rations of defaunated animals. Based on some studies, ruminal MDA and GSH can be affected by, at least, the following factors:

1. Urea reforms to ammonia in 1-4 h and is absorbed into the blood vessels (12)

2. Microbial proteins can be synthesized by rumen bacteria from ammonia (13,14).

3. In fauna-free ruminants, ruminal fungi numbers rise. In the case of urea supplementation, fungi cannot use urea. Therefore, neither the  $O_2$  consumption of the fungi nor their oxidation activity increases. On the other hand, in the case of protein addition to rations with defaunation, fungi can use the proteins. This may increase the oxygen consumption of fungi (15,16). The resulted elevated oxygen consumption may increase MDA levels.

4. An increase in the use of vitamins has also been reported in animals fed urea supplemented rations (13). Given that most vitamins are important antioxidants (4), it may be thought that urea supplementation supports the antioxidant defense system of defaunated animals.

5. Pond et al. (13) report that defaunation increases the absorption of minerals such as Cu, Zn and Fe. The elevated metal ion concentrations in the serum of defaunated animals may, in part, explain the increased MDA in the blood and ruminal fluid. Some authors suggest that unsaturated fatty acid levels are clearly higher in defaunated animals (14,17). Dietary oxidants such as Cu may also increase polyunsaturated fatty acids and fatty acid peroxidation (4).

Kim et al. (18) have shown, in rats, that GSH is important in protecting cells from the harmful effects of oxidant factors.

This article may be the first research indicating that defaunation and dietary urea can affect blood and ruminal fluid oxidant and antioxidant markers. It is impossible to reach a fair judgment on the MDA and GSH concentrations investigated in this study due to limited information. Similar experiments are needed to provide

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further information to explain exactly the effects of defaunation and urea supplementation.

In conclusion, defaunation possibly increases the oxidative stress marker MDA in blood and in ruminal fluid, as well as GSH levels. Urea supplementation leads to augmented GSH status in ruminal fluid. In the ruminal fluid of fauna-free animals, urea supplementation lowers MDA. That means that more work on the effects of urea supplementation on the oxidant-antioxidant balance of defaunated animals is needed.

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