

Evaluation of some oxidative stress markers in *ovis aries* during different experimental conditions

V. Monteverde,¹ M. Rizzo,² D. Alberghina,² F. Fazio,² E. Giudice,² G. Piccione²

¹Institute for Experimental Veterinary Medicine of Sicily, Palermo; ²Department of Veterinary Sciences, University of Messina, Italy

Oxidative stress can be regarded as an imbalance between prooxidant/free radical production and opposing antioxidant defenses. There is growing evidence that oxidative stress (OS) significantly impairs organic function and plays a major role in the aetiology and pathogenesis of several metabolic diseases in veterinary medicine.¹ In view of this, we evaluate some oxidative stress markers in ten Comisana ewes (3 years old, clinically healthy) at different experimental conditions: shearing, road transportation, different reproductive status. Moreover we evaluate daily rhythms of oxidative stress markers. On each subject, blood samples were collected by jugular venipuncture to assess oxidative stress markers during: shearing (before, after shearing and 8h, 1d, 2d, 3d, 4d, 5d post-shearing); road transportation for 6 h over a distance about 490 km with an average speed of 80 km/h (before, after transportation and 8, 24, 48 h post-transport); different reproductive status (days 1, 40 and 200 of lactation and during dry period); and circadian rhythm under natural photoperiod -sunrise 5:10, sunset 20:45- (every 3 h over a 24 h period, starting at 8:00 on day 1 and finishing at 8:00 on day 2). All blood samples were collected into vacutainer tubes with no additive and were centrifuged at 3000×g for 20 min. The sera obtained were immediately analyzed by means of a UV spectrophotometer (Slim SEAC, Firenze, Italy) for the assessment of dROMs, Oxy-adsorbent and SHp. These techniques are based on the *spin traps* system, molecules which react with free radicals, creating complexes revealed by spectrophotometry. During milking and dry period was also evaluate daily rhythm of oxidative stress markers (mean level, amplitude, acrophase and robustness) applying a trigonometric statistical model on mean values. All the results obtained were expressed as mean values±standard deviation (SD). Data were normally distributed ($P<0.05$, Kolmogorov-Smirnov test). One-way repeated measure analysis of variance (ANOVA) was performed to determine the statistical significance and Bonferroni's test was applied as post hoc comparison test. The data were analyzed using the software STATISTICA 8 (Stat Soft Inc.).

The lowest values of dROMs observed before shearing and a their subsequent increase, could be due to the energy deficiency that occurs in ewes after shearing. Ambient temperature, relative humidity and shearing can influence thermoregulatory mechanisms and the productive performance and welfare of ewes. The high values of oxy-adsorbent and SHp values further demonstrate the compensatory response of the organism to the increase of free radicals (dROMs) inducing oxidative stress after shearing (Table 1). Our results indicate that shearing causes a change in the ewes homeostatic balance that leads to oxidative stress. The road transportation in ewes appears to have an influence on the increase of catabolic reactions that leads to the onset of oxidative stress. The results of our study (Table 1) showed an increase of dROMs, Oxy-adsorbent and SHp values in ewes after the road transportation in comparison with basal level ($P<0.05$). The pattern of dROMs, characterized by a significant increase during post transport respect to, is in agreement with other researchers.² The increase of dROMs after 48 h rest time corresponds to approximately 95.85% and represents the energetic deficiency that occurs in ewes during transport period. The high values of Oxy-adsorbent and SHp during rest time further demonstrated the compensatory response of the organism to the increase of dROMs inducing oxidative stress successively to road transportation. During the different reproductive periods, lowest values of dROMs at the start of experimental period could be due to the energetic deficiency which occurs in ewes during the last period of pregnancy. The significant increase of dROMs on days 40 and 200 of lactation and during dry period compared to values obtained on day 1 of lactation shows high oxidative processes which occur during lactation in ewes. The pattern of dROMs characterized by low values at the beginning of lactation and by a significant increase at the mid-point of lactation was previously observed in lactating ewes.^{3,4} The high values of Oxy-adsorbent and SHp at the end of lactation document the compensative response of the organism to oxidative stress. Oxidative processes increased ($P<0.05$) at the end of milking period (Figure 1), together with a compensative response of the organism to this stress and suggest the important role of oxidative status in dairy ewes. Our result showed that the high energetic requirements of milking period in ewes are directly proportional to free radicals formation and oxidative stress. The trigonometric statistical model of the single cosinor procedure indicated the existence of daily rhythm of dROMs, Oxy-ads and SHp in ewes with a nocturnal acrophase (dROMs=21:00; Oxy-ads.=21:30; SHp=21:15). All three markers showed a rhythm robustness above significance line (dROMs=81.5%; Oxy-ads.=79.1%; SHp=81.70%). In conclusion we can claim that there is a synergism between oxidative stress markers and the circadian rhythm of anti-oxidant power in ewes. The right zootechnics management and the formulation of ration that totally satisfy the requirements of the lactating subjects, seem to be essential in order to guarantee the productive status and the animal welfare.

Correspondence: Francesco Fazio, Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy.
E-mail ffazio@unime.it

©Copyright V. Monteverde et al., 2015
Licensee PAGEPress, Italy
Journal of Biological Research 2015; 88:5161

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 3.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Table 1. Patterns of mean values (\pm SD) of dROMs, Oxy-ads. and SHp and statistical significance changes about sampling time from pre-treatments.

Sampling time	Parameters					
	dROMs (UCARR)		Oxy-ads. (μ mol/l)		SHp (μ mol/l)	
	Shearing	Transport	Shearing	Transport	Shearing	Transport
before	31.80 \pm 2.20	31.20 \pm 2.03	1.930 \pm 0.04	2.015 \pm 0.14	102.70 \pm 3.82	112.60 \pm 2.58
after	33.80 \pm 2.05	34.90 \pm 2.09	1.939 \pm 0.06	2.158 \pm 0.18	104.80 \pm 3.04	114.50 \pm 2.25
after 8h	34.60 \pm 1.93	36.40 \pm 2.50 [■]	1.941 \pm 0.05	2.289 \pm 0.18 [■]	105.70 \pm 5.06	125.70 \pm 1.96 [■]
after 1d	36.00 \pm 1.74	48.10 \pm 3.01 [■]	1.957 \pm 0.04	2.732 \pm 0.16 [■]	107.60 \pm 3.32	146.30 \pm 2.22 [■]
after 2d	42.00 \pm 1.49 [■]	62.00 \pm 2.94 [■]	2.035 \pm 0.04	2.892 \pm 0.13 [■]	117.80 \pm 3.55	169.10 \pm 2.95 [■]
after 3d	45.60 \pm 1.81 ^{■▲}		2.179 \pm 0.06 [■]		156.60 \pm 4.76 ^{■▲}	
after 4d	55.00 \pm 1.91 ^{■▲}		2.335 \pm 0.08 ^{■▲}		210.60 \pm 6.35 ^{■▲}	
after 5d	70.20 \pm 2.42 ^{■▲*}		2.882 \pm 0.08 ^{■▲}		343.60 \pm 4.72 ^{■▲}	

Significance (P<0.05): [■]vs before; [▲]vs after, after 8 h and after 1d; ^{*}vs after 4d.

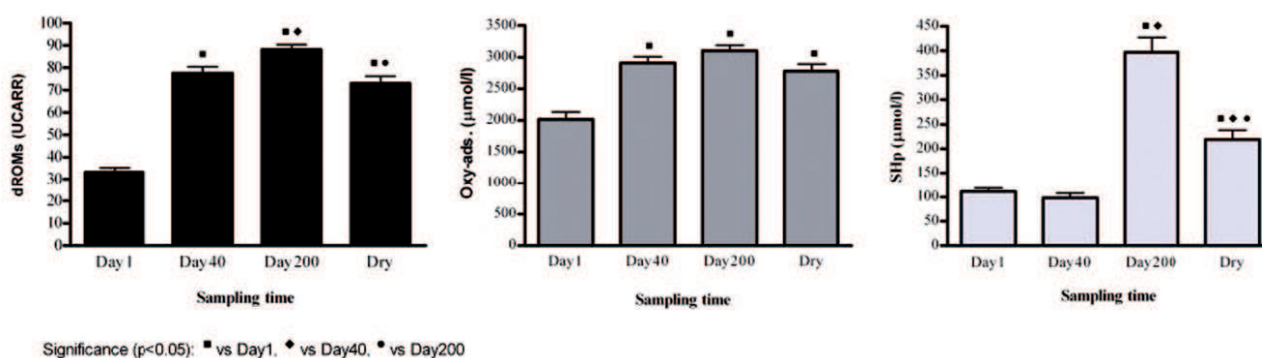


Figure 1. Mean values with relative standard deviations and statistical significance of daily patterns of dROMs, Oxy-ads. and SHp observed during different reproductive status.

References

- Castillo Rodríguez C, Wittwer Menge F, Cerón JJ. Oxidative Stress in Veterinary Medicine. *Veterinary Medicine International*. 2011;2011:812086.
- Halliwell B. Vitamin C: an antioxidant or pro-oxidant in vivo. *Free Radical Res* 1996;25:439-54.
- Piccione G, Borruso M, Fazio F, et al. Oxidative stress evaluation during milking period in the ewes. *J Appl Anim Res* 2006;29:109-12.
- Piccione G, Borruso M, Giannetto C, et al. Assessment of oxidative stress in dry and lactating cows. *Acta Agr Scan A-An* 2007;57:101-4.