Evaluation of serum homocysteine and oxidative stress during lactation in ewes

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ABSTRACT: The aim of the present work was to measure and evaluate the effect of the stage of lactation on serum homocysteine and oxidative stress in ewes. For our study ten Comisana ewes, 3 years old, clinically healthy, with the same level of production and during the same milking period were used and antioxidant barrier (Oxy-adsorbent), reactive oxygen species (dROMs), thiol antioxidant barrier (SHp) and homocysteine (sHcy) were investigated. One-way repeated measures analysis of variance (ANOVA), followed by Bonferroni's test, showed statistical differences ($P < 0.05$ was considered statistically significant). The statistically significant variations of dROMs, Oxy-adsorbent, SHp and sHcy during the experimental period concerned suggest an increase of oxidative processes at the end of lactation which are indicative of a response to this stress. The systematic analysis of oxidative stress and its influence on homocysteine levels are a valid instrument for the assessment of the health status of an animal and of good management procedures in a period such as lactation in which the homeostatic processes of organisms are altered.

Keywords: antioxidant barrier; reactive oxygen species; thiol antioxidant barrier; homocysteine; lactation; sheep

Oxidative stress, a particular kind of chemical stress, is caused by an imbalance between the production of free radicals and the capability of an organism to absorb their excess. It is extremely dangerous because it does not exhibit any symptoms and is recognizable with great difficulty by means of common methods of analysis (Piccione et al., 2007). The alteration of oxidative balance, if not adequately restored by the antioxidant barrier, induces an oxidative stress with cellular damage (Trevisan et al., 2001) which makes the organism sensitive to serious degenerative diseases (Fridovich, 1999; McCord, 2000). The formation of free radicals is a normal event in many pathological conditions and an overproduction is common during strenuous activities. Therefore, determination of free radicals is an index of oxidative stress, which is directly proportional to the condition of the organism. The exposure to oxidative stress has a very important role also in the metabolism of homocysteine (Zinellu et al., 2007).

Several studies have shown that plasma homocysteine levels were affected by dietary factors such as protein and vitamin deficiencies (Kalantar-Zadeh et al., 2003; Yeh and Yeh, 2006), by genetic background, and by several pathological conditions (Ueland et al., 2001). Some authors suggested protein restriction in pregnant- and lactating rats-induced oxidative stress and hypohomocysteinaemia also in their offspring (Fetoui et al., 2008). As previously observed by the high energetic requirements of lactation in sheep, the formation of free radicals is directly proportional to productive levels of the organism (Piccione et al., 2006). Given the remarkable impact of metabolic stress, it is interesting to establish the effects of the different lactation phases on the efficiency of antioxidant mechanisms. The objective of the present study was to measure and

evaluate the effect of the stage of lactation on serum homocysteine and oxidative stress parameters in ewes.

MATERIAL AND METHODS

Ten Comisana ewes, 3 years old, clinically healthy, with the same level of production and during the same milking period were used. During the experimental period, all ewes were not pregnant. They were taken to graze at 09:30 in the morning and to shelter at 16:30 in the afternoon. Ewes were milked in the morning from 07:00 to 09:00 and in the afternoon from 16:30 to 17:30 and were fed a supplementary diet once a day (with pellet feed, hay and water *ad libitum*).

Blood samples were collected by jugular venipuncture on days 1, 40 and 200 of lactation and during dry period, using vacutainer tubes (21 gauge × 1-inch needle, Vacutest 11030, Vacutest Klima s.r.l, Padova, Italy) with no additive at the same time in the morning (09:00). Blood samples were successively centrifuged at 3000 rpm for 20 min and the obtained sera were analyzed with a UV spectrophotometer (Slim SEAC model, Firenze, Italy) for the assessment of the following parameters: antioxidant barrier (Oxy-adsorbent), reactive oxygen species (dROMs) and thiol antioxidant barrier (SHp).

The assessment of the free radicals (dROMs) and the anti-oxidant power (Oxy-adsorbent and SHp) was done with the so-called "spin traps" system, which consists of molecules reacting with free radicals from complexes visible with a spectrophotometer.

The colorimetric d-ROMs test assesses the concentration of hydroperoxides (R-OOH), a class of reactive metabolites of oxygen, in a biological sample (serum, plasma, tissue and cells). The concentration of ROMs is expressed as Carratelli Units (1 CARR U = 0.08 mg (%) hydrogen peroxide).

The OXY-Adsorbent test assesses the anti-oxidant action of the plasmatic barrier by measuring its ability to contrast the oxidative action of hypochlorous acid. It is a highly oxidative compound that is synthesized in physiological situations from activated polymorphonucleated leucocytes and acts as an oxidant against bacterial attacks.

The colorimetric SHp test assesses the thiol antioxidant plasma barrier that contrasts the propagation of the peroxidative processes by inactivating both alkoxyl and hydroxyl radicals. This test is based on the ability of thiol groups to develop a coloured complex when reacted with DTNB (5.5-dithiobis-2-nitrobenzoic acid).

Serum total Hcy values were determined by high performance liquid chromatography (HPLC – Agilent 1100, BIO-RAD) with fluorimetric detection and isocratic elution (Pfeiffer et al., 1999). The fluorescence of the separated compounds was measured with a detector adjusted for excitation at 385 nm and emission at 515 nm. The total homocysteine concentrations were calculated by a calibration curve using the known amino acid concentrations and cystamine as the internal standard. All the results were expressed as mean ± SEM. In all ewes, the effect of sampling time on Oxy-adsorbent, dROMs, SHp and sHcy levels was examined using one-way repeated measures analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant. Bonferroni's multiple comparison test was applied for post-hoc comparison. Data were analyzed using the software STATISTICA 5.5 (StatSoft Inc., Tulsa, OK, USA).

RESULT AND DISCUSSION

Table 1 shows mean values (± SEM) of Oxy-adsorbent, dROMs, SHp and sHCy levels in ten Comisana ewes on different days of lactation and during dry period.

By applying one-way repeated measures analysis of variance, the following results were obtained:

dROMs ($F_{(3.27)}$ = 103.20, $P < 0.0001$);
Oxy-adsorbent ($F_{(3.27)}$ = 23.59, $P < 0.0001$);
SHp ($F_{(3.27)}$ = 58.51, $P = 0.008$);
sHcy ($F_{(3.27)}$ = 57.67, $P < 0.0001$).

dROMs showed a statistically significant increase on days 40 and 200 of lactation and during dry period compared to values obtained on day 1 of lactation, a statistically significant increase on day 200 of lactation compared to day 40 of lactation and a statistically significant decrease in dry period compared to day 200 of lactation.

Oxy-adsorbent showed a statistically significant increase on days 40 and 200 of lactation and during dry period compared to values obtained on day 1 of lactation.

SHp showed a statistically significant increase on day 200 of lactation and during dry period compared to values obtained on days 1 and 40 of lacta-

Table 1. Average values of dROMs, Oxy-adsorbent, SHp and sHCy, expressed in their conventional units of measurement with the related standard errors (± SEM) and statistical significance observed in ten Comisana ewes on days 1, 40 and 200 of lactation and during dry period

Parameters	Sampling time			
	day 1	day 40	day 200	dry
dROMs (U.Carr.)	33.30 ± 1.89	77.60 ± 2.85[1]	88.20 ± 2.20[1,2]	73.00 ± 3.18[1,3]
Oxy-ads. (µmol/l)	2 016.00 ± 115.7	2 912.00 ± 97.71[1]	3 105.00 ± 84.21[1]	2 777.00 ± 113.6[1]
SHp (µmol/l)	110.70 ± 7.46	98.70 ± 9.47	397.30 ± 30.10[1,2]	218.90 ± 19.40[1,2,3]
sHcy (µmol/l)	3.40 ± 0.17	6.02 ± 0.28[1]	5.73 ± 0.18[1]	7.27 ± 0.09[1,2,3]

[1]vs day 1; [2]vs day 40; [3]vs day 200 ($P < 0.05$)

tion and a statistically significant decrease in dry period compared to day 200 of lactation.

sHcy showed a statistically significant increase on day 200 of lactation and during dry period compared to values obtained on day 1 of lactation, a statistically significant increase on day 200 of lactation and in dry period compared to day 40 of lactation and a statistically significant decrease in dry period compared to day 200 of lactation.

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 (Piccione et al., 2006, 2007). Low 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 (Bertoni et al., 1984). The negative energetic balance promotes mobilization of lipids and gluconeogenesis, which induced an increase of free radicals, as shown by our results; although other authors demonstrated that seasonal rather than nutritional factors have a more pronounced effect on oxidative status markers in dairy goats (Di Trana et al., 2006).

The high values of Oxy-adsorbent and SHp at the end of lactation document the compensative response of the organism to oxidative stress. The power of the antioxidative agents is related to their capacity of interaction with several groups of free radicals (Chaudiere and Ferrari-Iliou, 1999).

As previously described by Memisođullari et al. (2008) during exposure to oxidative stress, the uptake and utilization of sulphur-containing amino acids, such as cysteine and methionine, increased. The transsulphuration and transmethylation pathways of methionine metabolism provide cysteine and methionine needed for the synthesis of antioxidants. Methionine is converted to S-adenosylmethionine (SAM), and SAM is converted to S-adenosylhomocysteine (SAH); ultimately, SAH is also converted to Hcy (Memisođullari and Akcay, 2004; Panayiotidis et al., 2004). Consequently, the oxidative stress produced by lactation increases serum Hcy levels. In humans, elevated Hcy levels indicate a risk factor of cardiovascular disease and of all pathologies associated with endothelial dysfunction (Zinellu et al., 2007; Moat, 2008). Then hyperhomocysteinaemia, which occurs during lactation, could lead to a similar disease in ewes.

Hence the systematic analysis of oxidative stress and homocysteine levels is a valid instrument for the assessment of the health status of an animal in a period such as lactation in which the homeostatic processes of organisms are altered. It is thus useful to consider the oxidative status as a reliable index of animal welfare to be able to demonstrate possible preventive alterations of the oxidative steady state before the beginning of degenerative pathologies.

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Received: 2008–07–02
Accepted after corrections: 2008–08–04

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