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Research

Livestock handling and road transport influence some oxidative stress parameters in ewes

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ABSTRACT

The aim of this study was to evaluate the influence of shearing and road transport on reactive oxygen species (dROMs), antioxidant barrier (Oxy-ads), and thiol antioxidant barrier (SHp) concentrations in healthy Comisana ewes. Thirty animals were divided into 3 equal groups: group A was used as the control, group B was shorn, and group C was transported by road for 6 hours. For animals in experimental groups (groups B and C), blood samples were collected before (T0) and immediately after (T1) the shearing and/or road transport procedures and 24 (T2) and 48 (T3) hours from the end of each procedure. For the control group, blood sampling was performed for the same time/data points as for the experimental groups. A 2-way repeated-measures analysis of variance showed significant higher dROMs, Oxy-ads, and SHp values ($P < 0.05$) in group B compared to control group at T3. Group C showed significant higher dROMs, Oxy-ads, and SHp values ($P < 0.05$) than control group at T1, T2, and T3. The results obtained in this study suggest that road transport mostly affected the oxidant/antioxidant status in ewes. In addition, the trend of dROMs values together with trend of Oxy-Ads and SHp values found in our study suggest that despite the increment of oxidative phenomena occurring following shearing and transport, oxidative homeostasis was maintained by the efficiency of the antioxidant defenses both in shorn and transported ewes.

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Introduction

The routine management procedures in farm animals, such as shearing and road transport, induce a stress response that is conditioned by a number of factors including individual sensitivity and relative intensity (Carcangiu et al., 2008, 2018). Psychological stress due to farm management or an inappropriate housing condition is thought to elevate oxidative stress in ewes (Pregel et al., 2005). Oxidative stress occurs when oxidative substances and, particularly, reactive oxygen species overwhelm antioxidant defenses (Pourova et al., 2010). The balance between antioxidants and pro-oxidant compounds at a cellular level represents an important determinant of various physiological processes. Maintenance is the main aim of so-called an integrated antioxidant system built in the animal body. In farm animals, oxidative stress is involved in a number of pathological conditions, including those associated with animal production, reproduction, and welfare (Lykkesfeldt and Svendsen, 2007). The alteration of the oxidative balance, if not adequately restored by the antioxidant barrier (Oxy-ads), induces an oxidative stress with cellular damage (Trevisan et al., 2001) that makes the organism sensitive to serious degenerative diseases (McCord, 2000). In the last few years, the evaluation of oxidative stress has become a field of research in veterinary medicine and particularly in ruminant health (Celi, 2010; 2011). Stressful conditions lead to an excessive production of free radicals resulting in an imbalance between oxidants and antioxidants in favor of oxidants at the cellular or individual level (Khadija et al., 2009). The formation of free radicals is a normal event in many pathological conditions. An overproduction of free radicals is common during strenuous activities. However, even under moderate stress conditions and without clinical problems, the induced inflammatory status is always accompanied by oxidative status. Road transport

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and shearing are critical phases and often are considered as causes of stress, raising considerable interest both in economic and animal welfare terms (von Borell, 2001). Some researchers have monitored the oxidative stress parameters in ruminants when the homeostasis is altered (Castillo et al., 2005; Stefanon et al., 2005; Mutinati et al., 2013). Particularly, many studies were carried out to assess the influence of shearing on homeostatic balance of sheep showing changes of oxidative stress parameters in shorn ewes respect to unshorn ewes (Piccione et al., 2003; Pennisi et al., 2004; Piccione et al., 2008). We know that road transport influences oxidative status biomarkers (Nazifi et al., 2007; Idrus et al., 2010; Onmaz et al., 2011; Piccione et al., 2012) and that transport may cause injury, increased morbidity and mortality rates, and reduce performance.

Knowing the extent of the oxidative stress to which the animals are subjected during the different management procedures could be useful for the design of correct food integration with antioxidants of natural origin that can counteract the rise of reactive oxygen species, thereby improving animal welfare and providing advantages of increasing productivity. Therefore, the aim of the present study was to evaluate and compare the modifications of reactive oxygen species (dROMs), Oxy-ads, and thiol antioxidant barrier (SHp) in ewes after routine livestock management procedure such as shearing and road transport.

Material and methods

Ethical statement

All experimental procedures were carried out in accordance with European legislation regarding the protection of animals used for scientific purposes (European Directive 2010/63), as recognized and adopted by the Italian law (DL 2014/26).

The animals enrolled in the study have not been subjected to stressful conditions experimentally, as all the procedures representing the experimental protocol of the current survey, including the shearing and the transport, fall within the routine management procedures of the farm. In particular, animals were monitored during routine shearing and transport to the buying farm because the ewes were intended for sale. Blood sampling was concurrently performed with permission and by the authorized veterinarian. In Italy, routine procedures, including sampling of biological samples such as blood or milk, do not require an authorization, an ID, or protocol number.

Animals

The study was done on 30 nonpregnant clinically healthy Comisana ewes during the mild-dry season of May-June in Sicily (Italy). All the animals enrolled in the study were defined clinically healthy with no evidence of disease and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart rate, respiratory profile, appetite, fecal consistency, and hematologic profile (data not showed). All the animals were fed with the same constant diet composed of good-quality alfalfa hay and a concentrate mixture that consisted of the following ingredients: oats 23%, corn 36%, barley 38%, and mineral and supplements 3%. About 200 g/animal of concentrate was distributed once daily, whereas hay and water were available ad libitum.

The animals were divided into 3 groups: group A (n = 10, aged 3-4 years and with a mean body weight of 55.40 ± 3.60 kg) was used as the control group, group B (n = 10, aged 3-4 years and with a mean body weight of 55.80 ± 4.2 kg) was shorn by means of hand shears, and group C (n = 10, aged 3-4 years and with a mean body weight of 55.00 ± 3.20 kg) was transported by road.

The experiment was carried out concurrently on the 3 studied groups, and each group was housed in an individual pen (10 × 7m) in the same farm located in Sicily, Italy (38°00′49″N 15°25′18″E, 80 m above sea level) under natural photoperiod during Summer 2015 (sunrise at 04.35 AM, sunset at 07.15 PM). For all experimental period, temperature and relative humidity were continuously recorded with a data logger (Gemini, Chichester, West Sussex, UK). Ewes of group C were transported within a single deck trailer 2.0 × 3.6 × 1.6 m (width × length × height) at a stocking rate of 0.27 m^2 per animal, in compliance with EC Regulation 1/2005 provisions. The route taken during each transport event included a mixture of principally flat main roads (speed limit 50 to 70 km/h, including stops for traffic lights only) and highways (continuous driving at the speed limit of 70 to 100 km/h). The length of the journey was approximately 510 km, and it took approximately 6 hours. Ewes of group C had access to feed until the beginning of loading procedures, but additional feed was not provided during travel. None of the animals had previous experience of truck transport nor were they familiar with the vehicle, loading, or unloading procedures.

The respective climatic conditions, together with the temperature-humidity index (THI), recorded at all sampling times for each group are showed in Figure 1. The THI, an indicator of thermal comfort for ewe, was calculated using the U.S. Weather Bureau's Temperature Humidity Index Formula for ruminant species (Potter and Jacobsen, 2000):

THI (°C) = T°ambient + (0.36 × point of steam condensation) + 41.5.

Blood sampling and laboratory analysis

From animals of experimental groups (groups B and C), blood samples were collected via jugular venipuncture into vacutainer tubes containing clot activators (Terumo Corporation, Tokyo, Japan) before (T0) and immediately after (T1) the shearing and/or road transport procedures and 24 (T2) and 48 (T3) hours from the end of the shearing and/or road transport procedures. Blood sampling from shearing sheep was synchronized to the shearing time of each animal.

On control group (group A), blood sampling was performed on the same data points of experimental groups. Blood samples were centrifuged at 3000 × *g* × 20 minutes, and the obtained sera was immediately analyzed by the same operator using a UV spectrophotometer (model Slim SEAC, Firenze, Italy) to assess the concentration of dROMs, Oxy-adsorbent (Oxy-ads), and SHp. These techniques are based on the "spin traps" system, in which molecules react with free radicals, creating complexes revealed by spectrophotometry.

The dROMs test (Alberti et al., 2000) is a colorimetric test that is based on the capacity of transition metal ions to generate in vitro alkoxyl and peroxyl radicals in the presence of hydroperoxides. A chromogenic reagent (N,N-diethyl-para-phenylendiamine) is then added to this solution. This chromogen possesses the feature of being oxidized by hydroperoxyl and alkoxyl radicals and transformed into a pink to red colored cation. The concentration of the colored complex is directly related to the hydroperoxide levels of the sample. The concentration of ROMs, that directly parallels with color intensity, is expressed as Carratelli Units (1 CARR U = 0.08 mg % hydrogen peroxide). Increased values directly correlate to increased levels of oxidative stress. The oxy-ads test (Gerardi et al., 2002) evaluates the ability of plasma to oppose the massive oxidant action of an excess of hypochlorous acid (HClO) in water solution by assessing photometrically the residual unreacted radicals of the acid. The sample is subjected to massive oxidation by HClO; the

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