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Shearing induces secondary biomarkers responses of thermal stress in sheep

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The study involved 30 non-pregnant, clinically healthy Comisana sheep in order to evaluate the effect of shearing on rectal temperature (RT), respiratory rate (RR), heart rate (HR), white blood cells (WBC), red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb) and total proteins (TP). During the spring season the animals were divided into two groups: twenty sheep, designated as the experimental group, were shorn (Group A), and ten sheep, designed as the control group, were left unshorn (Group B). All animals were weighed using a mechanical balance in the morning before shearing and after shearing. During the experimental period ambient temperature and relative humidity were recorded, while the temperature-humidity index was calculated. The measurements of studied secondary stress parameters were assessed before shearing (day 0), 1, 15, 30, 45 and 60 days after shearing in the experimental group and at the same time points in the control group. Two-way repeated measure analysis of variance (ANOVA), followed by Bonferroni's multiple post hoc comparison test, showed a significant effect of shearing and time on RT, RR, HR, RBC, Hct and Hb. These parameters play an important role in providing complementary information for the assessment of thermal stress, since they are affected in response to the stress of shearing, suggesting that they are useful secondary stress indices in shorn sheep. These modifications are useful to monitor the stressful conditions, but also to evaluate health and welfare of animals, and to improve their productivity.

KEYWORDS: secondary stress biomarkers / sheep / shearing / thermal stress

Environmental factors that directly and adversely affect health and welfare of animals include thermal irradiation and airspeed together with the temperature-

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humidity index (THI), which is the sum of the effects of ambient temperature and relative humidity, capable of estimating the degree of thermal stress [Shehab-el-deen et al. 2010]. In recent years many detrimental effects of thermal stressors on health of animals have been studied considering the relationships between behavioural, physiological and biochemical indicators in the evaluation of the adaptive capacity and consequently "welfare" of the animals [Sanger *et al*. 2011, Waltz *et al*. 2014]. In particular, studies on shorn sheep exposed to extreme environmental conditions or to a climatically mild atmosphere, have demonstrated that shearing induces adaptive thermogenesis in animals [Caola *et al.* 1998, Al.Ramamneh *et al*. 2014]. In fact, shearing is a necessary routine practice in the management of sheep, at the same time constituting a stressor that may compromise welfare [Dikmen *et al*. 2011, Sanger *et al*. 2011]. In sheep the maintenance of homeothermy is influenced by their fleece, which represents an insulating layer protecting the animal against both heat and cold [Sleiman and Abi Saab 1995, Pennisi *et al*. 2004]. It has an extremely low thermic conductivity (0.00091 kcal s^{-1} cm^{-1} $°C^{-1}$) and both in winter and summer it maintains a high thermic gradient between atmosphere and the skin [Piccione and Caola 2003]. In fact, the conditions of the outer coat layer are modified by fleece removal, resulting in a change of thermal conductance. The breadth of the thermoneutral zone, defined as the range of ambient temperature, within which the metabolic rate is at a minimum and temperature regulation is achieved by a non-evaporative physical process alone, is significantly correlated to fleece length in the sheep. In fact, it has been demonstrated that shearing in different seasons significantly affectsthermoregulation and some physiological and blood parameters [Suhair and Abdalla 2013, Piccione *et al.* 2006, 2008]. Moreover, shearing influences oxidative parameters, causing a change in the sheep's homeostatic balance that leads to oxidative stress [Piccione *et al*. 2011]. Thus, thermal stress due to shearing could evoke both a primary and secondary response. In vertebrates, the primary stress response involves the release of hormones and activation of the hypothalamic-pituitary-interrenal axis initiating a secondary stress response thorough variations of secondary indices of stress [Eslamloo *et al*. 2014, Wells *et al*. 1999] that may be used to assess the adversity of the thermal environment, which may affect the growth, lactation and reproduction of dairy cows [Hahn 1999, West 1999].

Thus, considering that climatic conditions and shearing can influence thermoregulatory mechanisms and welfare of sheep, the aim of this study was to evaluate not only the haematological and haematochemical profile, but also to study the pattern of some physiological and biochemical parameters that could be considered as secondary stress biomarkers in shorn sheep.

Material and methods

The present study, carried out during the spring season in Sicily (Italy) at an altitude of 230 metres above sea level (latitude: 37° 19' 19" and longitude: 13° 35' 23"), involved a laboratory component and a veterinary clinic component at the

Department of Veterinary Science, University of Messina. All treatments, housing and care of animals were reviewed and approved in accordance with the standards recommended by the US National Research Council's *Guide for the Care and Use of Laboratory Animals* and European Union's Directive 86/609 CEE.

The study involved 30 non-pregnant, clinically healthy Comisana ewes divided into two groups: subjects of Group A (n=20, aged 2-3 years old and weighted 50.30±2.6 kg), designated as experimental group, were shorn, and subjects of Group B (n=10, aged 2-3 years old and weiging 51.40±3.2 kg), designed as the control group, were left unshorn. In terms of feeding conditions, Groups A and B were fed daily on hay (2 kg), wheat straw (1 kg), wheat concentrate (0.5 kg) and water *ad libitum*.

All animals were weighed using a mechanical balance before shearing and after shearing (1, 15, 30,45 and 60 days from the shearing date). Before shearing the subjects of each group have been also subjected to the measurement of Body Condition Score (BCS, 0 to 5 scale). The sheep' BCSs were also measured 30 and 60 days after shearing. The measurements of secondary stress parameters were assessed before shearing (day 0), 1, 15, 30, 45 and 60 days after shearing in the experimental group and at the same time points in the control group.

Rectal temperature (RT) was measured using a digital thermometer (HI92704, Hanna Instruments Bedfordshire, UK), which probe was inserted into the rectum to a depth of 9 cm. Heart rate per minute was determined by auscultation with a stethoscope, and respiratory rate per minute was assessed by observation of chest and abdomen movements, and auscultation with a stethoscope.

From all sheep blood samples were collected via a jugular venipuncture using two different vacutainer tubes: one tube containing ethylenediamine tetraacetic acid (EDTA) as an anticoagulant agent for haematological analyses, while the other tube was without any anticoagulant agent for the assessment of total proteins using a standard kit. Particularly, blood samples containing EDTA, White blood cells (WBC), Red blood cells (RBC), Haematocrit (Hct) and Haemoglobin (Hb) were assayed using an automatic analyzer of haematology (HecoVet, SEAC, Florence, Italy). The tubes, used for the evaluation of total proteins, were centrifuged at 3.000 rpm for 10 minutes, providing serum which was stored at -20°C until analysed. The concentration of total serum proteins was determined by the biuret method using a UV Spectrophotometer (SEAC, Slim, Florence, Italy). All samples were refrigerated and analysed within 24 hours.

Ambient temperature (AT) and relative humidity (RH) for the entire experimental period were continuously recorded with a data logger (Gemini, Chichester, West Sussex, UK). The temperature-humidity Index (THI), used as an indicator of thermal comfort for sheep, was calculated from dry-bulb temperature and relative humidity according to the following equation:

The temperature-humidity index (THI) was calculated using the following equation:

$$THI_{[°C]} = AT - 0.55\ (1 - 0.01RH)\ (AT - 14.5)$$

where:

AT – ambient temperature (°C);

RH – relative humidity (%).

All the results were expressed as mean values ± standard deviation (SD). Data were normally distributed ($P<0.05$, Kolmogorov-Smirnov test). Two-way repeated measure analysis of variance (ANOVA) was performed to determine significant differences between Groups A and B, the statistically significant effect of sampling time and the interaction term Time x Groups. The level of significance was set at <0.05. Bonferroni’s multiple post hoc comparison test was applied. The data were analyzed using STATISTICA 8 (Stat Soft Inc.) software package.

Results and discussion

As shown in Figure 1, during the experimental period the environmental temperature remained almost constant with an average value of 27.5°C, while relative humidity proved to be quite variable with a peak at 62%.

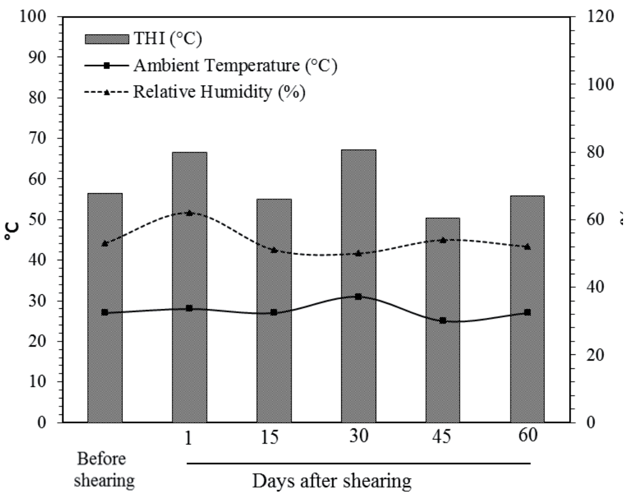

Fig. 1. Averages of ambient temperature, relative humidity and temperature-humidity index, expressed in their units of measurement, recorded during the experimental period.

The average values of body weight for Groups A and B are presented in Table 1. Two-way ANOVA showed no significant effect of shearing or time on body weight. The average BCS values (±SEM) obtained in sheep of Group A before shearing, 30 and 60 days after shearing and the average BCS values (±SEM) obtained in sheep of Group B, at the same time points as for Group A, are presented in Table 2. ANOVA was applied for BCS, showing that BCS did not significantly change.

Table 1. Averages (±SD) of body weight, expressed in kg, obtained in group A (shorn ewes) and group B (unshorn ewes) during the experimental period

Parameter	Group	Before shearing	Days after shearing				
			1	15	30	45	60
Body weight (kg)	A	49.20±5.12	47.60±4.94	47.48±4.95	47.60±4.80	48.60±4.74	48.92±5.02
	B	48.60±4.05	48.70±4.18	48.84±4.59	48.64±4.22	48.60±4.38	48.54±4.12

Table 2. Averages (±SD) of Body Condition Score in group A (shorn ewes) and group B (unshorn ewes) before shearing, 30 days and 60 after shearing

Body Condition Score	Group A		Group B	
	mean	SD	mean	SD
Before shearing	2.84	0.10	2.78	0.08
30 days after shearing	2.75	0.07	2.66	0.07
60 days after shearing	2.71	0.08	2.64	0.09

Table 3. Averages (±SD) of rectal temperature (RT), respiratory rate (RR), heart rate (HR), white blood cells (WBC), red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb) and total proteins (TP), expressed in their units of measurement, obtained in group A (shorn ewes) and group B (unshorn ewes) during the experimental period

Parameter	Group	Before shearing	Days after shearing				
			1	15	30	45	60
RT (°C)	A	38.97^{a}±0.07	39.40^{b}±0.06	39.31^{b}±0.06	39.14^{ab}±0.05	39.15^{ab}±0.07	39.02^{a}±0.04
	B	39.08±0.09	39.16±0.08	39.14±0.07	39.15±0.08	39.13±0.07	39.19±0.05
RR (breaths/min)	A	30.30^{a}±0.92	39.80^{b}±1.00	32.15^{a}±0.91	31.05^{a}±1.28	31.20^{a}±0.97	31.40^{a}±0.93
	B	30.86±1.02	30.92±0.99	30.48±0.87	30.54±1.15	30.22±1.22	30.42±0.89
HR (beats/min)	A	73.80^{a}±7.60	84.40^{b}±7.44	76.28^{a}±7.64	72.32^{a}±7.04	71.80^{a}±8.12	72.00^{a}±8.94
	B	74.00±8.72	74.80±7.94	74.60±9.40	76.02±9.42	72.88±9.28	74.10±8.80
WBC (K/μL)	A	11.88±0.52	11.53±0.62	11.34±0.57	11.77±0.45	11.80±0.69	12.07±0.72
	B	11.48±0.74	11.49±0.56	10.98±0.78	11.55±0.58	11.40±0.66	11.64±0.42
RBC (M/μL)	A	9.05^{a}±0.33	10.32^{b}±0.33	10.17^{ab}±0.24	9.82^{ab}±0.31	9.51^{ab}±0.28	9.79^{ab}±0.25
	B	9.14±0.32	9.22±0.34	9.46±0.23	9.09±0.13	9.22±0.24	9.12±0.25
Hct (%)	A	28.09^{a}±0.49	30.81^{b}±0.52	29.22^{b}±0.50	28.66^{a}±0.43	28.25^{a}±0.53	28.32^{a}±0.50
	B	28.16±0.52	28.89 ± 0.54	28.95±0.67	28.12±0.43	28.72±0.65	28.22±0.43
Hb (g/dl)	A	9.51^{a}±0.22	10.50^{b}±0.32	9.38^{ab}±0.23	8.52^{a}±0.27	8.60^{a}±0.28	9.69^{a}±0.26
	B	9.98±0.37	9.68±0.27	9.55±0.22	9.96±0.33	9.99±0.29	9.49±0.32
TP (g/dl)	A	7.70±0.26	7.50±0.23	7.65±0.26	7.80±0.21	7.07±0.32	7.24±0.39
	B	7.63±0.38	7.54±0.30	7.24±0.22	7.44±0.39	7.12±0.33	7.13±0.32

^{ab}Means bearing the same superscripts at different sampling times within the same parameter represent differ significantly at $P<0.05$.

Table 3 shows average values of secondary stress parameters during the experimental period in sheep of Groups A and B. At the beginning of the experimental period, all studied parameters did not show any significant differences between the Groups, confirming homogeneity of the sheep population. Moreover, a time x groups interaction was not significant. ANOVA showed a statistically significant effect of shearing ($P<0.001$) and a significant effect of time ($P<0.05$) on RT, RR, HR, RBC, Hct and Hb. The analysis of the results obtained during the experimental period in the present study suggests that these parameters could be considered as secondary stress indices in the Comisana sheep breed. Particularly, when exposed to thermal stress, animals showed responses including changes of RT, RR, HR, RBC, Hct and Hb between sheep of two groups, but also within the fleeced group.

The thermoregulation ability is a basic physiological attribute in mammals that is strongly influenced by the effectiveness of external insulation. In particular, shearing facilitates heat dissipation, which implies changes in body heat content and which ultimately affects body temperature. However, a change of heat production may be considered as an adaptive response and a capacity of sheep to adjust to the environment, because the benefits from shearing promoting thermoregulation depend largely on environmental conditions, considering the insulating properties of fleece [Piccione and Caola 2003, Beatty *et al*. 2008]. In fact, the physical stress of shearing and consequently heat stress produce a transient increase in rectal temperature, as stress-induced hyperthermia has been demonstrated in a variety of homeothermic species [Piccione *et al*. 2002, Silanikove 2000, Waltz *et al*. 2014].

After shearing the respiratory rate statistically significantly increases in shorn sheep, probably because it tends to closely follow heat loss by evaporation [Pennisi *et al.* 2004]. It has been observed that an increase of respiratory rate is a major method of thermal dispersion. Thermoregulatory heat loss by panting is achieved through an increase in respiratory frequency coupled to a reduction in respiratory volume [Piccione *et al.* 2008].

Heart rate shows a similar trend in respect to respiratory rate due to the direct effect of heat stress inducing haemodynamic adaptations, because the animals produce high quantities of heat dispersed by an enlarged peripheral vascular system [Pennisi *et al*. 2004; Piccione *et al.* 2008]. In agreement with previous results [Alieksiev 2008], the observed increase in heart rate in shorn sheep could reflect a relatively unessential enhancement of basal metabolic activity. Moreover, the gradual decline after the elevation in the heart rate after shearing could also indicate a downward shift in the temperature.

It is also evident by an elevation of haematological parameters that shorn sheep are highly sensitive to thermal stress. It has been shown that thermal stress increases skin blood flow to promote heat loss [Collin *et al*. 2001]. This blood redistribution to the skin causes a reduction of blood flow to other tissues; therefore, a significant increase of red blood cells, haematocrit and haemoglobin after shearing could be caused by a transient variation of blood circulation that occurs during heat stress conditions, as previously observed in other species [Waltz *et al.* 2014]. This hypothesis is supported by the fact that the haematological parameters had the same time course as the studied physiological parameters reflecting stress caused by heat exposure.

In conclusion, the results confirm that fleece removal is one of the most stressful events in sheep's life from the point of view of thermal stress caused by shearing. The findings showed that rectal temperature, respiratory and hear rate, red blood cells, haematocrit and haemoglobin are useful secondary stress indices in shorn sheep. These parameters play an important role in providing complementary information for the assessment of thermal stress, since they are affected in response to the stress of shearing, thus suggesting that these modifications are useful not only for the monitoring of the stressful conditions, but also to evaluate health and animal welfare, and to improve their productivity.

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