The effect of shearing procedures on blood levels of growth hormone, cortisol and other stress haematochemical parameters in Sarda sheep

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The aim of this research was to investigate how growth hormone (GH) cortisol and some haematochemical parameters could be modified by the stress caused by the stages of shearing in Sarda breed sheep. Five groups of 10 sheep each were formed. Group A, only separated from the flock; Group B, only tied; Group C, both tied and shorn (animals in these three groups were ewe lambs shorn for the first time); Group D, adult females both tied and shorn; and Group E, adult entire males both tied and shorn (animals in these two groups had been shorn previously). Five blood samples were taken from each animal: the day before treatment (first sample); at the start of the treatment (second sample); in the middle of shearing for Groups C, D and E, 10 min after separation in Group A and 10 min after tying in Group B (third sample); at the end of treatment (fourth sample); and on the day after treatment (fifth sample). Plasma GH levels showed a decrease (P < 0.01) in Groups A, B, C and D during treatment (third and fourth samples), while Group E only at the end of shearing (fourth sample). In the third sample, the highest GH levels were recorded for Group E (P < 0.05), while it was recorded in the fourth sample for Groups A and E (P < 0.05). Cortisol levels showed a clear increase (P < 0.01) in all groups during treatment, but Group A showed a decrease in the fourth sample in comparison to the third sample. Males in the second, third and fourth sample and Group A only in the fourth sample showed lower cortisol levels when compared with the other groups (P < 0.05). Plasma glucose levels showed an increase (P < 0.01) in all groups during treatment but Groups B, C and E showed the highest values (P < 0.05). Magnesium (Mg) showed an increase in all groups in the third and fourth sample, while sodium (Na), in the same samples, only in Groups B, C and D. Potassium (K) values showed a significant decrease (P < 0.05) only in Groups C and D at the end of shearing. These results show that GH secretion is influenced by all the stress procedure: separation, tying and shearing. Shearing, even if necessary for animals, causes a significant change of the blood parameters involved in the stress response.

Keywords: cortisol, GH, shearing, sheep, stress

Introduction

The usual management procedures in sheep breeding, such as transportation, foot treatment, pharmacological treatment, shearing, isolation, capture and so on, induce an endocrine and metabolic response known as stress (Balduck and Sibly, 1990; Hargreaves and Hutson, 1990a). The severity of the reaction is governed by a number of factors including individual sensitivity, unfamiliarity to the stimulus and the relative intensity (Grandin, 1997). The response is also conditioned by social position and by sex within the group, since dominant animals and males have a higher resistance to stress, as evidenced by a lower cortisol concentration in blood (McGlone et al., 1993; Turner et al., 2002). Furthermore, novelty produces a strong hormonal and metabolic reaction in animals; how quickly they get used to a particular stimulus will depend on its intensity, and a gradual approach to the stress factor reduces the response (Dantzer and Mormede, 1983; Grandin, 1997). The reaction to the above-mentioned stress factors is mainly centred in the activation of the sympathetic system and the hypothalamic–hypophysis–adrenal axis through catecholamine and glucocorticoid production (Miller and O’Callaghan, 2002). These hormones render the animals alert, thus giving them the
ability to react to environmental stimuli, in order to preserve organic homeostasis (Herman and Cullinan, 1997). Glucocorticoids and catecholamines also cause a rise in glycaemia, starting from glucidic and non-glucidic substrates such as proteins and an increase of non-esterified fatty acid (NEFA), derived from lipid mobilization (McMahon et al., 1988; Mormede, 1988). Taken together, these effects produce a greater availability of energy for the brain and muscles, and thereby a more efficient behavioural response to stress. Glucocorticoids and catecholamines provoke the increase of blood sodium (Na) and a decrease of potassium (K), modifying the electrolytic equilibrium (McDonald, 1975). Moreover, blood magnesium (Mg) is affected by cold-exposure stress (Achmadi et al., 2001), but little is known regarding the relation between the shearing procedure and this last mineral. Growth hormone (GH) is known regarding the relation between the shearing procedure and this last mineral. Growth hormone (GH) is among the various hormones involved in stress response, though its concentration during stress differs between species. Stress causes an increase in the plasma concentration of GH in humans (Greenwood and Landon, 1966), monkey (Meyer and Knobil, 1967) and pig (Machlin et al., 1968), while the opposite occurs in rodents (Muller et al., 1967). GH secretion is not modified by surgical stress in dogs, or by restraint in dark chambers in sheep (Lovinger et al., 1974; Cronin et al., 1981). The action of this hormone in a situation of acute stress, like shearing, is not yet known, but it plays an important role in the regulation of several physiological processes such as growth, lactation and other metabolic effects (Gluckman et al., 1987). The events leading to shearing must be among the most stressful, since the animal is penned for capture, separated from the flock, thrown down and finally shorn (Hargreaves and Hutson, 1990a). However, effects of tying of the animals on stress are not known as properly as the other procedure, but this practice is always utilized during shearing in Sardinian sheep management.

Therefore, in order to determine how the various stages of shearing affect GH secretion, the levels of this hormone in blood, along with cortisol concentrations and a number of blood parameters, were analysed in adult female and male sheep, and in ewe lambs with no previous experience of shearing. The different age and gender categories of animals were utilized to compare the effect of previous experience and sex on shearing procedure hormonal response.

Material and methods

Animals
The study was carried out on a flock of about 400 Sarda breed sheep. The location was in Sardinia, at 39° North Latitude, 600 m above sea level. During the day, the animals grazed on natural pasture, and were penned at night, when they received a concentrated commercial food-supplement of 200 g per head (crude protein (CP) 20.4% and 12.5 MJ metabolizable energy (ME)/kg dry matter (DM)). The sheep also had free access to hay (CP 11.1% and 7.2 MJ ME/kg DM) and water. According to the traditional techniques of Sardinian sheep breeding, the animals are shorn once a year, in late spring. Fifty sheep were randomly chosen from the flock, according to the following criteria: 30 previously unshorn ewe lambs, aged about 8.1 months; 10 adult ewes of about 4 years; and 10 entire adult males of about 4.7 years of age. Adult ewes and males had been shorn in the previous years.

Experimental procedures
The animals were divided into the following experimental groups:

Group A: 10 ewe lambs; they were separated from the flock, and kept together in a pen from which they could not see the rest of the flock.

Group B: 10 ewe lambs; they were separated from the flock, and their feet were tied. These were not shorn, but were kept tied and placed in the lateral decubitus position during the treatment.

Group C: 10 ewe lambs; they were separated from the flock, their feet were tied and subsequently shorn and kept in the shearing pen.

Group D: 10 adult ewes; they were separated from the flock, their feet were tied, they were shorn and kept in the shearing pen.

Group E: 10 entire males; they were separated from the flock, their feet were tied, they were shorn and kept in the shearing pen.

For each animal, treatment (separation, tying of the feet and shearing) was performed, on the same day, without any interruption. The whole treatment lasted approximately 20 min for each sheep, whether shorn or unshorn, and it took about 2 h to perform the treatments on all five groups. Shearing was performed by hand using traditional shearing scissors in a 15 × 10 m pen. The animals were led into a pen, where they were fed, before being led into the shearing area through a sliding door. After the process, the animals were released into an adjacent pen.

Blood samples
Five samples of blood were taken by one puncture from the jugular vein of each animal, using sample tubes containing heparin (Becton Dickinson, Plymouth, UK).

First sample: the day before shearing at 0900 h, for all the groups.

Second sample: just after the start of separation from the flock, at 0900 h, for all the groups.

Third sample: 10 min after the second sample. This sample was collected while animals of Groups B, C, D and E were kept tied, animals of Groups C, D and E were also being shorn and animals of Group A were only separated from the rest of the flock.

Fourth sample: 10 min after the third sample, just before the feet were untied in animals of Groups B, C, D and E.

Fifth sample: the day after, at the same time as the first sample (0900 h).
The blood was immediately centrifuged at 3000 rpm for 20 min at 4°C, and the plasma was frozen at −20°C until analysis.

Hormone analysis
Plasma concentration of GH was quantified by a heterologous double-antibody radioimmunoassay (RIA), according to Celi et al. (2003). The method was developed using a rabbit antiseraum to bovine GH (NHI-GH-B13) at a final dilution of 1 : 7000. The cross-reactivity was as follows: GH 100%; prolactin (PRL) 0.1%; LH 0.01%; and FSH, thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and caprine placental lactogen (cPL) < 0.01%. Assay sensitivity (90% B/B0) was 0.5 ± 0.12 ng per tube. Purified ovine GH (NIDDK-oGH) was used for radioiodination according to Salacinsky et al. (1981); specific activity was 27.6 μCi/μg. The same preparation (NIDDK-oGH) was used as the reference standard. The within-assay coefficient of variation (WACV) and the between-assay coefficient of variation (BAVC) were 9.7% and 13.8%, respectively. Plasma levels of cortisol were determined as reported by Tamanini et al. (1983) using a RIA method. The sensitivity (90% B/B0) of the cortisol antibody was 4.9 ng/ml, and the cross-reactivities were as follows: 20.4% with cortisone, 74.6% with deoxycortisol-11α, 1.1% with corticosterone and 0% with progesterone and oestrogens.

Analysis of metabolites
Plasma glucose and Mg were analysed using enzymatic colorimetric methods, GOD-POD-PAP and HK-G6PDH, respectively (Sentinel Chemical, Milano, Italy). Na and K concentrations were determined by a flame spectrophotometer (Corning Flame Photometer 410; Corning Medical and Scientific, Corning, NY, USA).

Statistical analysis
Hormone profile data were analysed by repeated measure analysis of variance (ANOVA) using MINITAB Statistical software (Minitab Inc. 2000, State College, PA, USA) for the comparison of plasma concentrations of the various hormones and metabolites over time. Sources of variation were group, sample and their interaction. All data are reported as mean ± SD; differences with P < 0.05 were considered significant.

Results
Mean plasma GH levels showed a progressive drop in all the groups during the treatments. Plasma GH levels decreased (P < 0.01) in Groups A, B and C during (third sample) or at the end of treatment (fourth sample), while at the beginning (second sample) in Group D, but only at the end of treatment in Group E (P < 0.01). GH levels in Group E were higher than those in the others during the third sample, whereas at the end of treatment (fourth sample) those in Group E were higher than those in the others during the third sample, whereas at the end of treatment (fourth sample).

Table 1 Mean ± s.d. values of growth hormone (GH) (ng/ml) in the groups during sampling

<table>
<thead>
<tr>
<th>Groups</th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Fourth sample</th>
<th>Fifth sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.3 ± 3.4a</td>
<td>5.8 ± 11.9b</td>
<td>2.5 ± 2.5bc</td>
<td>3.5 ± 2.1by</td>
<td>5.9 ± 2.7b</td>
</tr>
<tr>
<td>B</td>
<td>4.5 ± 2.9a</td>
<td>3.1 ± 3.8b</td>
<td>1.7 ± 1.7ac</td>
<td>1.2 ± 1.1bc</td>
<td>4.9 ± 2.3b</td>
</tr>
<tr>
<td>C</td>
<td>4.2 ± 3.8a</td>
<td>4.4 ± 4.8b</td>
<td>2.1 ± 1.1ac</td>
<td>1.5 ± 0.9ax</td>
<td>4.7 ± 2.7b</td>
</tr>
<tr>
<td>D</td>
<td>4.8 ± 3.1a</td>
<td>2.8 ± 2.1a</td>
<td>2.5 ± 2.2bc</td>
<td>2.2 ± 1.9ax</td>
<td>4.0 ± 2.3b</td>
</tr>
<tr>
<td>E</td>
<td>4.9 ± 4.1a</td>
<td>5.9 ± 4.1b</td>
<td>4.2 ± 1.8by</td>
<td>3.3 ± 1.7by</td>
<td>5.1 ± 3.8b</td>
</tr>
</tbody>
</table>

Group A: ewe lambs only separated from the flock; Group B: ewe lambs separated and tied; Group C: ewe lambs separated, tied and shorn; Group D: adult ewes separated, tied and shorn; Group E: entire adult males separated, tied and shorn.

First sample: the day before shearing; Second sample: at the moment of separation from the flock; Third sample: 10 min after the second sample; Fourth sample: 10 min after the third sample; Fifth sample: the day after shearing.

As means within a row with different capital letters differ significantly (P < 0.01) in sample comparison.

Mean values within a column with different lower-case symbols differ significantly (P < 0.05) in group comparison.
Shearing stress and hormonal response

Table 2 Mean ± s.d. values of cortisol (ng/ml) in the groups during sampling

<table>
<thead>
<tr>
<th>Groups</th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Fourth sample</th>
<th>Fifth sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.3 ± 5.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>38.9 ± 13.4&lt;sup&gt;By&lt;/sup&gt;</td>
<td>53.6 ± 8.6&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>40.2 ± 14.1&lt;sup&gt;Rx&lt;/sup&gt;</td>
<td>20.2 ± 6.5&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>10.4 ± 8.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>36.1 ± 18.9&lt;sup&gt;By&lt;/sup&gt;</td>
<td>53.6 ± 9.5&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>53.3 ± 8.6&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>18.5 ± 8.6&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>10.6 ± 6.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>24.1 ± 12.5&lt;sup&gt;My&lt;/sup&gt;</td>
<td>54.0 ± 13.7&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>60.4 ± 8.3&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>16.4 ± 8.2&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>9.2 ± 6.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>31.5 ± 13.8&lt;sup&gt;My&lt;/sup&gt;</td>
<td>47.4 ± 12.8&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>51.2 ± 6.6&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>16.6 ± 10.2&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>8.9 ± 7.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>15.2 ± 4.8&lt;sup&gt;My&lt;/sup&gt;</td>
<td>32.9 ± 4.5&lt;sup&gt;Rx&lt;/sup&gt;</td>
<td>37.4 ± 4.9&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>12.2 ± 8.6&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Group A: ewe lambs only separated from the flock; Group B: ewe lambs separated and tied; Group C: ewe lambs separated, tied and shorn; Group D: adult ewes separated, tied and shorn; Group E: entire adult males separated, tied and shorn.

<table>
<thead>
<tr>
<th></th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Fourth sample</th>
<th>Fifth sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55.3 ± 12.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>78.4 ± 14.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>83.6 ± 12.9&lt;sup&gt;RV&lt;/sup&gt;</td>
<td>88.5 ± 9.3&lt;sup&gt;R&lt;/sup&gt;</td>
<td>60.1 ± 14.2&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>58.4 ± 14.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>89.7 ± 17.2&lt;sup&gt;B&lt;/sup&gt;</td>
<td>118.9 ± 28.9&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>113.1 ± 34.7&lt;sup&gt;CV&lt;/sup&gt;</td>
<td>62.5 ± 12.9&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>52.7 ± 15.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>67.8 ± 8.7&lt;sup&gt;B&lt;/sup&gt;</td>
<td>111.4 ± 26.9&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>117.1 ± 28.1&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>64.4 ± 13.1&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>54.1 ± 10.8&lt;sup&gt;A&lt;/sup&gt;</td>
<td>55.5 ± 9.5&lt;sup&gt;B&lt;/sup&gt;</td>
<td>74.2 ± 16.4&lt;sup&gt;Rx&lt;/sup&gt;</td>
<td>88.9 ± 23.2&lt;sup&gt;R&lt;/sup&gt;</td>
<td>58.7 ± 12.3&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>54.4 ± 12.8&lt;sup&gt;A&lt;/sup&gt;</td>
<td>62.2 ± 26.9&lt;sup&gt;B&lt;/sup&gt;</td>
<td>89.7 ± 40.7&lt;sup&gt;Rx&lt;/sup&gt;</td>
<td>113.6 ± 41.5&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>62.3 ± 10.8&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Group A: ewe lambs only separated from the flock; Group B: ewe lambs separated and tied; Group C: ewe lambs separated, tied and shorn; Group D: adult ewes separated, tied and shorn; Group E: entire adult males separated, tied and shorn.

Table 4 Mean values ± s.d. values of magnesium (mg/dl) in the groups during sampling

<table>
<thead>
<tr>
<th>Groups</th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Fourth sample</th>
<th>Fifth sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.1 ± 0.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.1 ± 0.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.9 ± 0.8&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.9 ± 0.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.2 ± 0.5&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>1.9 ± 0.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.4 ± 0.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.9 ± 0.8&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.3 ± 0.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.0 ± 0.4&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>2.2 ± 0.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.1 ± 0.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.7 ± 0.7&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.1 ± 0.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.2 ± 0.5&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>2.2 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.3 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.2 ± 0.4&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.7 ± 0.7&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.3 ± 0.8&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>2.2 ± 0.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.3 ± 0.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2.9 ± 0.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.4 ± 0.7&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.3 ± 0.5&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Group A: ewe lambs only separated from the flock; Group B: ewe lambs separated and tied; Group C: ewe lambs separated, tied and shorn; Group D: adult ewes separated, tied and shorn; Group E: entire adult males separated, tied and shorn.

Discussion

The various treatments produced a drop in blood-plasma levels of GH in all the groups under observation. The greatest drop was observed in females separated from the group, separated and tied, as well as in those that had also been shorn. This fact demonstrates that somatotropin secretion is affected at the beginning by any stress, such as separation, tying and shearing, but it is later influenced by severe stress, such as tying and shearing in comparison to the separation from the flock. Indeed, in animals that were only separated from the group, plasma GH levels rose again in the fourth sample without reaching the level of the first sample, after a significant drop. The other three groups of
females suffered a further drop in GH levels, as highlighted by the significant differences, which is seen in a comparison of the groups. Cataldi et al. (1994) observed, on the contrary, that blood levels of this hormone rose significantly after an acute, short-lived period of stress, determined by isolation and manual immobilization. Similar results were also observed in bovines, after undergoing a brief period of isolation and manual immobilization. Similar results were also observed in bovines, after undergoing a brief period of stress, determined by isolation and manual immobilization. Cataldi et al. (1994) observed, on the contrary, that blood levels of this hormone rose significantly after an acute, short-lived period of stress, determined by isolation and manual immobilization. Similar results were also observed in bovines, after undergoing a brief period of isolation and manual immobilization.

Table 5  Mean ± s.d. values of sodium (mEq/l) in the groups during sampling

<table>
<thead>
<tr>
<th>Groups</th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Fourth sample</th>
<th>Fifth sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>145.3 ± 2.5</td>
<td>146.9 ± 2.1</td>
<td>148.2 ± 3.2</td>
<td>147.1 ± 3.3</td>
<td>145.8 ± 2.6</td>
</tr>
<tr>
<td>B</td>
<td>146.5 ± 3.2</td>
<td>146.9 ± 2.9</td>
<td>151.6 ± 3.9</td>
<td>150.6 ± 3.4</td>
<td>146.2 ± 2.9</td>
</tr>
<tr>
<td>C</td>
<td>145.6 ± 3.6</td>
<td>149.1 ± 3.7</td>
<td>149.3 ± 2.9</td>
<td>149.4 ± 2.9</td>
<td>145.5 ± 3.5</td>
</tr>
<tr>
<td>D</td>
<td>145.5 ± 2.7</td>
<td>143.4 ± 3.5</td>
<td>150.3 ± 4.9</td>
<td>149.5 ± 4.3</td>
<td>147.7 ± 3.0</td>
</tr>
<tr>
<td>E</td>
<td>148.3 ± 2.5</td>
<td>150.1 ± 3.5</td>
<td>148.3 ± 3.9</td>
<td>149.9 ± 3.8</td>
<td>148.8 ± 3.9</td>
</tr>
</tbody>
</table>

Group A: ewe lambs only separated from the flock; Group B: ewe lambs separated and tied; Group C: ewe lambs separated, tied and shorn; Group D: adult ewes separated, tied and shorn; Group E: entire adult males separated, tied and shorn.

First sample: the day before shearing; Second sample: at the moment of separation from the flock; Third sample: 10 min after the second sample; Fourth sample: 10 min after the third sample; Fifth sample the day after shearing.

Table 6  Mean ± s.d. values of potassium (mEq/l) in the groups during sampling

<table>
<thead>
<tr>
<th>Groups</th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Fourth sample</th>
<th>Fifth sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.1 ± 0.4</td>
<td>4.90 ± 0.2</td>
<td>5.0 ± 0.4</td>
<td>5.1 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>B</td>
<td>4.9 ± 0.18</td>
<td>4.92 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td>4.90 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>5.3 ± 0.2b</td>
<td>5.3 ± 0.3b</td>
<td>5.3 ± 0.2b</td>
<td>4.8 ± 0.2a</td>
<td>5.3 ± 0.3b</td>
</tr>
<tr>
<td>D</td>
<td>5.2 ± 0.4b</td>
<td>5.3 ± 0.3b</td>
<td>5.4 ± 0.2b</td>
<td>4.9 ± 0.3a</td>
<td>5.4 ± 0.2b</td>
</tr>
<tr>
<td>E</td>
<td>5.2 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td>5.1 ± 0.4</td>
<td>5.2 ± 0.3</td>
</tr>
</tbody>
</table>

Group A: ewe lambs only separated from the flock; Group B: ewe lambs separated and tied; Group C: ewe lambs separated, tied and shorn; Group D: adult ewes separated, tied and shorn; Group E: entire adult males separated, tied and shorn.

First sample: the day before shearing; Second sample: at the moment of separation from the flock; Third sample: 10 min after the second sample; Fourth sample: 10 min after the third sample; Fifth sample the day after shearing.

In the table, group A has mean values within a row with different lower-case letters as significant differences (P < 0.05) in sample comparison.

In conclusion, the observed effects of cortisol and stress on somatotropin secretion are not in agreement with the findings of Hargreaves and Hutson (1990a, 1990b). The lack of difference between the two groups indicated that tying the legs of the animals was as stressful as tying and shearing. This finding also agrees with those reported by Hargreaves and Hutson, (1990a). The change of corticosteroid levels in sheep that had only been isolated from the flock does not agree with the findings of Hargreaves and Hutson (1990a). They found that isolation produced a rise in cortisol levels only during the initial stages of observation, whereas in our experiment, when the animals were not isolated one by one but were kept together in a group separated from the main flock, the levels of this hormone remained high for the whole isolation period, in agreement with the findings of Cataldi et al. (1994).

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Furthermore, there was no difference in cortisol levels between the sheep that had been shorn in previous years and those subjected to shearing for the first time. This fact supports what Hargreaves and Hutson (1990d) reported, who shone the same animal twice, 2 weeks apart, without obtaining any change in response to the second shearing. This confirms that the animals do not become accustomed to stressful procedures, such as upending (the manipulation in preparation for shearing) (Grandin, 1997). Moberg and Wood (1982) found that though sheep may become accustomed to handling, there is only a minimal change, if any, in secretion from the adrenal cortex.

Males, on the contrary, show much lower plasma levels of cortisol than females, under the same treatment. This can presumably be attributed to an inhibiting effect of gonadal steroid on ACTH secretion (Dawood et al., 2005), although the fact that there is a direct cortisol-inhibiting effect of testosterone on the adrenal gland cannot be excluded (van Lier et al., 2003). However, cortisol haematic concentrations, recorded in all the animals involved in the trial, showed that shearing procedures, although they are necessary in sheep management, caused a severe stress.

Plasma levels of glucose rose in all groups during the experiment. This progressive rise is directly proportional to cortisol levels, and must therefore be attributed to the hyperglycaemic effect of this hormone, to which we may add the glucocorticoids, leading to Na excretion and K retention (Ali et al., 1987). The change observed by us may be linked to the shorter duration of stress-inducing stimuli; our experiment lasted only 20 min, while in the above-mentioned cases stress lasted much longer, producing Mg depletion.

As far as the change of Na and K levels is concerned, our results may be explained by the mineral-corticoid effect of glucocorticoids, leading to Na excretion and K retention (Debenedetti, 1998): the animals with the highest blood cortisol levels showed the greatest variation in the concentration of these two minerals.

**Conclusion**

The pattern of the blood parameters shows how the various stages of shearing all lead to a situation of stress. The change in blood GH levels suggests that, in sheep, GH secretion levels are influenced above all by tying, shearing and by sex. The observed high blood cortisol levels demonstrated that tying of the animals should be avoided, as this phase is the most stressful. Furthermore, alternative shearing methods, with the absence of feet tying, separation from the flock and waiting times, should be used to achieve stress reduction.

**References**


