Utility of acute phase proteins as biomarkers of transport stress in ewes and beef cattle

Francesco Fazio,1 Vincenzo Ferrantelli,2 Antonello Cicerò,2 Stefania Casella,1 Giuseppe Piccione1
1Dipartimento di Scienze Veterinarie, Università di Messina; 2Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri, Palermo, Italy

Abstract

The effect of transport on serum amyloid A (SAA), haptoglobin (Hp), Fibrinogen and white blood cells (WBC) was evaluated in 10 ewes and 10 beef cattle. All animals were transported by road for 6 h over a distance of about 490 km with an average speed of 80 km/h. Blood samples, collected via jugular venepuncture, were obtained before and after transport as well as after 12, 24 and 48 h rest time. One-way repeated measures analysis of variance showed a statistically significant effect of sampling time on SAA, Hp, and WBC in ewes and beef cattle. Based on these results, Hp and SAA levels, together with WBC, may be useful indicators of animal health and welfare and in predicting the risk assessment in meat inspection.

Materials and Methods

The study was carried out on 10 ewes and 10 beef cattle. All animals were clinically healthy. They were transported by road for 6 h over a distance of about 490 km with an average speed of 80 km/h, involving a combination of road surfaces ranging from small country lanes (10 km) through secondary roads (60 km) to motorways (420 km). All animals had no previous experience of road transport. The journey started at 08:00 a.m. and lasted 6 h. Particularly, transport took place during spring, with an outside temperature of 18-20°C and 50-60% relative humidity. After road transport the animals were confined to paddock and 50-60% relative humidity. After the transport the animals were kept for their welfare. In fact, transported animals are exposed to a variety of physical and psychological stimuli that disrupt their homeostasis and metabolism. Road transport, considered as one of the main causes of stress, may be more or less severe and affect a large number of systems. The effects of transport stress on animal health and welfare have been evaluated through behavioural, physiological and haematological variables (Adenkola and Ayo, 2010; Broom, 2003, 2008), mobilisation of energy and protein metabolism (Todd et al., 2000), activity of enzymes and hormones (Adenkola and Ayo, 2010; Stull and Rodiek, 2000), and the changes of immune system (Early and O’Riordan, 2006). There is great scientific interest aimed at ensuring the welfare of transported animals and identifying easily obtainable biomarkers in relation to transport stress. In fact, stress experienced by farm animals during the transport may influence the acute phase proteins (APPs) in beef cattle and ewes (Giannetto et al., 2011; Piccione et al., 2012) and also cause economic losses due to decreased carcass and meat quality (Teke et al., 2014). In the case of increased physiological stress or physical activity during the transport, muscle glycogen reserves may be used before slaughter. This can lead to higher ultimate meat pH, darker meat colour, tougher meat and greater water holding capacity (Gregory, 1998). Acute phase proteins are a group of blood proteins linked to stress because their concentrations decrease (negative APPs) or increase (positive APPs) in response to external or internal challenges (González et al., 2008; Petersen et al., 2004; Cerón et al., 2005; Eckersall and Bell, 2010). In particular, serum amyloid A (SAA), haptoglobin (Hp) and fibrinogen (Fbg), in health monitoring programmes in livestock are useful for the identification of diseases or subclinical diseases. After considering that the linkage among animal health, welfare and APPs becomes more and more important, the aim of this study was to evaluate the modifications of serum concentrations of Hp, SAA and Fbg, together with white blood cell (WBC), in order to identify the impact of transport on biomarkers. This will be increasingly useful to reduce transport stress that influences health, welfare and final quality of the meat of ewes and beef cattle.

Results

The application of ANOVA showed a statistically significant effect of sampling time (P<0.05) on SAA, Hp and WBC in ewes and beef cattle. The concentration of SAA was assessed on blood samples containing citrated sodium, after centrifugation, using a coagulometer (Clot 2S; SEAC, Florence, Italy). The WBC count was assessed on blood samples containing ethylenediaminetetraacetic acid using a multiparametric automatic analyser (HeCoVet; SEAC). The concentration of Fbg was assessed on blood samples containing citrated sodium, after centrifugation, using a coagulometer (Clot 2S; SEAC, Florence, Italy). The WBC count was assessed on blood samples containing ethylenediaminetetraacetic acid using a multiparametric automatic analyser (HeCoVet; SEAC). One-way repeated measure analysis of variance (ANOVA), followed Bonferroni’s multiple post-hoc comparison, was performed to determine the significant effect of sampling time in ewes and beef cattle. The level of significance was set at P<0.05. Data were analysed using the software STATISTICA 8 (Stat Soft Inc.).
beef cattle. Tables 1 and 2 show average values of all studied parameters, expressed in conventional units of measurement with standard deviations and statistical significances, measured during the experimental period in ewes and beef cattle.

**Discussion**

All data obtained before transport were within the physiological range referred to in the literature for ewes and beef cattle (Eckersall and Bell, 2010; Ganheim et al., 2003; Jain et al., 2011). The results of this study confirmed that a linkage between stress and APP response exists. Particularly, SAA and Hp increased both before transport, after road transport; °after 24 h vs before transport.

Consequently, the APPs represent important biomarkers of stress during the road transport of ewes and beef cattle. This is interesting not only to monitor the health status and welfare of transported animals but also to improve meat quality characteristics of ewes and beef cattle.

**Conclusions**

Modern veterinary medicine is increasingly focusing on prevention rather than cure and these biomarkers are important factors for the animal’s environment and welfare. In particular, the results of this study suggest that SAA and Hp levels, together with WBC, may be useful indicators of animal health and welfare and good predictors of risk assessment in meat inspection.

---

**Table 1. Average values of serum amyloid A, haptoglobin, fibrinogen and white blood cells (standard deviation) and statistical significances measured during the experimental period in ewes.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before transport</th>
<th>After road transport</th>
<th>After 12 h</th>
<th>After 24 h</th>
<th>After 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA (mg/L)</td>
<td>9.26±0.78</td>
<td>9.33±0.56</td>
<td>9.86±0.44</td>
<td>10.11±0.55*</td>
<td>10.73±0.57*</td>
</tr>
<tr>
<td>Hp (g/L)</td>
<td>0.09±0.02</td>
<td>0.11±0.03</td>
<td>0.20±0.06</td>
<td>0.29±0.08</td>
<td>0.44±0.07*</td>
</tr>
<tr>
<td>Fbg (g/L)</td>
<td>2.04±0.24</td>
<td>2.12±0.31</td>
<td>1.97±0.34</td>
<td>1.98±0.47</td>
<td>2.11±0.30</td>
</tr>
<tr>
<td>WBC (K/µL)</td>
<td>10.78±0.82</td>
<td>11.36±0.88</td>
<td>11.22±0.78</td>
<td>11.45±1.06</td>
<td>12.54±0.98</td>
</tr>
</tbody>
</table>

SAA, serum amyloid A; Hp, haptoglobin; Fbg, fibrinogen; WBC, white blood cells. *After 24 h vs before transport, °after 48 h vs before transport.

**Table 2. Average values of serum amyloid A, haptoglobin, fibrinogen and white blood cells (standard deviation) and statistical significances measured during the experimental period in beef cattle.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before transport</th>
<th>After road transport</th>
<th>After 12 h</th>
<th>After 24 h</th>
<th>After 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA (mg/L)</td>
<td>15.40±1.56</td>
<td>20.00±4.55</td>
<td>23.50±3.98</td>
<td>55.50±3.54*</td>
<td>101.90±15.26#</td>
</tr>
<tr>
<td>Hp (g/L)</td>
<td>0.18±0.03</td>
<td>0.21±0.03</td>
<td>0.22±0.20</td>
<td>0.23±0.03*</td>
<td>0.38±0.03*</td>
</tr>
<tr>
<td>Fbg (g/L)</td>
<td>4.00±0.81</td>
<td>4.54±0.49</td>
<td>4.34±0.59</td>
<td>4.16±0.35</td>
<td>4.26±0.50</td>
</tr>
<tr>
<td>WBC (K/µL)</td>
<td>8.49±1.01</td>
<td>9.08±0.89</td>
<td>9.36±0.88</td>
<td>9.52±0.61</td>
<td>10.21±0.81*</td>
</tr>
</tbody>
</table>

SAA, serum amyloid A; Hp, haptoglobin; Fbg, fibrinogen; WBC, white blood cells. *After 24 h vs before transport, °after 48 h vs before transport, #after 24 h vs before transport, after road transport and after 12 h; °after 48 h vs before transport, after road transport and after 12 h and after 24 h.

---

**References**


European Commission, 2005. Council


