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

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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.

Introduction

The transport is an inevitable husbandry practice that animals unexpectedly encounter in the livestock industry and can have implications 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 (Gonzàlez *et al*., 2008; Petersen *et al*., 2004; Ceron *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 of ewes and beef cattle.

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Materials and Methods

The study was carried out on 10 ewes and 10 beef cattle. Alla 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 where environmental temperature was between 18 and 23°C, and relative humidity was 50-65%. After the transport the animals were fed hay (2 kg), wheat straw (1 kg) and wheat concentrate (0.5 kg). Water was available *ad libitum*. All animals were transported in accordance with Directive 1/2005 CEE (European Commission, 2005).

Blood samples were collected from each animal by jugular venipuncture into evacuated glass tubes (Venoject; Terumo Europe, Leuven, Belgium) before and after the transport as well as after 12, 24 and 48 h rest time to determine the APPs. Each animal was sampled by experienced and skilled operators in less than 1 min to minimise handling stress affecting the results. The SAA concentration was performed with an enzyme-linked immunosorbent assay using ELISA kits (Tridelta Development, Maynooth, Ireland). The concentration of Hp was assessed using commercial colorimetric kits (Tridelta Development) in microplates, based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. The reading of absorbancies and the consecutive calculation of final concentrations of both APPs were performed on automatic microplate reader Opsys MR (Dynex Technologies, Denkendorf, Germany).

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 <0.05. Data were analysed using the software STATISTICA 8 (Stat Soft Inc.).

Results

The application of ANOVA showed a statistically significant effect of sampling time ($P<0.05$) on SAA, Hp and WBC in ewes and

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.

Parameters	Experimental conditions				
	Before transport	After road transport	After 12 h	After 24 h	After 48 h
SAA (mg/L)	9.26±0.78	9.33±0.56	9.86±0.44	10.11±0.55*	10.73±0.57$^{\Delta}$
Hp (g/L)	0.09±0.02	0.11±0.03	0.20±0.06	0.29±0.08	0.44±0.07$^{\Delta}$
Fbg (g/L)	2.04±0.24	2.12±0.31	1.97±0.34	1.98±0.47	2.11±0.30
WBC (K/μL)	10.78±0.82	11.36±0.88	11.22±0.78	11.45±1.06	12.54±0.98

SAA, serum amyloid A; Hp, haptoglobin; Fbg, fibrinogen; WBC, white blood cells. *After 24 h *vs* before transport; $^{\Delta}$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.

Parameters	Experimental conditions				
	Before transport	After road transport	After 12 h	After 24 h	After 48 h
SAA (mg/L)	15.40±1.56	20.00±4.55	23.50±3.98	55.50±3.54*	101.90±15.26$^{\#}$
Hp (g/L)	0.18±0.03	0.21±0.03	0.22±0.20	0.23±0.03°	0.38±0.03$^{\#}$
Fbg (g/L)	4.00±0.81	4.54±0.49	4.34±0.59	4.16±0.35	4.26±0.60
WBC (K/μL)	8.49±1.01	9.08±0.89	9.36±0.88	9.52±0.61	10.21±0.81$^{\bullet}$

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

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 in sheep and beef cattle during the rest time relative to changes from pre-transportation values. In sheep SAA increased significantly after 24 and 48 h of road transport, while Hp increased significantly after 48 h of road transport only. In beef cattle the results showed a statistically significant increase of Hp 24 h after transport and of SAA compared to the previous data points, and 48 h after transport. Both parameters reached statistically significant higher values. As previously demonstrated, the SAA concentration tends to increase rapidly, whereas Hp concentration increases at a slower rate during the acute phase response (Colditz *et al*., 2005; Eckersall *et al*., 2007), which is consistent with the findings of the present study. It has also been reported that the stimuli can induce different APPs response which may account for the different SAA concentration compared to the hp concentration observed (Lepherd *et al*., 2011). Lomborg *et al*. (2008) demonstrated marked SAA responses in healthy adult cattle after exposure to complex stressors as road transport. In fact, these results are in agreement with studies of other researchers who emphasised the role of APPs not only with inflammation but also with some conditions such as road transport which can be highly stressful and compromise welfare (Petersen *et al*., 2004). When ewes and beef cattle are stressed, there is a rapid relase of cathecolamines which results in glycogen depletion causing a lower rate of *post-mortem* lactic acid synthesis and hight ultimate pH, undesirable colour, making such dry beef (Marenčić *et al*., 2012). The APPs assay may have a potential role for monitoring adverse environmental and/or management stressors, thus enabling better animal welfare practice (Piñeiro *et al*., 2007; Murata, 2007). 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 veterinay 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.

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