

Haematological and Cortisol Changes after a 3 h Road Journey in Sheep

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Abstract: The effects of a short road journey on haematological variables and cortisol levels were studied in 138 healthy sheep. Animals were divided into two groups: group A (n = 93:54 pregnant ewes, 24 post-partum ewes and 15 rams) were transported 125 km by road for 3 h group B (n = 45:20 pregnant ewes, 15 post-partum ewes and 10 rams) was the control group. Blood samples were collected from both groups under basal conditions and in group A after transport and unloading. In group A pregnant ewes showed higher Red blood cell Distribution Width (RDW) ($p < 0.05$), Plateletcrit (Pct) ($p < 0.01$), Platelet (Plt) ($p < 0.01$) and cortisol levels ($p < 0.001$) after transport than basal values; post-partum ewes showed higher RDW ($p < 0.01$), Mean Platelet Volume (MPV) ($p < 0.05$) and cortisol levels ($p < 0.001$), rams showed higher values of RDW ($p < 0.05$), Plt ($p < 0.05$) and cortisol levels ($p < 0.05$). Pregnant ewes also showed lower values of White Blood Cell (WBC) ($p < 0.01$) and higher values of Red Blood Cell (RBC) ($p < 0.01$) than post partum ewes. In addition, pregnant ewes and rams showed higher cortisol levels ($p < 0.05$) before transport than control group ($p < 0.05$). In group B pregnant ewes showed higher WBC and RBC values ($p < 0.01$) than post-partum ewes. Evaluation of haematological variables and circulating cortisol after a short journey is therefore, effective in evaluating short term stress in sheep and provides an additional tool for distinguishing between control group and transported animals.

Key words: Transport, sheep, haematological variables, cortisol, stress, Italy

INTRODUCTION

The effects of transport stress on animal health and welfare have been evaluated through behavioural and physiological variables (Tarrant and Grandin, 2000; Fazio and Ferlazzo, 2003; Broom, 2003a, 2008). Several blood constituents, chemical and hormonal parameters have been used as indicators of poor welfare in sheep e.g., Packed Cell Volume (PCV), total protein, glucose, lactate, creatine kinase, cortisol (Broom, 2003b; Ali *et al.*, 2006; Bornez *et al.*, 2009; Tadich *et al.*, 2009).

Numerous studies have been carried out to determine the amount of long distance transport stress in sheep (Knowles *et al.*, 1995; Knowles, 1998; Hall *et al.*, 1999) and lambs (Knowles *et al.*, 1993, 1996, 1998; Tadich *et al.*, 2009). Thus, it is well known that long term stress can affect a large number of systems in this species including the immune (Archer *et al.*, 2007; Krawczel *et al.*, 2007; Fisher *et al.*, 2010) and reproductive systems (Phogat *et al.*, 1999; Smith *et al.*, 2003b).

The extent to which short transport stress affects the haematological and adrenocortical responses of sheep has not been sufficiently studied and there are limited scientific data available to demonstrate its effect on coping strategy (Knowles *et al.*, 1995; Broom *et al.*, 1996; Ruiz-de-la-Torre *et al.*, 2001).

Physiological cortisol concentrations reported for sheep have a wide range of values because many variables can influence their levels and these include: age (Parrott *et al.*, 1994; Bornez *et al.*, 2009), different genotypes (Hall *et al.*, 1998), pregnancy (Smith and Dobson, 2002), breeding season (Dobson *et al.*, 1999; Phogat *et al.*, 1999), daily rhythms (Bell and Wood, 1991), diet (Simonetta *et al.*, 1991), previous experience of being handled (Fell *et al.*, 1985) and individual emotional reactivity (Deiss *et al.*, 2009). Cortisol represents an important marker of stress which is commonly used in sheep to assess the effects of non cognitive and cognitive stimuli (Dousek *et al.*, 2002; Smith *et al.*, 2003a). Research has been carried out to address issues such as

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changes in cortisol concentrations after isolation or transport simulation (Parrott *et al.*, 1994), after continuous and intermittent transport (Broom *et al.*, 1996; Kent, 1997; Krawczel *et al.*, 2007), after a novel environment post-transport (Cockram *et al.*, 2000) and after different loading procedures (Parrott *et al.*, 1998). However, the strongest stimulus for cortisol release has been observed during the transfer of sheep from their individual holding pens to vehicles and the commencement of vehicle motion (Broom *et al.*, 1996).

Hence, the objective of the present study was to evaluate the haematological and cortisol changes after a short road journey in sheep, taking into account any differences arising between pregnant and post-partum ewes and rams.

MATERIALS AND METHODS

Animals: The study was carried out on 138 healthy Comisana sheep before and after transport. The animals were divided into two groups. Group A (n = 93:54 pregnant ewes, 24 post-partum ewes, 15 rams) was designated as observational group; these animals had no previous experience of truck transport and were transported by road for 3 h over a distance of 125 km. Group B (n = 45:20 pregnant ewes, 15 post-partum ewes, 10 rams) formed the control group. Subjects of both groups were aged 3-4 years and weighed 45.00 ± 10.00 kg.

The pregnant ewes were at the late stage of pregnancy (130 ± 5 days), the post-partum ewes had delivered 3 days earlier and were suckling their lambs the rams were destined for meat production. The subjects were transported over the exact same distance and route. None of the animals had previous experience of truck transport nor were they familiar with the vehicle, loading and unloading procedures.

Animals were weighed before loading and re-weighed after unloading. Subsequently, they were confined to paddock where they were provided with food (hay, clover, broad beans, oats and barley) and water *ad libitum*. Haematological values, serum cortisol levels and body weight were measured.

All methods and the procedures used in this study were reviewed and approved by the Messina University Institutional Board for the Care and Use of Animals.

Blood sampling: The animals were held in a communal pen adjacent to the farm with other sheep of the same flock. They were restrained manually by a person when passing through the race and two blood samples, one with sodium heparin (2.5 mL) and the other without anticoagulant (5 mL) were collected directly into vacuum

tubes (Venoject, Terumo®, Belgium) from the jugular vein. Blood samples were collected on the farm between 12:30 and 14:00 under basal conditions (group B) and immediately before loading and transport (group A). Loading took place between 14:00 and 15:30. The journey began at 15.30 and ended at 18.30 unloading took no >60 min. At this point the final blood samples were collected (between 19.30 and 20.45), again on the farm. All sheep were then allocated to the pen.

During the sampling period the mothers were separated from their lambs. Lambs were confined in an adjacent pen and were able to make visual contact.

Experimental design: The single two-deck rigid livestock vehicle (FIAT 142 IVECO) used was a 136 quintal standard with metal sides and floor. The sheep were confined within 144000 cm^2 :60 subjects (i.e., space allowance was $0.24 \text{ m}^2/\text{animal}$). The floor of the vehicle was covered with straw, neither food nor water was provided. A distance of 125 km was covered; predominantly on motorways and smooth roads (80 km) and in part on bumpy secondary roads (45 km) by the same professional driver. Driving speeds ranged from $0\text{-}80 \text{ km h}^{-1}$ and the route took 3 h to complete, journey speed ranged between $25\text{-}50 \text{ km h}^{-1}$.

Temperatures were recorded outside and inside the vehicle before transport. Journeys took place in winter (December), the weather was mild but wet (temperatures fluctuating between $13.5\text{-}15.0^\circ\text{C}$).

Temperature and relative humidity inside the vehicle at start and after transport ranged from $15.5\text{-}18.6^\circ\text{C}$ and from $30.2\text{-}33.5\%$, respectively. These parameters were monitored continuously with a Hygrothermograph ST-50 (Sekonic Corporation, Tokyo, Japan), placed near the centre of the trailer.

The number of animals per load, the available floor area, distance travelled and time between loading and unloading were recorded.

Data analysis: Haematological analyses were performed on the 2.5 mL individual blood samples collected into a vacuum plastic tube containing sodium heparin as an anticoagulant.

The venous blood samples were analyzed (within 1 h) using an automatic photometer SLIM for haematological assays (SEAC, Rome, Italy), WBC, RBC, Plt, Hb, PCV, MCV, MCH, MCHC, RDW, MPV, Pct, PDW. The remaining blood was then centrifuged at $1,500 \times g$ for 15 min at 4°C and the resultant plasma samples were harvested and stored at -20°C pending assay for cortisol levels, using a commercially available immunoenzymatic kit supplied by RADIM (Pomezia, Rome, Italy). The

hormone assay utilised had a cortisol detection range of 0-1380 nmol L⁻¹. Intra and interassay Coefficients of Variation (CV) were 4.6 and 6.9%, respectively.

Statistical analysis: Data are presented as mean values± Standard Deviation (SD) of duplicate measurements. A one way Repeated Measures Analysis of Variance (RM-ANOVA) was performed to determine whether transport stress had any effect. Significant differences between group A and B were established using the Student's unpaired t-test. The level of significance was set at <0.05. All calculations were performed using the PRISM package (GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

In group A, pregnant ewes showed higher values of RDW (p<0.05), Pct (p<0.01) and Plt (p<0.01), post-partum ewes showed higher values of RDW (p<0.01) and MPV (p<0.05), rams showed higher values of RDW (p<0.05) and Plt (p<0.05) after the short journey compared to basal values (Table 1). Compared to post-partum ewes, pregnant subjects showed lower values of WBC (p<0.01) and higher values of RBC (p<0.001) before transport and lower MPV values (p<0.05) after transport (Table 1). No significant differences in haematological variables were observed between rams and ewes.

Cortisol levels of group A ranged between 53.95-85.25 nmol L⁻¹ in basal conditions and between 127.74-164.96 nmol L⁻¹ after transport. Pregnant ewes and rams showed higher cortisol levels (p<0.05) before transport than control group (Fig. 1).

In group B pregnant ewes showed lower values of WBC (p<0.01) and higher values of RBC (p<0.01) than post partum ewes (Table 2). No significant differences in haematological variables were observed between control rams and ewes.

Cortisol levels in the control group (B) ranged from 44.14-58.20 nmol L⁻¹ in basal conditions as follows: pregnant ewes 58.20±20.83 nmol L⁻¹; post-partum ewes 44.14±20.05 and rams 51.04±19.48 nmol L⁻¹.

The results (Fig. 1) showed a significant increase in circulating cortisol levels after the short road journey, compared to levels before transport both in pregnant (+95%; p<0.01) and post-partum (+190%; p<0.001) ewes and also in rams (+50%; p<0.05). No significant differences in cortisol levels between pregnant and post-partum ewes and rams were observed, neither before transport nor after transport. No significant differences in body weight loss after transport were observed.

Basal haematological values observed in sheep are in agreement with physiological ranges reported in this species by Kaneko (1989), Phogat *et al.* (1999) and Bornez *et al.* (2009). The slight decreases in haemoglobin, haematocrit and MCV values observed in sheep after transport confirm previous data reported after transport in

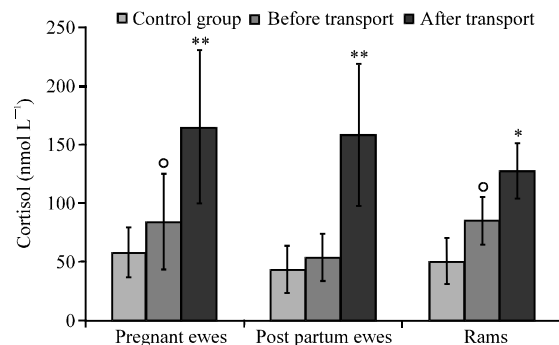


Fig. 1: Circulating cortisol levels (M±SD) of ewes and rams in control group (group B) and before and after transport (group A); Asterisks (*p<0.05; **p<0.001) indicate significant differences (*p<0.05; ** p<0.001) vs. before. Symbol indicates significant differences (°p<0.05) vs. control group

Table 1: Haematochemical variables (M±SD) in ewes and rams before and after transport (group A)

Variables	Pregnant ewes (54)		Post partum ewes (24)		Rams (15)	
	Before	After	Before	After	Before	After
WBC (thousands μL^{-1})	9.17±2.3700 ^b	10.36±3.350	11.27±2.1200	11.98±4.1200	10.37±2.100	10.88±3.1200
RBC (millions μL^{-1})	9.14±1.2800 ^b	9.49±1.590	8.59±1.6000	8.97±1.4400	8.99±1.500	9.17±1.5400
Plt (thousands μL^{-1})	238.53±104.58	340.2±152.83 ^b	308.47±166.25	261.93±141.67	248.4±106.25	361.90±131.57 ^a
Hb (g dL ⁻¹)	10.60±1.3600	10.57±1.260	10.07±1.6800	10.29±1.3600	10.00±1.880	10.19±1.5600
PCV (%)	36.68±4.3000	37.32±5.110	35.59±6.3300	36.34±4.7000	36.39±4.320	37.34±3.7000
MCH (g dL ⁻¹)	11.63±0.6200	11.25±1.130	11.76±0.5700	11.53±0.7100	11.26±0.550	11.63±0.8100
MCV (fL)	40.33±2.4600	39.46±2.520	41.41±1.6200	40.76±3.3600	41.00±1.120	40.56±2.1600
MCHC (g dL ⁻¹)	28.87±0.8800	28.46±2.300	28.38±0.8800	28.35±1.3800	28.28±0.580	28.15±1.8000
RDW (%)	14.85±1.7800	15.67±1.010 ^a	13.77±2.5700	15.50±0.9600 ^b	14.77±1.570	15.55±0.5600 ^b
MPV (fL)	8.83±1.3800	8.83±1.400 ^a	8.50±0.8700	9.93±1.8400 ^a	8.70±0.570	9.83±1.6400 ^a
Pct (%)	0.20±0.0800	0.30±0.160 ^b	0.26±0.1400	0.25±0.1500	0.28±0.100	0.30±0.1500
PDW (%)	11.74±1.7200	11.96±1.310	11.28±3.0800	12.53±2.1300	11.38±2.080	12.73±2.0300

In brackets number of subjects. Significant difference compared with before values: ^a(p<0.05), ^b(p<0.01). Significant difference compared with post partum ewes: ^a(p<0.05), ^b(p<0.01). WBC = White Blood Cell; RBC = Red Blood Cell; Plt = Platelet; Hb = Haemoglobin; PCV = Packed Cell Volume; MCH = Mean Corpuscular Haemoglobin; MCV = Mean Corpuscular Volume; MCHC = Mean Corpuscular Haemoglobin Concentration; RDW = Red Blood Cell Distribution Width; MPV = Mean Platelet Volume; Pct = Plateletcrit; PDW = Plateletcrit Distribution Width

Table 2: Haematochemical variables (M±SD) in control ewes and rams (group B)

Variables	Basal		
	Pregnant ewes (20)	Post partum ewes (15)	Rams (10)
WBC (thousands μL^{-1})	9.05±2.1700*	10.37±2.220	10.07±2.000
RBC (millions μL^{-1})	9.10±1.2000*	8.50±1.600	8.79±1.760
Plt (thousands μL^{-1})	230.13±100.08	300.1±168.25	240.4±100.24
Hb (g dL^{-1})	10.00±1.2600	10.05±1.600	10.06±1.800
PCV (%)	36.38±3.3000	35.55±6.300	36.32±3.420
MCH (g dL^{-1})	11.33±0.6300	11.36±0.560	11.23±0.500
MCV (fL)	40.73±2.3600	41.40±1.600	41.20±1.100
MCHC (g dL^{-1})	28.67±0.8000	28.36±0.860	28.38±0.520
RDW (%)	14.80±1.7000	13.70±1.570	14.67±1.530
MPV (fL)	8.82±1.3500	8.60±0.860	8.75±0.560
Pct (%)	0.23±0.0600	0.26±0.160	0.28±0.010
PDW (%)	11.54±1.7300	11.38±2.080	11.48±2.030

vs. post partum: *($p < 0.01$)

lambs (Bornez *et al.*, 2009). However, the higher RDW values observed after transport do not confirm previous data obtained in this species (Bornez *et al.*, 2009). The significant changes in Plt in both pregnant ewes and in rams after transport showed that the stimulatory effect of transport is probably significantly present after 3 h. In addition, the higher values of Pct after transport only in pregnant ewes and of MPV only in post partum ewes showed that different physiological states induce significant differences in haematological variables in this species.

The researchers may conclude that in sheep there are different physiological indicators of animal welfare and these could vary as a result of transport stress. However, not all haematological variables are useful as indicators of stress.

In relation to the hormonal results, the basal cortisol levels observed in sheep are in agreement with physiological ranges reported for this species by Kaneko (1989) and Phogat *et al.* (1999). Hence, cortisol concentrations can be utilized as an absolute marker of stress therefore when basal values are known, the relative changes can also be useful. The results observed document how the adrenocortical response, evaluated by means of the modifications in circulating cortisol levels, actively modulates the response to short road transport stress with a large and significant release of cortisol in pregnant and post-partum ewes and in rams.

The higher cortisol levels observed after transport confirm data previously obtained in sheep (Smith *et al.*, 2003a; Hogan *et al.*, 2007; Krawczel *et al.*, 2007) with the greater changes in cortisol release occurring during the first 3 h, when the stimulatory effect of transport is probably greater as previously reported (Knowles *et al.*, 1995; Broom *et al.*, 1996; Ruiz-de-la-Torre *et al.*, 2001).

The influence of circadian rhythms on cortisol levels were not investigated because single measurements are insufficient and inadequate to interpret the results. In addition, many endogenous and exogenous factors can

affect the adrenocortical function including loading, unloading, environmental changes and modify diurnal rhythms. It remains to be proven how these factors may influence the hormonal rhythms.

Moreover, cortisol levels were lower under basal conditions than values after transport, both in ewes and rams. These findings confirm the significant effect of transport stress on cortisol levels, irrespective of the existence of periodicity obtained for cortisol in the late-gestation foetal sheep and in pregnant ewes (Bell and Wood, 1991; Simonetta *et al.*, 1991). In addition, the higher cortisol levels before transport than basal values showed that the novelty stimuli and/or their predictability (pre-transport) also produced a stimulatory adrenocortical response in sheep. However, the higher cortisol levels observed after transport between 19:30 and 20:45 showed that transport stress may mask the physiological pattern of this hormone and may modify the course and amplitude of rhythms.

In addition, the energy costs of standing during short road transport were not considerable and posture movements were probably reduced with the quality and duration of transport. In this sense, the increase in cortisol levels could be a contribution to moderate energy requirements, comprising reactions related to intermediary metabolism although, protein turnover, plasma osmolality and substrate cycles were not done.

These results indicate that short road journey can affect cortisol patterns more severely but that effective compensatory mechanisms restore normal physiological adaptation to stress protecting the body against exaggerated catabolism as shown by the higher cortisol levels after transport and by the absence of significant body weight loss.

CONCLUSION

This study has shown haematological variables such as RDW, MPV, Pct and Plt and circulating cortisol changes after short transport to be effective in evaluating short term stress in sheep and to provide an additional tool for distinguishing between pre and post transport effects.

These findings also suggest that the cortisol changes of sheep may play an important role in providing complementary information for the assessment of transport stress in pregnant and post-partum ewes and rams.

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