

Influence of the short-term road transport stress on blood parameters in cows

RAMIN ALI-GHOLI, ASRI-REZAEI SIAMAK, *MOHAMMADI DARYOUSH

Clinical Science, Veterinary College, Urmia University, *Veterinary Graduated, Urmia University

Ali-Gholi R., Siamak A.-R., Daryoush M.

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Summary

Blood parameter changes have been investigated after short-term road transport stress in 18 crossbred dairy cows in northwest of Iran. Cows were transported in 4 groups of four cows and one group of two cows in five different days. Each group was transported by truck up to a 40 km round trip for an hour. Blood was taken in 5 ml amounts from the jugular vein from each cow before transport and then repeated bleeding at 1.5 hours intervals up to 7.5 hours after transportation. Blood samples were assessed for cortisol, (ELISA), glucose, calcium (Ca), phosphorus (IP), magnesium (Mg) (spectrophotometer method), sodium (Na), potassium (K) (flame Photometer), total protein (TPP), fibrinogen (Refractometer method), leucocytes and differential count. Blood cortisol, glucose and leucocytes count increased up to 340%, 48.6% and 23% within 1.5, 7.5 and 6 hours after transport, respectively. Haematocrit decreased up to 17% after 7.5 hours transport. The mean differences (Wilk's Lambda) for these parameters before and after transport were significant ($P < 0.05$). The mean differences for Ca, IP, Mg and eosinophil count were also significant ($P < 0.05$). The highest changes were observed for glucose, hematocrit after 7.5 hours, Ca and the leucocytes count after 6 hours, cortisol after 1.5 hours and the lymphocyte count after 3 hours of transportation. There were significant ($P < 0.05$) relationships between cortisol&Mg ($r = -0.54$), cortisol&TPP ($r = -0.55$), cortisol&fibrinogen ($r = -0.52$), cortisol&neutrophil ($r = -0.52$) cortisol & lymphocyte count ($r = 0.79$) 3 hours after transport. It is concluded that short-term road transport stress increased blood cortisol, that changes in the blood parameters are mainly due to glucose, hematocrit, and leucocyte count and blood electrolytes. Although these changes were within the reference range for cattle, since these parameters are known as health parameters in a ruminant's life they should be considered during the transportation.

Keywords: transport stress, glucose, cortisol, leucocytes, hematocrit, calcium

The concern on stress study in ruminants is based on maintaining the animals' welfare, improve production and reproduction performances. The preliminary response to stress is reflected by a change in physiological parameters by releasing of ACTH from the anterior pituitary and corticosteroid from the adrenal cortex bringing about metabolic and other changes that are characteristic of stress effects in animals (18). Factors such as heat (2), cold (29), diseases and fasting (18), water deprivation (31), calf weaning (8) and transport (23) are known as the main stressors in ruminants.

Transport stress has been considered as a significant stressor in ruminants due to the purposes of the animals' export or import by airplane, boat, truck and train, grazing in long distance pastures, presence in animal exhibitions, transport to the animal hospital for treatment and prophylaxis. Thus different types of transport and their effects on the physiological responses (1), growth and weight gain (12), meat quality (27),

milk yield (28) in cattle (22), sheep (11), goats (13) and calves (15) have been investigated by numerous authors. It has been reported that in the majority of types of stresses blood cortisol increases (25), serum glucose might increase (27), decrease (21) or remain unchanged (7) and also various changes are observed in some other blood parameters based on short-term transport (25), long duration transport (17) or transport with a combination of the other stresses (26).

In accordance with the information about the importance of transport stress, its effect on the physiological changes in animals, and the changes occurring mainly in the initial stage of transport, it would be useful to investigate the stress effects in adult cows, since there are many reports that have been published on calves, steers and sheep. Secondly, in particular regions in which healthy or sick cows are transported to animal clinics for veterinary objectives such as checkups and treatment, it could be possible to take into account the

changes that occur during transport stress as a part of the disease stage. Therefore, this study was designed according to a one hour transportation of dairy cows for 40 km. The objectives were: 1) to determine the blood biochemical and hematological changes during transport; 2) to compare the mean blood parameter changes up to 7.5 hours after transport; and 3) to find the correlations between blood parameters before and after transportation.

Material and methods

Animals and route of transport. A number of 18 cross-bred, non-lactating and non-pregnant dairy cows were selected in 2004. They were over 4-years-old, examined for health parameters and located in the veterinary college hospital of Urmia University. Cows were fed lucerne and water before transport. They were classified in four groups of four cows and one group of 2 cows. Each group was separately transported by truck in different days, early in the morning for a 40 km round trip in a combination of gravel and muddy roads and returned to the initial location after an hour. Transport was carried out in the spring, the climatic condition was semi-rainy and the temperature and humidity were around 25°C and 65-75%, respectively. A 5 ml Jugular blood vein (2 ml including EDTA for hematological parameters and 3 ml without EDTA for biochemical tests) was first taken by syringes before transport (control) and then at 1.5 hour intervals up to 7.5 hours (treatment). Therefore, they were bled 6 times and the number of transports for the whole experiment was 5 trips.

Blood sampling. Blood samples were taken before transport and 10 minutes after arrival. The cows were bled at 1.5 hour intervals up to 7.5 hours after transport (5 bleedings). Blood samples with EDTA were tested for packed cell volume (PCV), fibrinogen, total protein (TPP) and leucocytes evaluation. Blood serum was separated by centrifugation in 3000 g for 15 minutes and used for cortisol, glucose, calcium (Ca), magnesium (Mg), inorganic phosphorus (IP), sodium (Na), and potassium (K) assessments.

Laboratory measurements. Blood cortisol was assessed by ELISA using a commercial cortisol kit (GMBH, Germany). Serum glucose, Ca, IP and Mg were evaluated by spectrophotometer using relevant commercial kits (Pars Azmon, IR). Serum Na and K were measured by flame Photometer using Na and K standards. Hematocrit was assessed by microhematocrit centrifugation. Fibrinogen and TPP were evaluated by the Refractometer method. Leucocyte and differential counts were also expressed as absolute counts.

Analysis. An SPSS statistical program was used to analyse the data. Case summary tests was carried out to determine the mean, standard deviation and error for blood parameters under study in different sampling times.

Tab. 1. Mean \pm SE of the blood parameters before and 7.5 hours after transportation in cows (n = 18)

Parameters	Before transport	1.5 hrs after transport	3 hrs after transport	4.5 hrs after transport	6 hrs after transport	7.5 hrs after transport
Sodium (mmol/l)	133.3 \pm 3.6	141.9 \pm 2.76	146.8 \pm 2.21	145.7 \pm 5.24	147.2 \pm 3.9	140.9 \pm 3.26
Potassium (mmol/l)	5.74 \pm 0.22	5.46 \pm 0.24	5.55 \pm 0.19	5.44 \pm 0.23	5.17 \pm 0.21	5.44 \pm 0.36
Total Protein (g/dl)	6.9 \pm 0.25	6.8 \pm 0.15	6.7 \pm 0.24	6.7 \pm 0.25	6.7 \pm 0.28	6.9 \pm 0.25
Fibrinogen (mg/dl)	488 \pm 64	509 \pm 55	519 \pm 61	463 \pm 41	478 \pm 41	431 \pm 55
Neutrophil (x/ml)	5964 \pm 1020	5340 \pm 428	5780 \pm 489	5222 \pm 502	6114 \pm 684	4918 \pm 528
Lymphocyte (x/ml)	5844 \pm 420 ^a	6060 \pm 547 ^a	5011 \pm 349 ^{ab}	5190 \pm 442 ^a	6154 \pm 403 ^{ac}	5936 \pm 508 ^a

Explanations: all parameters except for lymphocyte count ($P < 0.1 > 0.05$) were insignificant

Repeated measures ANOVA (Wilks' Lambda) were applied to compare the mean parameters among sampling times. Pairwise comparisons were used to find the mean effects after transportation. Pearson correlation tests were used to establish the relationships between cortisol at certain sampling times with the parameters under study.

Results and discussion

Table 1 shows the mean and standard error of Na, K, TPP, fibrinogen concentrations and absolute neutrophil and lymphocyte counts before and 7.5 hours after transportation in cows. The concentrations were varied within the sampling times, but the results of Wilks' Lambda were not significant except for lymphocyte count ($P < 0.1 > 0.05$).

Blood cortisol, glucose and leucocytes increased up to 340%, 48.6% and 23% after 1.5, 7.5 and 6 hours transport, respectively. The comparison of the mean cortisol, glucose and leukocyte count (Wilks' Lambda) showed significant differences ($P < 0.01$) within the sampling times (fig. 1, 2, 4). Mean hematocrit concentration decreased up to 17% after 7.5 hours transport (fig. 3). The results of repeated measure ANOVA showed differences ($P < 0.05$) within the sampling times. Blood Ca partially decreased while IP and Mg increased. The mean differences for all parameters were significant ($P < 0.05$, fig. 5, 6, 7). The difference for eosinophil count was significant ($P < 0.05$, fig. 9) and for lymphocyte count it was at the level of $P < 0.10 > 0.05$ (fig. 8).

Pairwise comparison analysis was carried out to indicate the highest mean effects after transport in cows. Glucose, hematocrit, cortisol and leucocyte count were the main parameters that statistically did not reach to the initial values after 7.5, 7.5, 1.5 and 6 hours, respectively. Pearson correlation results among cortisol concentration and other parameters in different sampling times showed significant ($P < 0.05$) relationships between cortisol and Mg ($r = -0.54$), cortisol and TPP ($r = -0.55$), cortisol and fibrinogen ($r = -0.52$), cortisol and neutrophil ($r = -0.52$) cortisol and lymphocyte count ($r = 0.79$) 3 hours after transport.

Serum cortisol concentration in welfare cows has been reported as 32.8 $\mu\text{g/dl}$ (30), which supports the

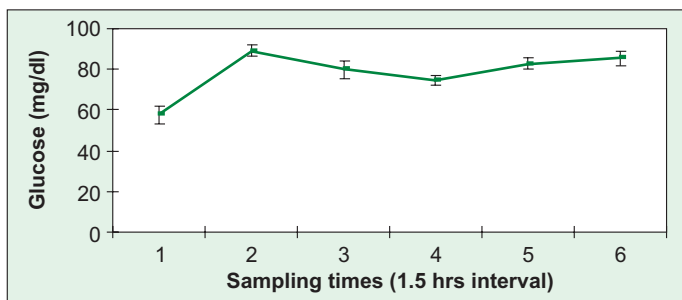


Fig. 1. Blood glucose concentrations before and 7.5 hours after transport ($P < 0.01$) ($\bar{x} \pm SE$)

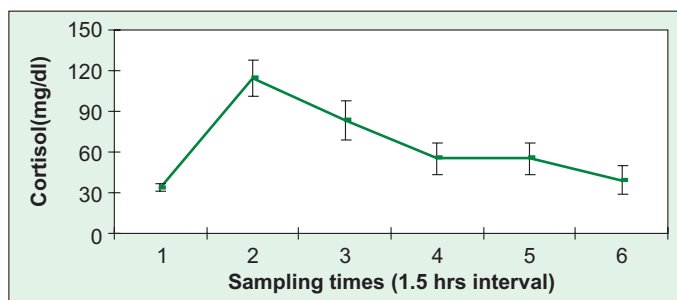


Fig. 2. Cortisol concentrations before and 7.5 hours after transport ($P < 0.01$) ($\bar{x} \pm SE$)

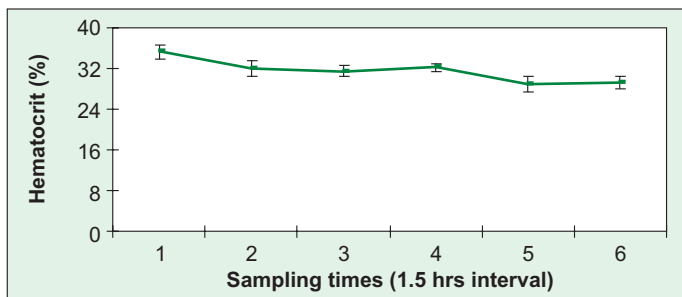


Fig. 3. Hematocrit concentrations before and 7.5 hours after transport ($P < 0.01$) ($\bar{x} \pm SE$)

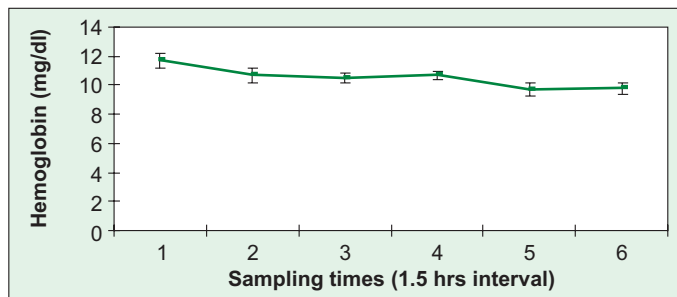


Fig. 4. Hemoglobin concentration before and 7.5 hours after transport ($P < 0.01$) ($\bar{x} \pm SE$)

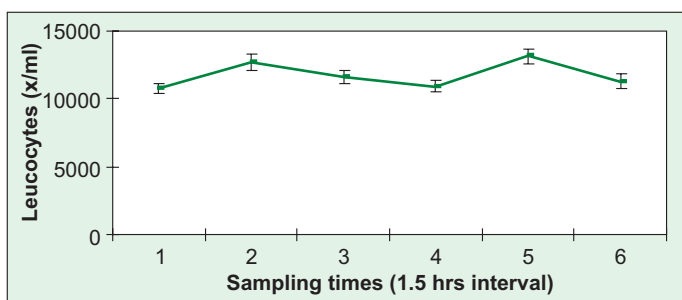


Fig. 5. Leucocytes before and after transport ($P < 0.01$) ($\bar{x} \pm SE$)

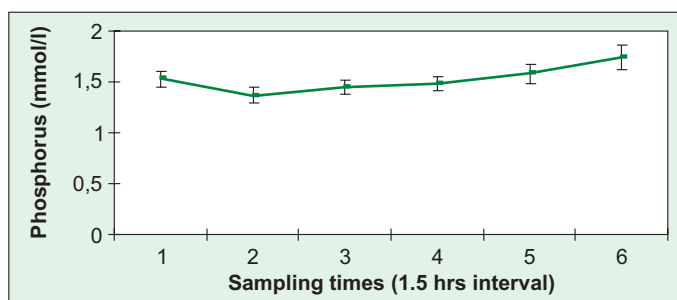


Fig. 6. Phosphorus concentration before and 7.5 hours after transport ($P < 0.1 > 0.05$) ($\bar{x} \pm SE$)

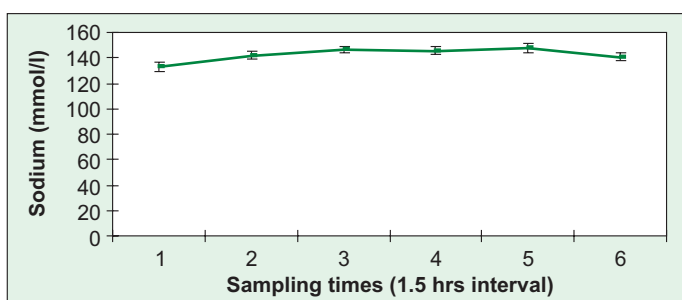


Fig. 7. Sodium concentration before and after 7.5 hours interval ($P < 0.0 > 0.05$) ($\bar{x} \pm SE$)

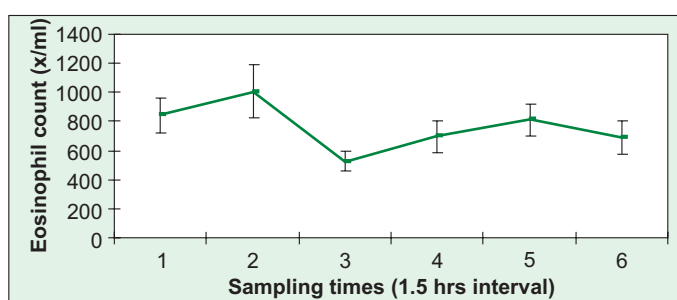


Fig. 8. Eosinophil counts before and 7.5 hours after transport ($P < 0.05$) ($\bar{x} \pm SE$)

result of this study for cows before subjected to transport. It was greater than reported by Dobson (6) up to 55 $\mu\text{g/dl}$ following transport stress. An increase of cortisol concentration up to 114 $\mu\text{g/dl}$ indicates the influence of transport as a major stressor in dairy cows. Steinhardt (23) reported that cortisol increases up to 10 fold following transport stress. Many authors reported increases in cortisol concentration subjected in animals transported up to 30 minutes (16), 45 mi-

nutes (13), one hour (20), 2 hours (19) and 4 hours (26) in ruminants. Physiological responses to stress were demonstrated firstly by hypothalamus, pituitary and adrenal reactions which were mainly related to environmental stressors such as noise and, secondly, by sympathetic, adrenal and medulla reactions in which they are related to nervous stressors such as transport (14). Thus, the effects of stressors on the physiological status are associated with negative effects on

weight gain, growth rates and fitness parameters (1). Knowles et al. (10) believes that long-term transport stress is responsible for low weight gain while Sartorelli et al. (20) places emphasis on short-duration transport stress as applied in this study. The transportation of animals may be accompanied by the presence of other stresses such as crowding, heat, cold, physical and excitement stress, therefore these factors could account for the high cortisol level in this study.

Hyperglycemia following transport stress has been reported by different authors (5, 27), while Schaefer et al. (21) mentioned hypoglycemia and in some others there was no variation observed in glucose concentration (19, 20). Hyperglycemia in transport stress could be related to increase in gluconeogenesis, glycogenesis, and gluconeogenesis, low glucose consumption in body and high carbohydrate metabolism and for hypoglycemia vice versa (18). Although a relationship between blood glucose and cortisol concentrations has been shown, the discrepancies in the reported results for glucose indicates that it might not be a reliable factor in transport stress; in other words, different types of stress and their severity in different animals have a significant effect on blood glucose variation (18).

A significant decrease in PCV concentration (fig. 3) was consistent with the results of Brealey et al. (5) but differed from those that observed an increase in these parameters (15, 23, 25). Ramin (18) did not observe any changes in PCV following ACTH injection in calves. These results show that changes in PCV following transport were firstly within the reference range and secondly could not be related to an increase in cortisol concentration. It is believed that high PCV concentration following transport is more likely related to an increase in circulatory red blood cells than hemoconcentration or dehydration. Probably the first bleeding produced an increase of the PCV value and after transport these tended to normalize. A gradual and continued fall in PCV value in transport has a deleterious effect on animal health and should be quickly compensated by administration of minerals, tranquilizers and Phenobarbital.

Variation in leucocyte counts including an increase (3, 15), no changes (9), and decrease following transport stress was recorded (18) so far. The increase in leucocyte counts (fig. 4) was related to neutrophil cells as demonstrated after 6 hours transportation here. Cortisol is known to have an immuno-suppressive activity, with a decline in lymphocyte blastogenesis responses and an increase in the occurrence of disease (3). This mechanism varies according to the stress type, its severity and animal species. Thus, not only did leucocyte counts not fall at this juncture but also increased up to 23%, which would be a useful index in stress. The presence of lymphopenia and eosinopenia was also observed in cows (8), calves (15) and goats (13). Cortisol decreases interleukin 2 results, lymphocyte reduction and lymphopenia and induces the migration of

eosinophils to lymphoid organs and eosinopenia (18). Adrenalin would also be the cause of neutrophilia and eosinophilia by producing a decrease in the mobilization of leucocytes from the blood to other tissues and also an increase in the migration of marginal neutrophils located in the wall of the blood vessels. However, adrenalin was not an index in this study.

The significant decrease in calcium concentration was consistent with other findings (25), but not with Steinhardt (23) in cows and Kent and Ewbank (9) in calves. In spite of calcium decline in transport, it was nevertheless within the reference range, but one of the main reasons for Ca decline is probably related to increased urinary excretion of calcium due to aldestrone. Thus calcium consumption before transport would be useful during transport (21). An increase in Na concentration after transport would not be as important as calcium, but variations in Mg and IP concentrations have been seriously considered by Kent and Ewbank (9) as also demonstrated in this study.

According to the results, some parameters were significantly influenced by transport stress. These changes were varied from 1.5 to 7.5 hours after transport. The highest effects were observed in glucose, hematocrit and eosinophils in that they did not return to the initial values after 7.5 hours transportation. The effects of cortisol on the immune system, including lymphocyte and leucocyte counts, were transient, while glucose, hematocrit and calcium which are known as health and production parameters were significantly influenced even more than 6 hours, and should be considered following short-duration transportation. The short duration road transport stress was presented in relationships between cortisol and certain parameters just 3 hours after transport as reported in some studies. These correlations were between cortisol and glucose, total protein and hemoglobin (18, 25). In those studies the type of stress, animal species and the severity of stress were quite different from this study.

The results of this study showed that short-term road transport stress increased blood cortisol that was responsible for affects on some blood parameters. The longest affect was observed in glucose, hematocrit and leucocytes and the shortest was on lymphocytes and minerals. The major correlations were found between cortisol and blood parameters 3 hours following transport. It is concluded that short duration road transport stress has influences on blood glucose, hematocrit, leucocytes and electrolytes in that they were known as health and production parameters and must be considered before transportation in cows.

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Author's address: Ali-Gholi Ramin, Associate professor of the Large Animal, Internal Medicine, Veterinary College, Urmia University, College of Veterinary Medicine, Urmia University, P.O.Box 1177, Urmia, Iran; e-mail: aligholiramin@yahoo.com