EFFECT OF ROAD TRANSPORTATION ON BLOOD AND SERUM PARAMETERS AND THYROID ACTIVITY IN SYMPTOMATICALLY HYPERGLYCAEMIC FEMALE DROMEDARY CAMELS

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ABSTRACT

Ten animals were divided into two equal groups according to short (150–200 km) or long (250–400 km) transportation distance. Compared to 10 clinically healthy control animals, the glucose level, globulin concentration, neutrophil count, catalase and total antioxidant capacity activity were significantly higher in the camels after both the short- and long-distance lorry transportation, while the eosinophil count and super oxide dismutase activity were significantly lower. Long-distance transportation resulted in elevated creatinine, but lowered albumin in the camels. The long-distance group also exhibited a significant increase in total T3 and total T4 levels; however, their TSH levels were significantly lower than in the control camels. The short-distance lorry transportation of camels led to an elevation of the white blood cell count and haemoglobin concentration, but lower haematocrit % and lymphocyte count. Thus, the altered thyroid hormone levels and changes in the physiological metabolic profiles taken together may be effective biomarkers of transportation stress in this species. Finally, it was cleared that the obtained pathophysiological changes may be resulted from the transportation-induced stress in hyperglycaemic animals.

Key words: Blood, dromedary camel, hyperglycaemic lorry transportation, thyroid

In the Kingdom of Saudi Arabia (KSA), camels are commonly transported for a variety of purposes including clinical examination, slaughter and sale. Animal transportation has been linked to health disorders and economic loss (Padalino *et al*, 2015). Previous studies have reported that the long duration (Chacon *et al*, 2005) and density of animals in the lorry during transportation (Waas *et al*, 1997) lead to a stressful condition in the animals.

Ideally, blood glucose levels in camels range between 70 and 90 mg/dL (Poonia *et al*, 2016). Under stress conditions, the body shifts physiologically into fight-or-flight mode, hence elevating the blood glucose. It is well known that transportation involves many stressful factors for large animals, including manipulation, foreign environments, noise and fasting. These responses can change depending on the duration of the transportation (Padalino *et al*, 2015).

As an endocrine response to stress, thyroid hormones in particular are known as important modulators of energy metabolism (Kaneko *et al*, 2008; Eshratkhah *et al*, 2010). The alteration in thyroid hormone concentrations following different stresses has been reported in some domestic animals (Saeb *et al*, 2010). In addition, these hormones may have the capability to regulate the activities and metabolic pathways of the anti-oxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). In the serum of camels, these enzymes are changed during environmental stress (Kataria *et al*, 2010).

To the best of our knowledge, no study has been conducted to date describing the possible relationship between thyroid hormone status, antioxidant enzymes and serum and blood chemical profiles of symptomatically hyperglycaemic dromedary camels after short and long-distance lorry transportation. This study was therefore, undertaken to investigate the influence of lorry transportation on thyroid hormones, blood and serum biochemistry and antioxidant enzymes in the symptomatically hyperglycaemic camels admitted to Qassim University Veterinary Teaching Hospital.

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Materials and Methods

This study was carried out at the Qassim University Veterinary Teaching Hospital and the Qassim University Farm in the Qassim region, KSA, during the November and December 2017. The experimental protocol was approved by the Animal Ethical Committee of Qassim University Deanship for Scientific Research.

Animals

Ten non-pregnant, non-lactating, clinically healthy female camels (Camelus dromedarius) aged 3-6 years were used as non-lorry-transported control camels in the present study. These camels were selected from the Qassim University farm based on the absence of any disease. Their body condition scores were considered normal, ranging between 3 and 3.5. Another ten female animals were admitted to the clinic after lorry transportation suffering from long- standing decrease in body weight and progressive weakness and had high glucose levels as measured by glucometer. These camels were divided into two equal groups according to the distance of transportation: short (150-200 km.) and long (250-400 km.).The body condition scores of these animals ranged between 2 and 3.5.

Blood samples

Five-mL blood samples were collected by sterile jugular venepuncture into a tube containing EDTA and used for the haematological parameters. Another 5-mL were collected and serum was separated by centrifugation at $3000 \times \text{g}$ for 20 min and kept in a deep freezer at -20°C till assayed.

Haematological, biochemical and hormonal analyses

Haemogram and leukogram evaluations were carried out using an automated analyser (Vet Scan HM5, Abaxis, California, USA). The haemogram included red blood cell (RBC counts, × 10⁶/ μ L), haemoglobin (HGB) concentration, g/dL), haematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/dL). The leukogram included total leukocyte (WBC) count (× $10^3/\mu$ l), lymphocyte (LYM) count (× $10^3/\mu$ l), monocyte (MON) count (× $10^3/\mu$ l), neutrophil (NEU) count (× $10^3/\mu l$) and eosinophil (EOS) count (× $10^3/\mu$). The serum samples were tested using an automated biochemical analyser (Vet Scan VS2, Abaxis, California, USA.) to determine the protein profile of total protein (TP, g/dL), albumin Serum concentrations of total T_3 , T_4 and thyroid stimulating hormone (TSH) were assayed by ELISA procedures using purchased kits (Immunotech Corporation, 90 Windom St., Boston, MA 02134, USA). For the antioxidant enzyme activity, Calorimetric measurement was performed using kits (Biodiagnostic) to determine total antioxidant capacity (TAC), super oxide dismutase (SOD) and catalase (CAT).

Statistical analyses

All values were expressed as mean and standard error (SE) with P<0.05 and P<0.01 seen as statistically significant. For comparison between the groups, the data were analysed by the Mann-Whitney U test and two-way analysis of variance (ANOVA).

Results and Discussion

Glucose level

The effects of short- and long-distance lorry transportation on the glucose level of the dromedary camels are shown in Table 1. Compared to the control group, the glucose levels were significantly higher in the camels after both short (P<0.05) and long (P<0.01) distance lorry transportation.

Protein profile

The effects of short- and long-haul lorry transportation on the protein profile of the symptomatically hyperglycaemic dromedary camels are shown in table 1. The ALB concentrations of the camels after long lorry transportation were significantly lower (P<0.05) than central group; however, non- significant difference was seen in the short-distance transportation group. In addition, there was no significant difference between the total protein in either the short- or long-distance transportation group compared to the control group of camels. Moreover, GLOB concentrations were significantly higher in both the short (P<0.05) and long (P<0.01) distance transport groups than in the control group.

ALP, CREA, BUN and Amylase

The effects of short- and long-haul lorry transportation on ALP, CREA, BUN and amylase

in the symptomatically hyperglycaemic dromedary camels were shown in table 1. The data revealed no significant difference in ALP and BUN levels of the camels in either group subjected to lorry transportation compared to the control group. Likewise, no significant difference in BUN was seen in either transported group compared to the control group. The CREA level of the short-haul transport group did not show any significant difference; however, the CREA levels of the camels in the longhaul transport group were significantly higher (P<0.05) than in the control group. Finally, the data showed that amylase activity in the camels under both short- and long-distance lorry transportation conditions were significantly lower (P<0.05) compared to that of the control group.

Leukogram

Table 2 shows the effects of short- and longdistance lorry transportation on the leukograms of the symptomatically hyperglycaemic dromedary camels. The WBC count of the camels after short-distance lorry transportation was significantly higher (P<0.05) compared to the control group, while after long lorry transportation, there was no significant difference in the WBC. There was a significantly greater decline (P<0.05) in the lymphocyte count of the camels after short lorry transportation than in the control group. On the other hand, compared to the control camels, there was no significant difference in the count after long-haul lorry transportation than lymphocyte group. In addition, no significant differences were observed in the monocytes count in any of the three camel groups. However, the netvoyage count was significantly higher in the camels in both the short (P<0.01) and long (P<0.05) distance transportation groups compared with the control group, while the eosinophil count was significantly lower in both the short (P<0.01) and long (P<0.05) distance transportation groups.

Haemogram

The effects of short- and long-distance lorry transportation on the haemograms of the symptomatically hyperglycaemic dromedary camels are shown in table 2. The data revealed no significant differences in the RBC count of camels in either the short-or long-distance lorry transportation groups compared to the control group. The hemoglobin concentration of camels after short-distance transportation was significantly higher (P<0.05) than that of the control group; similarly, their hematocrit % was also significantly higher (P<0.05). However, there was no significant difference in HGB concentration or the HCT % between the camels after long-distance transport and the control group. No significant differences were found in the MCV, MCH, or MCHC among all 3 groups.

Electrolyte profile

The data revealed no significant differences in calcium concentration in either the short- or longdistance lorry transport groups compared to the control camels. Similarly, neither lorry transport group showed any significant differences in the P levels, nor in Na and K, compared to the control group (Table 3).

Thyroid hormones

There was a significant increase (P<0.01) in the total T3 levels of the camels after long-distance lorry transportation compared to the control group. Similarly, total T4 was significantly higher (P<0.05) in camels after long-distance lorry transportation;

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
GLU (g/dL)	114.40	4.51	170.53 ^a	15.81	224.82 ^b	35.10
ALB (g/dL)	4.68	0.16	3.75	0.26	3.55 ^a	0.51
GLOB (g/dL)	1.64	0.12	2.82 ^a	0.41	3.03 ^b	0.38
TP (g/dL)	6.18	0.16	6.57	0.29	6.58	0.35
ALP (U/L)	106.90	6.57	116.0	18.5	120.73	13.96
CREA (g/dL)	0.94	0.06	1.27	0.11	1.48 ^a	0.29
BUN (g/dL)	26.31	0.84	20.23	2.74	26.97	2.73
AMY (U/L)	616.7	41.7	418 ^a	69.0	412.2 ^a	25.6

 Table 1. Effect of short and long lorry transportation distance on biochemical profile of symptomatically hyperglycaemic dromedary camels.

^{a, b} Mean values of the short and long lorry transportation distance groups differed significantly from the value of the control group in the same row at P < 0.05 and P < 0.01, respectively.

however, their TSH levels were significantly lower (P<0.05) than those of the control camels (Table 4).

Antioxidant enzymes

A significant decrease was observed (P<0.05) and (P<0.01) in the SOD activity after short- and long-distance lorry transportation, respectively, compared to the control camels. Meanwhile, the CAT activity was significantly increased in the camels after short (P<0.05) and long (P<0.01) distance lorry transportation. However, the TAC activity in both transported camel groups was significantly higher (P<0.05) than in the control group (Table 5).

Animals exposed to unfavourable environments due to transportation experience very stressful conditions with a variety of physiological changes. These changes may have unfavourable effects on the camel's productivity and well-being.

Camels have developed resistance to harsh environments and found sustenance from sources unutilised by other species (Kadim *et al*, 2009). The complex physiological changes caused by lorry transportation stress can differ according to species and breeds within the same species (Ingram and Matthews, 2000). Different parameters, including metabolic, hormonal and behavioural changes, have been used to measure the physiological changes occurring during stress (Saeb *et al*, 2010; Nazifi *et al*, 2009).

Compared to the control group, glucose levels were significantly higher in the camels after shortand long-distance lorry transportation. This is in agreement with previous works conducted by Tadich et al (2005) and El Khasmi et al (2013) and with studies done on goats (Kannan et al, 2007; Minka and Ayo, 2010) and Holstein calves (Bernardini et al, 2012). However, results of other investigators did not support these findings, particularly those on cattle and goats (Earley et al, 2012). The elevated glucose level during lorry transportation may result from glycogenolysis in the muscle and liver due to an increased catecholamines from the sympathetic nervous system (Sanders and Straub, 2002; Almundarij et al, 2017) and glucocorticoids from the adrenal cortex (Tadich et al, 2005); however, these factors were not assessed in this study. In

Table 2. Effect of short and long lorry transportation distance on haematological profile of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance 1	orry transportation	Long-distance lorry transportation	
Parameters	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
WBC (×10 ³ /µL)	15.04	1.41	21.06 ^a	3.79	16.37	4.22
LYM (×10 ³ /µL)	3.21	0.54	1.53 ^a	0.22	1.82	0.27
MON (×10 ³ /µL)	0.100	0.01	0.14	0.02	0.13	0.04
NEU (×10 ³ /μL)	8.81	1.04	18.80 ^b	3.40	14.68 ^a	3.73
EOS (× $10^3/\mu$ L)	2.92	0.53	0.58 ^b	0.16	0.61 ^a	0.33
RBC (×10 ⁶ /µL)	8.69	0.32	10.53	0.76	8.77	0.92
HGB (g/dL)	13.60	0.49	17.00 ^a	1.44	13.27	1.17
HCT (%)	24.14	0.66	28.81 ^a	2.85	23.83	1.72
MCV (fl)	27.70	0.65	27.25	1.44	28.00	1.83
MCH (pg)	15.67	0.18	16.15	0.42	15.36	0.71
MCHC (g/dL)	56.31	1.18	59.45	2.04	55.42	1.09

^{a, b} Mean values of the short- and long-distance lorry transportation groups differed significantly from the value of the control group in the same row at P<0.05 and P<0.01, respectively.

Table 3. Effect of short and long lorry transportation distance on electrolyte profile of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance 1	orry transportation	Long-distance lorry transportation	
Parameters	Mean	SE(±)	Mean	SE (±)	Mean	SE(±)
CA (g/dL)	9.72	0.15	9.57	0.19	9.60	0.21
P (g/dL)	4.90	0.66	4.97	0.69	5.32	0.77
NA (mmol/L)	154.31	1.46	143.75	2.90	144.33	1.50
K (mmol/L)	5.57	0.09	4.92	0.22	4.56	0.30

contrast, a similar study in goats demonstrated that the elevation of blood glucose was due to the activation of the sympathetic nervous system rather than the plasma cortisol (Aoyama et al, 2008). In the present study, the elevated glucose levels resulting from the short-haul distance requiring a 2-3 h drive to the clinic could have been caused by a sympathetic outflow and increase in plasma cortisol either directly (O'Malley, 1971), or through ACTH (Maejima et al, 2006) mediated stress. On the other hand, both catecholamine administration and transportation have been shown to increase plasma glucose levels within 10-15 min in sheep (Parrott et al, 1994; Bassett, 1970). Additionally, a link was found between transportation stress, expression of the *c-fos* protein in the adrenal medulla and the increase in plasma glucose levels (Maejima et al, 2006). However, the blood glucose showed no significant change due to the length/duration of the transportation. Even when it increased from 170.5 g/dL to 224.8 g/dL, this might have been due to the variations of age among the animals, as previously mentioned by Eskandarzadeh et al (2014).

The increase in ALB levels during transportation may indicate the degree of stress in response to stressors (Križanović et al, 2008). The effects of shortand long-distance lorry transportation on the protein profiles of the symptomatically hyperglycaemic camels were recorded. Compared with the control group, the ALB concentration of the camels after long-haul lorry transportation was found to be significantly lower; however, no significant difference was seen in the short-haul transportation group. In addition, there was no significant difference between total proteins after either short- or long-distance lorry transportation compared to the control camels, whereas the GLOB concentrations were significantly higher in both groups of transported camels. As a result of lorry transportation, there was an elevation of GLOB and lowering of ALB without changes in the total protein. On the other hand, short lorry transportation had no effect on the ALB and total protein in the current study, suggesting that no dehydration occurred during the 2-3 h lorry transportation stress in the dromedary camels. The long-haul lorry transport of over 5-6 h had no significant effect on total protein concentration in the dromedary camels, as has been previously reported (Kataria and Kataria, 2004). It was concluded that no signs of dehydration had occurred in the dromedary camels during transportation, which may be a reflection of their ability to adapt to dehydration (Parker et al, 2003).

The data revealed that there was no significant difference in the ALP activity in either group of transported camels compared to the control group. The CREA and BUN levels of the camels after shorthaul transportation were not significantly different; however, the CREA levels of the camels in the long-haul transportation group were significantly higher than those of the control group. This result is consistent with a previous study on different species (Hartung, 2003). Therefore, the elevated levels of CREA may have been due to the catabolism of protein by cortisol after food deprivation (Wensvoort et al, 2004). On the other hand, a different study showed no significant effect on CREA levels during drought stress (Kataria and Kataria, 2004). The present data indicated that neither short-nor longhaul transportation had extensive adverse effects on the protein catabolism of the transported camels.

The haemogram is one of the physiological stress indicators for transportation in camels (El Khasmi et al, 2013). The values of the control group were consistent with haematological values obtained in camels in Saudi Arabia (Al-Sultan, 2008). Camels have small, elliptical RBCs and a lower HCT % compared with other mammalian RBCs (Farooq et al, 2011). The data showed that the HCT % was significantly higher in the camels transported shortdistance than in the control group. Moreover, the HCT % of the camels in the long-distance transport group was not significantly different from that of the control group. A previous study reported that the elevation of HCT % in animals might be attributed to haemoconcentration associated with splenic contraction induced by catecholamines (Tadich et al, 2005). It has been observed that HCT % may be increased during the handling and manipulation of animals (Tadich et al, 2005). The camel blood HGB concentration recorded in this study is in accordance with the values previously reported in Saudi Arabia byAl-Busadah (2007), in Iraq by Alsaad (2009) and in Iran by Ahmad et al (2004).

The blood index values (MCV, MCH and MCHC) and RBC count of the camels were similar to the reference values of Farooq *et al* (2011). Neither short-nor long-distance lorry transportation showed any significant difference from the control group. The HGB concentration of the camels after short-distance lorry transportation was significantly higher than that of the control group. However, there was no significant difference in HGB concentration between the camels after long transportation and the control

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	Mean	SE(±)	Mean	SE (±)	Mean	SE(±)
Total T3 (ng/dL)	144.60	10.40	107.21 ^a	33.91	214.2 ^b	59.51
Total T4 (ug/dL)	10.46	0.76	9.98	2.46	18.52 ^a	4.32
TSH (uIU/mL)	0.05	0.04	0.05	0.04	0.04 ^a	0.02

Table 4. Effect of short and long lorry transportation distance on total T3, T4 and TSH of symptomatically hyperglycaemic dromedary camels.

^{a, b} Mean values of the short and long lorry transportation distance groups differed significantly from the value of the control group in the same row at P <0.05 and P <0.01, respectively.

 Table 5. Effect of short and long lorry transportation distance on serum antioxidant enzymes of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	Mean	SE(±)	Mean	SE (±)	Mean	SE(±)
SOD (U/mL)	305.51	5.89	232.72 ^a	17.65	175.70 ^b	31.50
CAT (u/L)	288.0	23.11	380.12 ^a	36.11	602.32 ^b	66.86
TAC (mM/L)	1.767	0.260	3.118 ^a	0.528	2.701 ^a	0.426

^{a, b} Mean values of the short and long lorry transportation distance groups differed significantly from the value of the control group in the same row at P <0.05 and P <0.01, respectively.

group. The blood index values are in agreement with the previous works of Al-Sultan (2008) and Alsaad (2009) on camels. The variation in blood index values might be attributed to variable RBC size, age and physiological state.

The mean WBC count values recorded for the control animals in the present study were comparable to those reported by Ahmad et al (2004) and Alsaad (2009) and lower than those reported by Al-Busadah (2007) and Al-Sultan (2008). The WBC count of camels after short-distance lorry transportation was significantly higher than in the control group, which may be attributed to transportation stress, as reported in early studies (Tadich et al, 2005). However, there was no significant difference in WBC count after longhaul lorry transportation and in the control camels. Similar findings were presented by Minka and Avo (2010). El Khasmi et al (2013) found no significant changes in WBC counts in camels transported under heat stress. Al-Wabel (2010) reported that the total WBC count in camels was not affected by the stressful conditions of transportation.

Differential leukocyte counts in the present study indicated that in the control group, lymphocyte were the most predominant leukocytes, followed by neutrophil, as has been previously reported (Al-Busadah, 2007) and contradictory to AL-Sultan (2008) and Al-Busadah and Osman (2000). Variation in the white cell values could be attributed to the differences in breeds or the stress of sample collection (Higgins and Kock, 1984). There was a significant decline in the lymphocyte count of the camels after shorthaul lorry transportation when compared to the control group. On the other hand, no significant difference was seen between lymphocyte counts in the camels after long-haul lorry transportation and in the control camels. In addition, the monocyte count did not significantly differ among all three camel groups. However, the neutrophil count was significantly higher in the camels after both shortand long-distance lorry transportation compared with the control group. In contrast, the EOS count was significantly lower in both short- and longdistance lorry transported animals than in the control group. The observation of neutrophilia and lymphocytopeniain camels transported by lorry in the present study is supported by a previous study (Minka and Ayo, 2007). Al-Wabel (2010) found that transportation stress had no effect on the neutrophil counting camels, whereas it caused a slight reduction in the lymphocyte counts.

Endocrine responses constitute late response to stressors after nervous reflexes. Stress can affect the endocrine balancing of the essential parameters of production, metabolism, immunity and even growth. Hormonal signals play a pivotal role in homeostasis. Appropriate thyroid function and hormonal activity perform an essential task in the mechanisms that allow domestic animals to live and breed effectively (Todini, 2007). Many external factors are capable of altering thyroid hormone concentrations by acting on the hypothalamus, pituitary and/or thyroid gland itself (Todini, 2007). A change in the thyroid blood concentrations has been used as an indicator of the stress response (Saeb et al, 2010). The serum concentrations of T4 and T3 in the control group parallel values reported earlier (Nazifi et al, 2009). The data obtained showed a significant increase of T3 and T4 levels in the serum of the symptomatically hyperglycaemic dromedary camels after longdistance lorry transportation compared to the control group. However, TSH levels in the camels after long-distance lorry transportation were significantly lower than in the control camels. Physiologically, as blood concentrations of T3 and T4 increase, both T3 and T4 inhibit TSH and as a result, the TSH level decreased during the long-distance transportation. These results are consistent with other studies which have shown that after long-distance lorry transport, T4 levels were elevated in Limousin calves (Fazio et al, 2001) and cattle (Fazio et al, 2005). In addition, Hartung (2003) stated that transportation stress might reduce animal fitness by inducing dysfunction of the pituitary and thyroid glands. The T3 and T4 variation during transportation may suggest the possible effect of stress on thyroid hormones (Saeb et al, 2010). Saeb et al (2010) found no significant change in serum T3 in camels; however, T4 increased after 1h of transportation, while T3 and T4 showed a significant increase after 5h of transport.

Antioxidant enzyme activities are major markers of oxidative stress. Results showed the effects of short- and long-haul lorry transportation on the activity of SOD, CAT and TAC in the symptomatically hyperglycaemic dromedary camels. A significant decrease was seen in SOD activity in camels after short- and long-distance lorry transportation compared to the control group. Meanwhile, CAT and TAC activity were significantly higher in the camels after short- and long-haul lorry transportation than in the control group. It has been suggested that the increase in both CAS and TAC acted as a compensation factor to balance the production of free radicals during the stress of transportation (Padalino et al, 2017). Both elevated and lowered antioxidant enzyme activities have been reported under different conditions as a result of enhanced reactive oxygen species (ROS) production, either by up-regulation of enzyme activity or depletion due to fighting the ROS during stress (El Khasmi et al, 2015). Pronounced deviations in the levels of ROS scavengers and free radical generation following or during stress have been noted in the serum of camels (Kataria et al, 2010).

Transportation stress in camels leads to an increase in catalase activity (Nazifi *et al*, 2009). However, further studies should be focused on understanding the character of particular antioxidant enzymes in the tissues and under different stressful conditions.

In conclusion, this study has confirmed that the stress of long-distance lorry transport can be considered somewhat similar to severe stress. The altered thyroid hormone levels and changes in the physiological metabolic profiles taken together may be effective biomarkers of transportation stress in this species. Present study conclude that pathophysiological changes may result from the transportation-induced stress in hyperglycaemic animals.

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