

# Evaluation of plasma cortisol and TBARS levels in calves after short - term transportation

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## SUMMARY

In the present study calves were subjected to stressful conditions that occur during short - term transportation. In order to describe the influence of transport stress to lipid peroxidation intensity as well as stress parameters plasma cortisol concentration and the indicator of lipid peroxidation processes were estimated.

The experiment was carried out on 9 clinically healthy calves, which were transported on a truck for a duration of 2 hours. The blood samples were taken the day before the transportation and on the 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 16<sup>th</sup> and 22<sup>nd</sup> day after transport. In the obtained plasma, the concentration of cortisol by use of enzyme immunoassay (EIA) was measured. The concentration of thiobarbituric acid-reactive substances (TBARS) was determined using colorimetric method.

The analysis of cortisol concentration in the samples collected from calves from the 1<sup>st</sup> to the 22<sup>nd</sup> day after transportation showed typical dynamics with massive increase followed by gradual decrease. The highest values were observed on the 1<sup>st</sup> and 3<sup>rd</sup> day after transportation. The analysis of lipid peroxidation processes with respect to TBARS content, showed significant increase on 1<sup>st</sup>, 3<sup>rd</sup> day ( $P=0.01$ ) and significant decrease on 6<sup>th</sup> day after transportation when compared to results in plasma obtained before transportation.

These results suggest that short term transportation can induce stressful conditions for cattle leading to lipid peroxidation and oxidative stress which can be evidenced by the variations of plasma cortisol.

**Keywords :** transport - stress - cattle - plasma cortisol - lipid peroxidation (TBARS).

## Introduction

The particular role of stress factors in pathology of bovine diseases has been observed. In many cases these factors have been estimated as main predisposing factors to infectious diseases [6]. Exposure to stress conditions (e.g. weaning, transport, remixing of animals, high temperature) has been associated with activation of the hypothalamic-pituitary-adrenal axis. The result of these processes is the stimulation of the steroid hormones secretion by adrenal cortex. The increased release of adrenocorticotrophic hormone (ACTH), which leads to the elevation of glucocorticoid concentrations is thought to be the primary agent mediating negative effects of stress on the biochemical processes [28]. Therefore, glucocorticoids (especially cortisol) concentrations in blood and its metabolites in feaces (11.17 DOA - 11.17 - Dioxoandrostanes) are widely used as a measure of stress in cattle [4, 17, 18, 19].

The stress reaction can influence various metabolic processes. The effects of these changes include among others the increase of the body temperature, heart and respiration rate and also the decrease of body weight. It is possible to

## RÉSUMÉ

Au cours de cette étude, la cortisolémie et le mécanisme de peroxydation lipidique ont été évalués sur des veaux soumis au stress d'un transport de courte durée. Par A. Wernicki, R. Urban-Chmiel, M. Kankofer, P. Mikucki, A. Puchalski et S. Tokarzewski.

L'expérience a porté sur 9 veaux cliniquement en bonne santé, transportés dans un camion pendant 2 heures. Les prélèvements sanguins ont été réalisés la veille et le 1<sup>er</sup>, 3<sup>ème</sup>, 9<sup>ème</sup>, 16<sup>ème</sup> et 22<sup>ème</sup> jours après le transport. La concentration plasmatique de cortisol a été mesurés par EIA (Enzyme Immuno Assay) et les concentrations de TBARS (Substances Réagissantes avec l'Acide ThioBarbiturique) par une méthode colorimétrique.

Le profil de la cortisolémie du 1<sup>er</sup> au 22<sup>ème</sup> jour après le transport a montré une évolution caractéristique avec des valeurs maximales obtenues le 1<sup>er</sup> et le 3<sup>ème</sup> jour. Les concentrations en TBARS ont également augmentées entre le 1<sup>er</sup> et le 6<sup>ème</sup> jour après transport par rapport aux concentrations initiales (avant transport) ( $P<0.01$ ) reflétant l'existence d'un processus de peroxydation lipidique.

Ces résultats montrent que le transport en lui-même correspond à des conditions stressantes pour les veaux se traduisant par une perturbation de l'homéostasie.

**Mots-clés :** Transport - Stress - Cortisolémie - Peroxydation lipidique (TBARS) - Veau.

notice the increase of total serum protein content, the decrease of the concentration of unsaturated fatty acid, urea, nitrogen and many others substances [2, 11, 12, 26].

These metabolic processes accompany oxidative stress which appears as the consequence of the excess in reactive oxygen species (ROS) production. The significant changes were observed in homeostatic mechanisms between production and neutralization of the following reactive oxygen species : Superoxide- $O_2^-$ , Peroxyl -  $R-O_2^-$ , Alkoxy -  $R-O$  Hydroperoxyl -  $HO_2$ , Hydroxyl -  $\cdot OH$ , Hydroperoxide -  $R-OO$ , radicals and reactive nitrogen (Nitric oxide -  $NO\cdot$ , Nitrogen dioxide -  $NO_2\cdot$ ) radicals [2, 7, 16]. The best described consequence of the generation of ROS is lipid peroxidation, which involves 3 steps : initiation, propagation and termination. In many pathologic cases the connection between the increase in ROS concentration, lipid peroxidation processes and/or decreased concentration of exo- and endogenous antioxidants has been observed. It is well known that the intensity of lipid peroxidation may be evaluated by the determination of its intermediates (conjugated dienes, lipid hydrocarbons) - substances which react with thiobarbituric

acid [3, 27]. The intensity of lipid peroxidation can be evaluated with respect to the increase of thiobarbituric acid-reactive substances (TBARS) in plasma [2, 14].

However very little information is available about the relationship between cortisol concentrations and the lipid peroxidation process in cattle subjected to the stress conditions induced by short-term transportation. Some authors suggested that cortisol increasing during transportation may directly influence the increase of free fatty acids, urea,  $\beta$ -hydroxybutyrate and total plasma albumin [6, 26].

The present study was undertaken to test the causal link between transportation stress - and production of ROS leading to lipid peroxidation.

The purpose of this study was to determine plasma cortisol and TBARS concentrations as main indicators of the stress reactions occurring directly after short-term transportation in calves. These determinations would help in establishing the relationship between both parameters and in elucidation of transportation stress mechanisms.

## Materials and methods

The experiment was carried out on 9 clinically healthy heifer calves of 4 months of age. They weighed about 100 kg and derived from different breeding environments. Animals were transported at the same time, by truck for about 2 hours (a distance of 130-140 km). The experiment was performed in April and the ambient temperature was 12°C. After the transportation the animals were kept for feeding in the traditional cattle-house. The blood samples were collected on heparine as anticoagulant, in the morning (at about 9.00 a.m.) by jugular venipuncture the day before and on the 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 16<sup>th</sup> and 22<sup>nd</sup> days after the transportation. Plasma was stored at -20°C until assayed.

Cortisol concentrations after extraction with diethylether were determined by use of enzyme - immunoassay (EIA). Chemicals were kindly obtained from E. Möstl, University of Veterinary Medicine, Vienna, [18]. The procedure was carried out according to Palme and Möstl [18].

The plasma concentrations of lipid peroxidation products were evaluated by the determination of the TBARS concentrations based on the method proposed by Ledwozyw [13].

In brief, 2.5 ml of 20% trichloroacetic acid in 0.6 mol/dm<sup>3</sup> HCl was added to 0.5 ml of plasma and allowed to stand for 10 min. Then 1.5 ml of 0.67% TBA in 1 mol/dm<sup>3</sup> NaOH was added and the mixture was heated for 20 min in a boiling water bath. After cooling to room temperature, 4 ml of n-butanol was added, the mixture was shaken for 3 min and centrifuged for 10 min at 1500xg. The absorbance of the butanol layer was measured at 532 nm (Ultrospec 2000, Pharmacia, Sweden). The results were calculated using a standard curve prepared with different dilutions of malondialdehyde and expressed in  $\mu\text{mol/g}$  protein.

Total protein concentration in examined samples was measured according to the manufacturer directions using biuretic reagent (Cormay Total Protein 60, Romania) and photometer LP 400 with filter OD= 595 nm (Dr Bruno Lange GmbH, D).

The influence of additional stress connected with new environment, although the same for all animals, was not taken into consideration in present study. This topic, however, will be analysed in coming experiment.

The obtained results of cortisol and TBARS concentration were statistically analysed by use of ANOVA test ( $P=0.01$ ) (Statistica 6.0). The correlation coefficient between cortisol and TBARS concentrations in examined samples was analysed statistically (Pearson's linear correlation coefficient) with use of Statistica 6.0. P value  $<0.05$  was considered as significant.

## Results

Plasma cortisol concentration measured at 1<sup>st</sup> to 9<sup>th</sup> day after the transportation in samples obtained from calves exposed to short-term transportation stress was significantly higher ( $P \leq 0.01$ ) as compared to values obtained before transportation (day 0). (Table I)

The highest mean cortisol concentrations were observed directly after the transportation (153.02 nmol/l). Cortisol concentrations remained markedly elevated during the first 3 days after transportation. The values were about 3 times higher than the cortisol concentration in samples obtained from calves before the transportation (50.19 nmol/l). At a later period, from 6<sup>th</sup> day to 22<sup>nd</sup> day after the transportation

Day of samples collecting	Cortisol concentration (nmol/l)	TBARS (mmol/g protein)
<b>Before transportation</b>	50.19 ± 8.3	0.125 ± 0.013
<b>Day 1 after transportation</b>	153.03* ± 25.2	0.178* ± 0.0001
<b>3</b>	145.99* ± 16.1	0.208* ± 0.003
<b>6</b>	111.73* ± 17.9	0.193* ± 0.002
<b>9</b>	91.96* ± 17.9	0.129 ± 0.012
<b>16</b>	54.87 ± 17.4	0.131 ± 0.013
<b>22</b>	42.8 ± 12.0	0.129 ± 0.012

\*) Significant difference in comparison to Day before transportation,  $P \leq 0.001$ .

TABLE I. — The mean values of plasma cortisol and TBARS concentrations in samples obtained from calves before and after transportation.

the cortisol concentrations measured in transported animals showed a significant decrease ( $P \leq 0.01$ ) as compared to values obtained at 1<sup>st</sup> and 3<sup>rd</sup> day. The result on the 16<sup>th</sup> day (54.87 nmol/l) was similar (with no significant differences) to the initial concentration. (Figure 1).

Analysis of the TBARS content - an index of lipid peroxidation intensity - showed statistically significant increase ( $P \leq 0.01$ ) on the 1<sup>st</sup> and 3<sup>rd</sup> day as well as significant decrease on 6<sup>th</sup> day after the transportation in comparison to initial values (before transportation). The highest values were observed on the 1<sup>st</sup> (0.178 μmol/g protein), 3<sup>rd</sup> (0.208 μmol/g protein) and 6<sup>th</sup> (0.193 μmol/g protein) days after the transportation. (Figure 1).

The analysis of the overall correlation between plasma cortisol variations and TBARS detected a significant and positive correlation ( $r=0.70$ ) at  $P < 0.05$  (Figure 2). The specific correlation coefficient between cortisol and TBARS concentration for a particular day of experiment did not show significant correlation, except from day 16<sup>th</sup> ( $r=-0.72$ ,

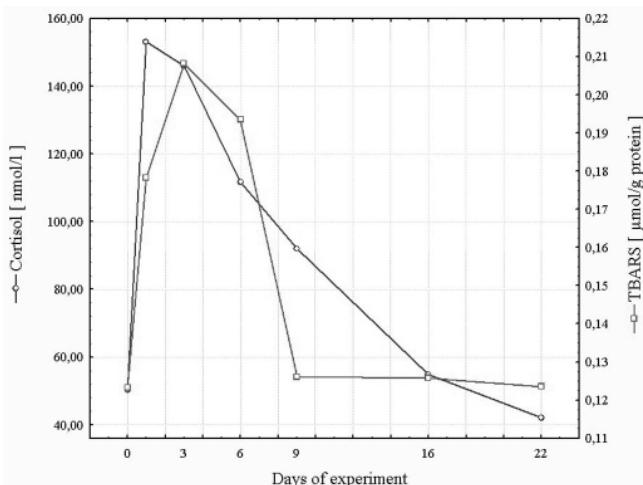


FIGURE 1. — The mean values of plasma cortisol and TBARS concentrations in samples obtained from calves before and after transportation.

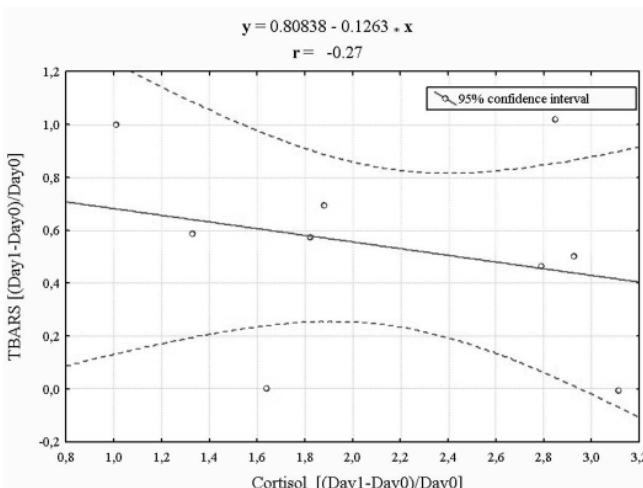


FIGURE 3. — Correlation coefficient between cortisol and TBARS concentration 1<sup>st</sup> day after transportation.

(Figure 7). It was possible to notice the negative correlation at every examined day as follows : 1<sup>st</sup> day -  $r=-0.27$ , 3<sup>rd</sup> day -  $r=-0.50$ , 6<sup>th</sup> day -  $r=-0.57$ , 9<sup>th</sup> day -  $r=-0.43$  and 22<sup>nd</sup> day -  $r=-0.47$  (Figure 3 - 6, 8).

## Discussion

The analysis of plasma cortisol concentrations obtained from calves from the 1<sup>st</sup> through 22<sup>nd</sup> days after transportation showed dynamic changes. Many authors [23 - 25, 28] have stated that transportation increased plasma and serum cortisol concentrations in cattle. The highest values obtained at the 1<sup>st</sup> to 3<sup>rd</sup> day after the transportation, suggest a significant influence of stress connected with transportation on the homeostatic mechanisms of the living organisms. The plasma cortisol concentrations at the 9<sup>th</sup> days after transportation, suggest a long adaptation period of the animals to the new environment and possible two stressful and combined events. However, this problem will be analyzed in the near

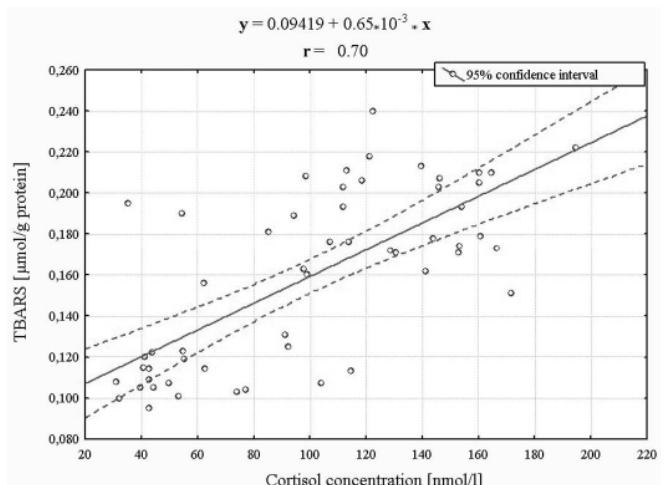


FIGURE 2. — Correlation coefficient between cortisol and TBARS concentration.

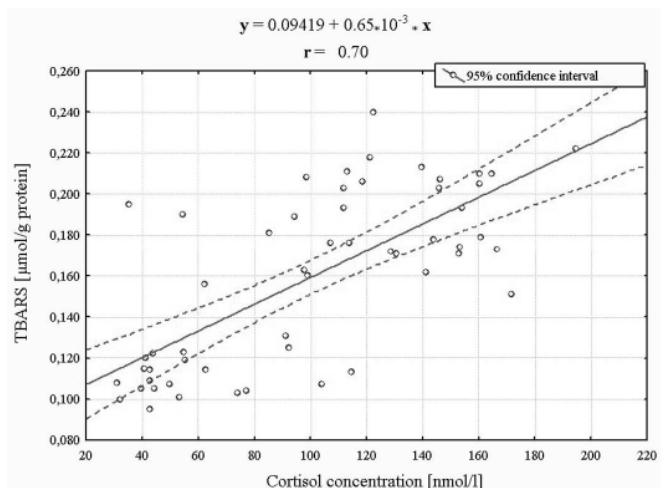


FIGURE 4. — Correlation coefficient between cortisol and TBARS concentration 3<sup>rd</sup> after transportation.

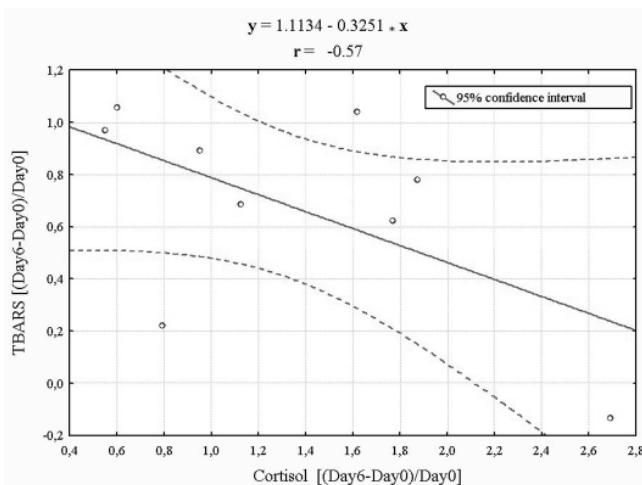


FIGURE 5. — Correlation coefficient between cortisol and TBARS concentration 6<sup>th</sup> day after transportation.

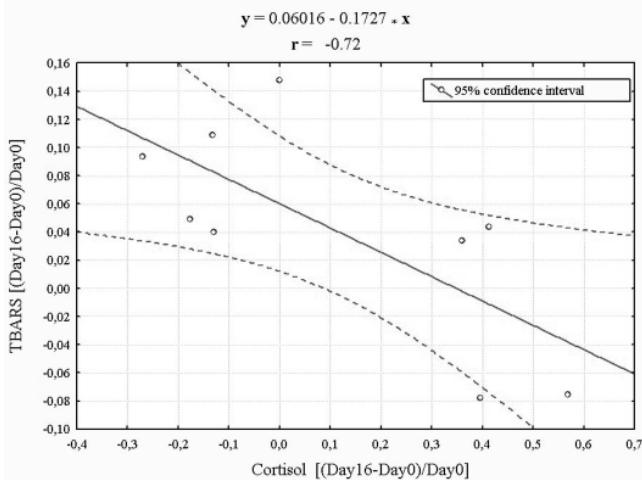


FIGURE 7. — Correlation coefficient between cortisol and TBARS concentration 16<sup>th</sup> day after transportation.

future experiment. The experiments carried out by [5] showed similar results ; a rapid growth from 20-40 nmol/l to 130-260 nmol/l in cortisol concentrations in cattle after different types of stress (weaning, transportation).

Many publications showed a correlation between stress reaction and increase of lipid peroxidation intensity in tissue such brain. The increase of TBARS concentration in serum is an indicator of this process [15, 21]. The positive and significant correlation obtained in the present study may confirm that transport induces stressful conditions evidencing by plasma cortisol and TBARS concentrations.

Some authors suggesting that cross talk between neuroendocrine control of stress response and cellular antioxidant systems may be essential for the adaptation processes. The cascade of biochemical events leading to systemic stress response starts from the activation of neuroendocrine pathways such as: sympathetic nervous system, hypothalamic pituitary axis as well as renin-angiotensin system and is followed by the release of the corresponding stress hormones (i.e. catecholamines, corticosteroids, growth hormone, glucagone

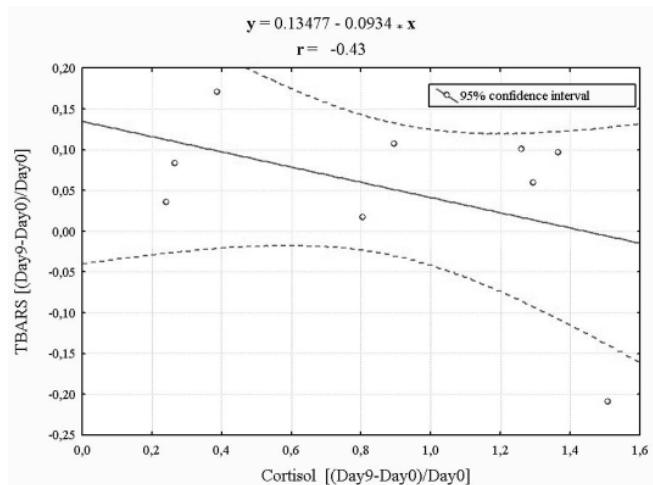


FIGURE 6. — Correlation coefficient between cortisol and TBARS concentration 9<sup>th</sup> day after transportation.

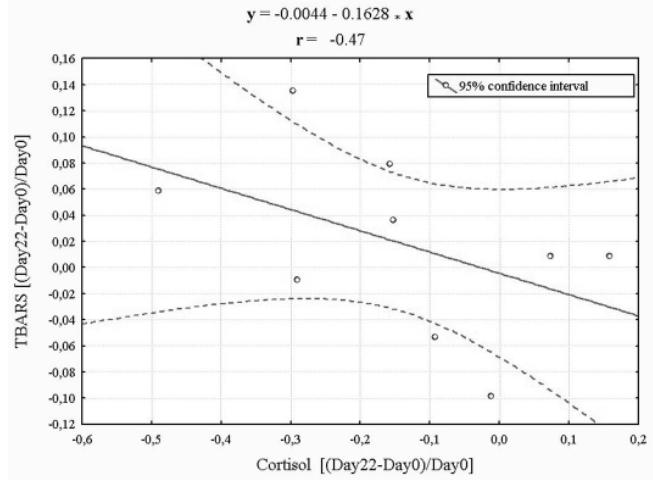


FIGURE 8. — Correlation coefficient between cortisol and TBARS concentration 22<sup>nd</sup> day after transportation.

and renin). These processes together with cytokines, which are induced by stress, initiate acute phase response as well as induce acute phase proteins which are essential mediators of inflammation. Stress-induced increase of lipids may be one of indispensable factors for macrophage activation [8, 15].

Cattle stress is a main predisposing factor for respiratory diseases especially causing *shipping fever* syndrome, which also has influence on economic losses in the beef production. It is common knowledge that this respiratory syndrome is caused by many infectious agents ; viruses (e.g. BRSV, PI-3), and bacteria (especially *Mannheimia haemolytica*). In some articles, authors suggest that viruses can also affect the host cell pro/antioxidant balance by increasing cellular pro-oxidants. Some of the viruses were shown to activate monocytes and polymorphonuclear leukocytes to generate free radicals [20, 22]. The results obtained in our experiment are mostly similar with results of other authors who evaluated the influence of different stress factors like psychological stress, hypoxia on TBARS concentrations. The occurrence of increasing TBARS concentration in examined plasma showed the significant role of transporting stress reaction on

lipid peroxidation processes. [15, 29]. These observations suggest that transport stress induce lipid peroxidation processes in animal tissues. Probably the physical effort during transport, which is connected with standing on the platform, keeping the balance and lack of room for changing of position can have great influence on the metabolic processes observed in muscles. Indeed the presence of these processes was observed during physical efforts and exercise in human and animals [1, 9, 10, 25]. In horses, after the transportation and training exercises a significant increase of lipid peroxidation was observed [8]. Probably the same or similar processes occur in transporting calves. Muscle immobilization can induce directly the release of catecholamines in CNS and also can initiate a local inflammatory response which lead to cytokine production. Catecholamines and cytokines (IL1, IL6 and TNF) contribute to activation of hypothalamic-pituitary axis and to glucocorticoids synthesis. Moreover during weaning and mixing of calves [23, 24, 28] animals react by activation neuroendocrine pathways and develop stressful reactions. These physical and psychological factors have an influence on the increase of catabolic reactions, which may cause an increase of ROS production.

A significant increase of cortisol concentrations and of lipid peroxidation may suggest a significant influence of transport stress on homeostatic mechanisms in calves. The estimation of direct relationship between pathology and lipid peroxidation caused after stress needs further examinations like respiratory diseases frequently observed in calves.

In conclusion, we suggest that measurement of plasma cortisol and TBARS concentration can be used to provide a better understanding of stress reaction in transported cattle.

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## References

- ASTHON T., YOUNG I.S., PETERS J.R., JONES E., JACKSON S.K., DAVIES B., ROWLANDS C.C. : Electron spin resonance spectroscopy, exercise, and oxidative stress : an ascorbic acid intervention study. *J. Appl. Physiol. Occup. Physiol.* 1998, **6**, 2032-2036.
- CLARKSON P.M., THOMPSON S.S. : Antioxidants : what role do they play in physical activity and health ? *Am. J. Clin. Nutr.* 2000, **72**, 637-646.
- CORONGIU F.P., POLI G., DIANZANI M.U., CHEESEMAN K.H., SLATER T.F. : Lipid peroxidation and molecular damage to polyunsaturated fatty acids in rat liver : recognition of two classes of hydroperoxides formed under conditions in vivo. *Chem. Biol. Interact.* 1986, **59**, 147-155.
- DANTZER R., MORMEDE P. : Stress in farm animals : A need for re-evaluation. *J. Anim. Sci.* 1983, **57**, 6-18.
- EWBANK R., PARKER M.J., MASON C.W. : Reactions of cattle to head restraint at stunning : A practical dilemma. *Anim. Welfare* 1992, **1**, 55-63.
- GRANDIN T. : Assesment of stress during handling and transport. *J. Anim. Sci.* 1997, **75**, 249-257.
- INANAMI O., SHIGA A., OKADA K., SATO R., MIYAKE Y., KUWABARA M. : Lipid peroxides and antioxidants in serum of neonatal calves. *Am. J. Vet. Res.* 1999, **60**, 452-457.
- ISHIDA N., HOBO S., TAKAHASHI T., NANBO Y., SATO F., HASEGAWA T., MUKOYAMA H. : Chronological changes in superoxide-scavenging ability and lipid peroxide concentration of equine serum due to stress from exercise and transport. *Equine Vet. J. Suppl.* 1999, **30**, 430-433.
- JI L.L., LEUWENBURGH C., LEICHTWEIS S., GORE M., FIEBIG R., HOLLANDER J., BEJMA J. : Oxidative stress and aging. Role of exercise and its influences on antioxidant systems. *Ann. N. Y. Acad. Sci.* 1998, **854**, 102-117.
- KANTER M. : Free radicals, exercise and antioxidant supplementation. *Proc. Nutr. Soc.* 1998, **1**, 9-13.
- KNOWLES T.G. : The effects of transport in slaughter weight lambs. *Br. Soc. Anim., Winter Meeting summary*, 1995, Paper 43.
- KNOWLES T.G. : A review of the road transport of cattle. *Vet. Rec.* 1999, **144**, 197-201.
- LEDWOZYK A., MICHALAK J., STEPIEN A., KADZIOLKA A. : The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human arteriosclerosis. *Clin. Chim. Acta* 1986, **155**, 275-284.
- MACHLIN L.J., BENDICH A. : Free radical tissue damage : protective role of antioxidant nutrients. *F.A.S.E.B. J.*, 1987, **6**, 441-445.
- MATSUMOTO K., YOBIMOTO K., HUONG THU THI N., FATTAH-ABDEL M., HIEN T.V., WATANABLE H. : Psychological stress - induced enhancement of brain lipid peroxidation via nitric oxide systems and its modulation by anxiolytic and anxiogenic drugs in mice. *Brain Res.* 1999, **839**, 74-84.
- MORICILLO E.J., ESTRELA J., CORTIJO J. : Oxidative stress and pulmonary inflammation pharmacological intervention with antioxidants. *Pharm. Res.* 1999, **40**, 393-404.
- MÖSTL E., MESSMAN S., BAGU E., ROBIA C., PALME R. : Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. *J. Vet. Med. A*, 1999, **46**, 621-632.
- PALME R., MÖSTL E. : Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Int. Mammal. Biol.* 1997, **62**, Suppl II, 192 - 197.
- PALME R., ROBIA C., BAUMGARTNER W., MÖSTL E. : Transport stress in cattle as reflected by increase in faecal cortisol metabolite concentrations. *Vet. Rec.* 2000, **146**, 108-109.
- RIBBLE C.S., SHOUKRI M.M., MEEK A.H., MARTIN S.W. : Clustering of fatal fibrinous pneumonia (shipping fever) in feedlot calves within transport truck and feedlot pen groups. *Prev. Vet. Med.* 1994, **21**, 251-261.
- SCARPELLINI F., SBRACIA M., SCARPELLINI L. : Psychological stress and lipoperoxidation in miscarriage. *Ann. N. Y. Acad. Sci.* 1994, **709**, 210-213.
- SCHWARZ K. B. : Oxidative stress during viral infection : A review. *Free Rad. Biol. Med.* 1996, **21**, 641-649.
- STEINHARDT M., THIELSCHER H.H. : Reactions of young dairy calves and young suckling calves from the mother cow herds to transportation induced stress-effects of age and rearing conditions. *Dtsch. Tierärztl. Wochenschr.* 1998, **105**, 17-24.
- STEINHARDT M., THIELSCHER H.H. : Quality of development and adaptation reactions of dairy calves at specific age periods in early life. Effect of rearing variations on proteins and minerals and on metabolic variables of blood. *Dtsch. Tierärztl. Wochenschr.* 2000a, **12**, 510-518.
- STEINHARDT M., THIELSCHER H.H. : Reactions of dairy calves exposed to transport stress at 60 days of age. Effects of various rearing conditions in the developmental quality of calves on physiological variables and their changes. *Dtsch. Tierärztl. Wochenschr.* 2000b, **107**, 59-65.
- WARRIS P.D., BROWN S.N., KNOWLES T.G., KESTIN S.C., EDWARDS J.E., DOLAN S.K., PHILLIPS A.J. : Effects on cattle of transport by road for up to 15 hours. *Vet. Rec.* 1995, **136**, 319-323.
- YU B.P., SUESCUN E.A., YANG S.Y. : Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2 : modulation by dietary restriction. *Mech. Ageing Dev.* 1992, **65**, 17-33.
- ZAVY M.T., JUNIEWICZ P.E., PHILLIPS A.A., VON TUNGELN D.L. : Effect of initial resistant, weaning, and transport stress on baseline and ACTH - stimulated cortisol responses in beef calves of different genotypes. *Am. J. Vet. Res.* 1992, **53**, 551-557.
- ZHANG J., PIANTADOSI C.A. : Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J. Clin. Invest.* 1991, **90**, 1193-1199.