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MUSCULAR DYSTROPHY IN DAIRY COWS FOLLOWING A CHANGE IN HOUSING TECHNOLOGY

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Abstract

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The objective of the study was to monitor the state of health (in particular damage to muscular tissues) in dairy cows suffering from a marked selenium deficiency. The investigations were conducted in a herd of Bohemian Red Pied cattle at the time of a transfer from a stanchion barn into loose boxes. Biochemical tests were done in nine clinically normal dairy cows before the transfer and thereafter on days 1, 3, 7, 14, and 28. Apart from selenium deficiency, only moderate increases of creatine kinase (CK) and lactate dehydrogenase (LD) were found before the transfer. Significant increases of CK, aspartate aminotransferase (AST) and LD activities and potassium concentration in blood plasma found on day 1 were indicative of a muscular damage. The activities of CK and LD, the concentration of potassium, and less markedly the activity of AST decreased at the time of the subsequent sampling (day 3). Another significant increases in CK, LD, and AST activities, indicative of persisting muscular damage, were observed, along with a decrease in glutathione peroxidase activity in whole blood, on day 28. Clinical muscular dystrophy, manifested by general weakness, subcutaneous oedema, and downer syndrome, and accompanied by marked increases in CK, LD, and AST activities was observed in four dairy cows of the transferred group. The results are an evidence of muscular dystrophy in adult selenium-deficient dairy cows in response to increased locomotor activity and stress associated with the change in housing conditions.

Selenium, cattle, glutathione peroxidase, creatine kinase, aspartate aminotransferase, lactate dehydrogenase

Muscular dystrophy associated with selenium and vitamin E deficiencies ranks with frequently occurring disorders described in many animal species. In his monograph, Shamberger (1983) described nutritional muscular dystrophy in chicks, rats, swine, mice, dogs, monkeys, sheep, goats, and calves and attached importance rather to selenium than to vitamin E supplementation in the prevention of this disease in sheep, goats, and calves. Nutritional muscular dystrophy in large farm animals is described as a disease occurring in America, Europe and Australia. In the Czech Republic muscular dystrophy in young cattle was described by Kursa (1969) and a massive outbreak also in young cattle was reported from Slovakia by Vrzgula (1972).

It has been generally accepted that muscular dystrophy affects most frequently young, rapidly growing calves, lambs and foals (Blood and Radostits 1989; Maas et al. 1996). Enzootic muscular dystrophy due to selenium and vitamin E deficiencies in horses and white muscle disease in cattle, sheep, goats and camels have also been included into a review of musculoskeletal disorders published by Doherty and Mulville (1992). Muscular dystrophy in cattle develops mostly in milk-fed calves, in young cattle at the beginning of the grazing period, and in late pregnant and postparturient cows. In addition to selenium and vitamin E deficiency, a significant role in the development of the disease is attributed to a high intake of unsaturated fatty acids increasing the concentration of hydroperoxides in the

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organism. Further factors participating in the development of muscular dystrophy are physical strain and unfavourable weather which increase the necessity for energy sources. These contributing factors may induce the development of clinical muscular dystrophy (McMurray and McEldowney 1977). Accumulation of toxic peroxides gives rise to cell membrane and tissue damage resulting in myopathy. This peroxidative damage is associated with increased release of lysosomal enzymes the activity of which can increase up to a hundredfold at the beginning of the clinical disease. Relevant to the diagnosis of muscular damage are creatine kinase, aspartate aminotransferase, and lactate dehydrogenase, of which creatine kinase is the most sensitive and specific indicator of muscular damage. Lactate dehydrogenase and aspartate aminotransferase are less specific, because they are released also from other damaged cells, in particular hepatocytes (Duncan and Prasse 1997; Cardinet 1997; Meyer and Harvey 1998).

Our clinical and biochemical investigations were focused on muscular damage, specifically on the development of muscular dystrophy in selenium-deficient dairy cows at the time of increased physical strain and stress resulting from a change in housing technology. The investigations included determination of activities of muscular cell enzymes in blood plasma, monitoring of further biochemical parameters, and observation of the development of clinical nutritional myodystrophy in dairy cows compromised by selenium deficiency and exposed to unusual challenge.

Materials and Methods

The investigations were conducted in a herd of Bohemian Red Pied cattle in north-east Bohemia at the time of transfer from a stanchion barn into loose boxes. Insufficient selenium supply had been repeatedly demonstrated in the herd on previous examinations (whole blood selenium < 10 $\mu g.l^{-1}$). The investigations were conducted at the time when the cows were transferred from a stanchion barn with a capacity of 178 heads into new premises for 400 dairy cows with loose boxes and a herring bone milking parlour. Monitoring of the state of health was oriented on locomotor disorders and other signs of muscular damage in the transferred cows. Biochemical tests were done in a selected group of nine cows. Attention was paid also to animals with pronounced manifestation of non-traumatic locomotor disorders.

a) Examination before the transfer

Nine clinically normal cows aged 4 to 7 years in the 2nd or 3rd month of lactation were selected. Blood samples were collected from *v. jugularis* into glass tubes and disposable heparinised plastic tubes. Blood plasma was separated by centrifugation within 2 h after sampling. Blood serum was separated from clotted blood after 24 h. Blood plasma, blood serum and whole heparinized blood were kept in a cool place until processing. Blood plasma was analysed for the contents of total protein, urea, creatine, glucose, triacylglycerols, cholesterol, activities of aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LD), γ-glutamyl transferase (GMT), sorbitol dehydroghenase (SD), alkaline phosphatase (ALP), and concentrations of sodium, potassium, magnesium, calcium, phosphorus, and vitamin E. Blood serum samples were tested for the concentrations of nonesterified fatty acids (NEFA) and bilirubin, and whole blood for the activity of glutathione peroxidase (GSH-Px). The concentration of vitamin E was determined fluorimetrically using the fluorescence spectrophotometer Perkin Elmer 204 (Bouda et al. 1980), that of NEFA photometrically (Dieterle et al. 1968) and those of minerals by flame atomic absorption spectrophotometry. The activity of GSH-Px in whole blood was determined as described by Paglia and Valentine (1967) using the Ransel Randox set. All the remaining biochemical values were determined using the automatic analyser Cobas Mira.

b) Examination after transfer

Blood samples collected at post-transfer days 1, 3, 7, 14, and 28 were tested specifically for damage to muscular cells (AST, LD, CK, potassium) and for hepatic functions (bilirubin, GMT, SD) to allow correct interpretation of changes in activities of nonspecific enzymes relevant to the diagnostics of muscular damage. Further the GSH-Px activity was measured.

c) Examination of sick animals

Four of the transferred cows developed pronounced clinical signs of a disease of the locomotor system, or even the downer cow syndrome from post-transfer day 5. All the affected cows were older than 6 years and in the 1st to 4th month of lactation. The cows were examined clinically and blood samples collected from two of them at day 7 were analysed for the full range of biochemical parameters tested as before the transfer.

The results were processed using the two-tailed *t*-test and the Excel software.

Results and Discussion

a) Examination before the transfer

Clinical examination did not reveal any health disorder in the cows to be transferred. However, the activity of GSH-Px (33.61 ± 12.62 μkat.l^{-1}) along with data from previous examinations indicated a serious selenium deficiency. The obtained value was considerably lower than the activity recommended by our laboratory (> 665 μkat.l^{-1}) which is equivalent to whole blood selenium concentration of 100 μg.l^{-1} (Pavlata et al. 2000). Further a moderate increase in CK activity in blood plasma (1.96 ± 0.78 μkat.l^{-1}) and LD (39.09 ± 6.57 μkat.l^{-1}) and marginal activity of AST (1.38 ± 0.22 μkat.l^{-1}) were found. Our data were interpreted with regard to reference ranges for CK (0.12 to 0.60 μkat.l^{-1}) suggested by Vrzgula and Sokol (1990) and AST (0.72 to 1.41 μkat.l^{-1}) and LD (16.31 to 29.05 μkat.l^{-1}) recommended by Pechová (1992). All the remaining parameters were within the respective reference ranges. It can therefore be concluded that the biochemical examinations did not discover any health disorder except for subclinical muscular dystrophy associated probably with a poor selenium status due to a low selenium content in the ration. The probable primary cause is a low selenium content in soil in the foothill area and hence in crops grown there. The mineral supplement fed with concentrates was selenium-free. More serious damage to the muscular tissue was probably prevented by abundant supply of vitamin E, as indicated by a high concentration in blood plasma (12.04 ± 2.90 μmol.l^{-1}).

b) Examination after the transfer

Data showing dynamics of parameters indicating damage to muscular tissue (activities of CK, AST and LD and blood plasma potassium concentration), including statistical significance of changes observed after the transfer are given in Figures 1 to 4 and Table 1.

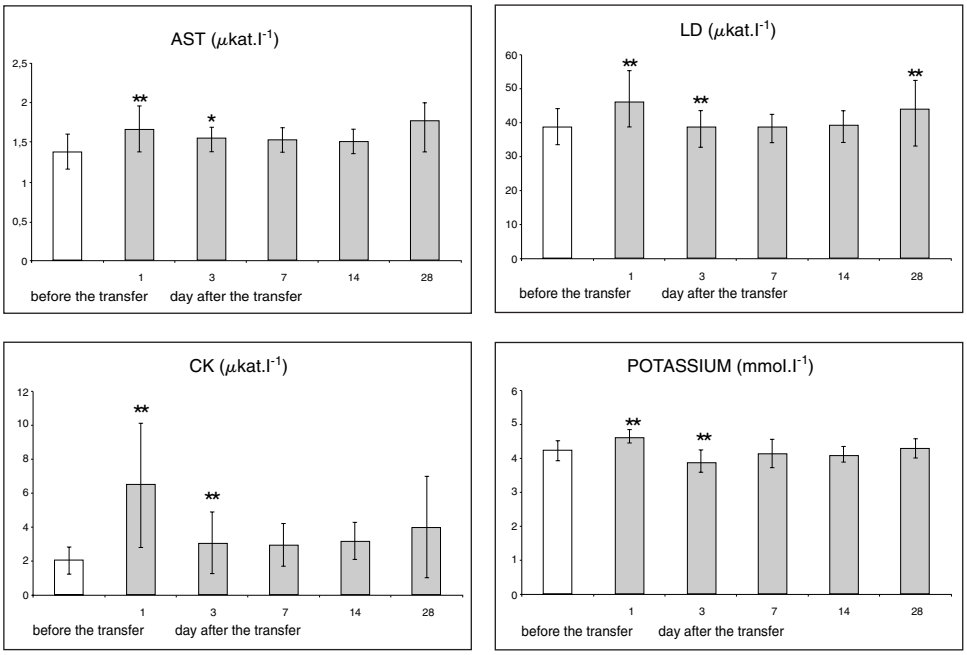

Fig. 1 to 4: Dynamics of parameters indicating muscular damage (mean ± S.D.) during the observation period. Data on significance of differences (** $p < 0.01$, * $p < 0.05$) pertain to the immediately preceding value.

Table 1
Changes in biochemical values after the transfer in terms of percentages of the values found before the transfer. Data on statistical significance of differences (** $p < 0.01$, * $p < 0.05$) pertain to values found before the transfer.

Day after the transfer	1	3	7	14	28
AST (%)	120	111	110	107	127
	**	*	-	-	**
LD (%)	118	99	99	102	112
	**	-	-	-	**
CK (%)	323	152	145	156	238
	**	-	-	*	*
POTASSIUM (%)	110	93	98	97	101
	**	-	-	-	-

The transfer was immediately followed by significant increases in blood plasma activites of CK (6.34 ± 3.57 μkat.l^{-1}), AST (1.66 ± 0.29 μkat.l^{-1}), and LD (46.18 ± 8.67 μkat .l^{-1}), and potassium concentration (4.59 ± 0.19 mmol.l^{-1}). It can be concluded from the obtained data that the transfer resulted in a damage to muscle cells manifested by increased activities of enzymes and potassium concentration in blood plasma. As the activities of GMT and SD and concentration of bilirubin did not change, it is evident that the increase in the activities of the nonspecific enzymes AST and LD was attributable also to muscular damage. The activity of LD and potassium concentration returned to initial values within three days after the transfer. Significant decreases were also observed for the activities of AST and CK, but initial values were not reached throughout the observation period. The decrease in enzymatic activities continued at day 7 and another marked increase, indicative of persisting muscular damage, was found at day 28. The dynamics of the parameters under study allow the conclusion that the muscular damage was associated with the stress induced by sudden environmental change and increased physical strain. After a transient improvement, the activities of enzymes indicating muscular damage increased again. This change can be interpreted either as a continuing moderate muscular dystrophy, or a relapse thereof due to selenium deficiency as indicated by low GSH-Px activity. However, the changes in enzymatic activities were not accompanied by clinical manifestations.

Dynamics of GSH-Px activity during the observation period indicating the selenium status are shown in Fig. 5.

The activity of GSH-Px was very low already before the transfer and decreased thereafter, amounting to about 70 % of the initial value in the period between days 7 and 28. We can assume that the low antioxidant capacity could also have been involved in the continuing damage to the muscular tissues. Graphic presentation of the data shows an inverse relationship between the activities of CK, AST and LD on the one hand and GSH-Px on the other hand. In other words, the increase in the activity of the former three enzymes is accompanied by a decrease of the latter.

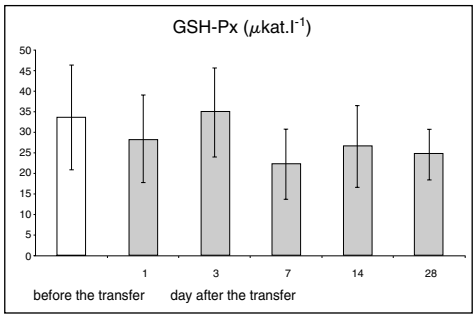

Fig. 5: Activity of GSH-Px (μkat.l^{-1}) in whole blood of dairy cows during the observation period.

The results of our investigations are consistent with the data published by Duncan and Prasse (1986) who characterised CK as a very rapidly reacting indicator of muscular damage with a relatively short degradation

half-time. Its activity reaches peak value 6 to 12 h after the rise of muscular damage. If this damage is transient only, the activity of CK returns to normal values within 24 to 48 h. Hence permanent increase of CK activity indicates persisting active muscular damage. A similar, if less pronounced, activity pattern was observed for LD. Unlike CK and LD, the activity of AST increased only slowly, but this change persisted for several days after the repair of muscular damage. Similar results were obtained by Cardinet (1997) who interpreted permanent increase in CK activity as a sign of continuing active myonecrosis and an increase in AST activity accompanied by decreasing or normal CK activity as a sign of a discontinued process.

The acute muscular damage, which developed immediately after the transfer of the herd under study into loose boxes, apparently resulted from increased physical strain, while that observed later during the observation period was due to selenium deficiency, or to the decrease in GSH-Px activity. This interpretation is supported by the development of clinical muscular dystrophy.

Another factor involved in the changes of enzymatic activities was stress induced by the change in housing conditions, increased locomotor activity, new milking technique, and rearrangement of the social hierarchy. Generally, stress is associated with increased production of highly reactive cell-damaging oxygen radicals and peroxides. Stress can also result in a loss of metals and proteins necessary for the synthesis of specific metalloenzymes (including GSH-Px) which degrade free radicals. Hence, this loss increases and prolongs their adverse effects on cells (Nockels 1994). This mechanism could also have underlain the GSH-Px-mediated muscular damage observed in our investigations. The tissue damage was manifested by increased activity of enzymes released from damaged cells. Very high activities of CK and AST in stressed cattle were described by Bennet et al. (1989). However, their results did not allow them to distinguish accurately between the roles of high levels of free radicals and of damage to tissues.

c) Examination of clinically sick animals

The affected dairy cows developed marked general weakness, reluctance to move, staggering when standing up or inability to stand, and eventually the downer cow syndrome without loss of consciousness. The onset of the first clinical manifestations was followed after two days by the development of subcutaneous oedema. Neither feed intake nor ruminal peristalsis were altered and body temperature and pulse and breathing rates were normal. Laboratory tests done in two of the affected cows showed very high activities of CK (106.2 and 69.8 $\mu kat.l^{-1}$), AST (6.04 and 5.24 $\mu kat.l^{-1}$), and LD (88.7 and 59.58 $\mu kat.l^{-1}$). None the remaining biochemical values exceeded the reference range. The observed clinical pattern along with laboratory findings documented the development of clinical muscular dystrophy, which probably resulted from combined action of selenium deficiency and strong stress, or more specifically, poor adaptability of older cows to the change in housing conditions. Considering the continuing aggravation of the state of health, all the cows developing clinical muscular dystrophy were emergency slaughtered.

Reports on clinically apparent muscular dystrophy in adult cattle are published rather seldom. Most of the described cases were associated with parturition (Kováč and Vrzgula 1990). A similar clinical pattern of myopathy (reluctance to move, hind leg weakness, downer syndrome), accompanied by “flying scapulas” and confirmed by necropsy and microscopic findings, was described by Gunning and Walters (1994) in Friesian heifers aged 18 to 24 months. Buergelt et al (1996) reported nutritional myodegeneration accompanied by “flying scapulas” in grazing selenium-deficient beef heifers. Illek et al. (2000) observed death of some beef cows with strong deficiency of selenium during a pasture.

Our results have identified sudden increase in physical strains and stress resulting from a change in housing technology as additional factors involved in the aetiology of muscular dystrophy which can develop into a clinically apparent form and eventually the downer cow syndrome in selenium-deficient animals.

Vznik svalové dystrofie u dojnic při změně technologie ustájení

Cílem práce bylo monitorovat zdravotní stav (především svalové poškození) u dojnic s výraznou karencí selenu v období změny technologie ustájení. Sledování se uskutečnilo v chovu dojnic českého strakatého skotu v období přesunu zvířat ze stáje s vaznou technologií do stáje s volným ustájením. U devíti vybraných klinicky zdravých dojnic bylo před přesunem a 1., 3., 7., 14. a 28. den po přesunu provedeno biochemické vyšetření. Před přesunem bylo kromě karence selenu zjištěno jen mírné zvýšení kreatinkinázy (CK) a laktátdehydrogenázy (LD). První den po přesunu bylo zjištěno svalové poškození na základě statisticky významného nárůstu aktivity CK, aspartátaminotransferázy (AST), LD a koncentrace draslíku v plazmě. Při dalším odběru byl zjištěn pokles aktivity LD, CK a koncentrace K, pozvolněji i AST. Další statisticky významný vzestup AST, LD i CK dosvědčující přetrvávající svalové poškození, byl zaznamenán 28. den po přesunu (při současně se snižující aktivitě glutathionperoxidázy v plné krvi). Dále byl popsán výskyt klinické formy svalové dystrofie u 4 dojnic přesunutého stáda doprovázený jejich celkovou slabostí, edematizací podkoží a ulehnutím, při současném výrazném vzestupu CK, AST i LD. Tyto zaznamenané výsledky dokládají vznik svalové dystrofie (včetně její klinické formy) u dospělých dojnic s karencí selenu po zvýšení pohybové aktivity a stresové zátěže v souvislosti se změnou technologie ustájení.

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â€œSustained fertility in dairy cows: problems and suggestionsâ€. genetics, housing, management, nutrition, immunology, molecular biology, endocrinology, metabolic and reproductive physiology, ethology, and animal welfare [18]. The present paper reviews the state-of-the-art of the multifaceted problem of infertility and decreased reproductive performance in high-producing dairy cattle and suggest short-, medium- and long-term solutions available to ameliorate it or aid to its permanent solution. Dairy herds undergoing no expansion in cow numbers exist as a steady state. Cow departure and entry is in balance. As heifers enter the milking string, mature cows are displaced from the herd.Â Following calving, initiation of reproductive function involves an orches- tration of physical, endocrine, and histologic events that consummate in uter- ine repair, ovulation, and resumption of estrous cycles. 4- 6,21,30,32,35,36,59,60,62 Similar events occur at puberty,41,47 except in lieu of uterine repair, develop- ment and growth of reproductive organs take place. The dairy industry depends on reproductive performance of their dairy cows in order to meet the growing demand products. The lactation cycle is dependent on the cowâ€™s ability to become pregnant since the hormones released during and after pregnancy are necessary for the development of the mammary gland and increase production of milk and milk products [8].Â It has also been shown that low reproductive efficiency hinders genetic improvement in dairy cow and cause direct economic loss.