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ACTIVITY OF ALKALINE PHOSPHATASE IN CATTLE BLOOD PLASMA ACCORDING TO STAGE OF PREGNANCY

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ABSTRACT

The project was aimed at determining physiological values in activity of alkaline phosphatase (ALP) in different stages of the reproduction cycle of cows. Blood samples were collected from the two herds (organic and conventional) over a period of two years. In both, the organic (A) and conventional herds, the highest ALP activity was found in cows pregnant for more than 140 days. Statistically significant differences were found only in the organic herd, specifically between the group of cows pregnant for more than 140 days and the group of cows after calving ($P < 0.05$) and non-pregnant cows ($P < 0.01$). Difference between herds A and B was also recorded ($P< 0.05$).

Key words: cow, enzyme, gestation

INTRODUCTION

Enzymes are a group of proteins of particularly great biological importance. The presence, or rather the activity, of these catalysts (sometimes also called ferments) facilitates chemical processes in the organism, the sum of which is referred to as metabolism (Rochling, 2001; Berg et al., 2006).

Enzymes are evaluated by enzymatic profile tests. A reduced enzymatic activity has no diagnostic significance. An increase in activity is directly proportional to the degree of damage suffered by an organ (Kawashima et al., 2007).

Alkaline phosphatase (ALP) belongs to hydrolases that cause hydrolysis of monoesters of phosphoric acid (Carlson, 1996; Mohri et al., 2007). ALP is made up of four isoenzymes – the placental, carcinoplacental, intestinal and a combined group of liver, renal and bone isoezymes producing real isoenzymes. It is a bone proliferation marker, and increased levels are found in cases of bone metabolism disturbances (Sato et al., 2005). ALP is localized mainly in the cellular membrane of hepatocytes (Valocky et al., 2007).

Physiologically increased levels are found in the growth period, during pregnancy (Sato et al. 2005) and also after phenobarbital administration (Bock, 1994). Different ALP reference values in bovine blood plasma have been reported: up to 3.3 $\mu kat.l^{-1}$ (Bock, 1994), and from 0.6 to 3 $\mu kat.l^{-1}$ (Jelinek et al., 2003). Pathologically increased levels: cholestasis (post-, intrahepatitic), liver poison intoxication, steroid - hepatosis, tumour metastases in the liver, hyperparathyroidism, hyperthyreosis, osteodystrophy, malignant bone tumours, fractures, rachitis, osteomalacia, periostitis (Bock, 1994; Boonprong et al., 2007). Serum ALP activity increases in case of hepatitis, biliary disorders, or during growth due to active bone metabolism (Carlson, 1996).

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MATERIAL AND METHODS

Two herds were selected for the experiment, an organic herd from a farm in Svojse (Herd A, altitude of 750 - 1070 m; consisting of the breeds Czech spotted, limousine, and hereford; without milk production; breeding with bull all year) and a conventional herd from a farm in Zaton (Herd B, altitude of 800 – 850 m; consisting of the breeds Czech spotted and galloway; 14.5 litres of milk per day; breeding with bull from May to August). Cows of both groups kept out-door throughout the year, with feeding only pasture and during winter with *ad libitum* hay and were bred.

In each of the herds, blood samples were collected four times in two years (in spring and in autumn of each year). A total of 45 and 43 samples were collected in Herds A and B, respectively. Blood samples were collected in the morning from the *vena jugularis* using disposable needles and test tubes with heparin, and were stored at a constant temperature. To obtain plasma, blood samples were centrifuged for 10 min at 1500 rpm. To determine blood plasma ALP [$\mu kat.l^{-1}$] activity, Bio-la-test (Lachema Brno, Catalogue No. 1300250) and Specol photometer (Carl Zeiss, Jena) were used. The ALP activity values ascertained were categorized according to stages of the cows' reproductive cycle, into ALP levels in non-pregnant cows, pregnant cows and cows after calving. The group of pregnant cows was further subdivided into cows within their first 140 days of pregnancy and those above that limit, and also whether they came from the ecological or the conventional herds.

The ALP activity levels were then statistically processed using the ANOVA. The data are presented as means with standard deviations.

RESULTS

Mean and standard deviation of ALP activity values in blood plasma of cattle from herds A and B are given in Table 1. In herd A (organic), the highest mean values were found in cows pregnant for more than 140 days, followed by cows in their first 140 days of gestation, cows after calving and non-pregnant cows. A comparison between the groups in different stages of reproductive cycle from herd A showed statistically significant differences ($P < 0.05$) between cows after calving and cows pregnant for more than 140 days, and statistically highly significant differences ($P < 0.01$) between non-pregnant cows and cows pregnant for more than 140 days.

Differential ALP values were found also in different stages of the reproductive cycle of herd B cows. It is evident from the data that the highest mean values were found in cows pregnant for more than 140 days, followed by cows in their first 140 days of gestation, cows after calving and, lastly, non-pregnant cows. In this case, no statistically significant differences between individual groups of cows were recorded.

DISCUSSION

Significantly higher level of ALP activity in all groups of cows was found in the organic herd A compared with the conventional herd B. The difference may be due to a higher metabolic rate in cows from the organic herd caused by their higher movement activity. It may have also been caused by an increase in bone metabolism caused by the absence of mineral feed additives in the feeds of organic herd compared with the conventional herd.

The highest level of ALP activity in herd A was found in cows pregnant for more than 140 days, followed by cows in their first 140 days of pregnancy, cows after calving and, lastly, non-pregnant cows. Bock (1994) considered this situation as normal and thus physiological. Jurajdova and Trcala (1990), however, reported no changes in ALP activity during pregnancy. A statistically non-significant increase was reported by Sato et al. (2005) between non-pregnant and pregnant

Table 1: Comparison of ALP values in the cows of both herds

Index	Herd A			Herd B			Significance
	N	$\bar{x}$	sd	N	$\bar{x}$	sd	
NP	15	1.81	1.1	9	1.43	0.9	N.S.
to 140 d	10	2.67	2.1	15	1.83	0.6	N.S.
from 141 d	8	3.52	1.5	9	2.00	1.2	$^{*}P<0.05$
AC	12	2.09	1.3	10	1.57	0.4	N.S.
total	45	2.38	1.1	43	1.72	0.81	$^{*}P<0.05$

Significance within herd: A (NP:141**.141:AC*)
NP = non pregnant; AC = after calving

cows. Statistically significant differences were also found between the group of cows pregnant for more than 140 days and two other groups, the group of non-pregnant cows ($P < 0.01$) and cows after calving ($P < 0.05$). This may be due to higher metabolic rates in high-pregnant cows as a result of nourishing the foetus.

Similar to herd A, the highest level of ALP activity in herd B was found in cows pregnant for more than 140 days, followed by cows in their first 140 days of pregnancy, cows after calving and, lastly, non-pregnant cows. Between the groups, however, no statistically significant difference was observed. The last step was a statistical comparison between peer groups from the two herds, i.e. non-pregnant cows from herd A were compared with non-pregnant cows from herd B, etc. The only statistical difference found was between cows pregnant for more than 140 days in the two herds, which might be explained by higher metabolic rates in the ecological herd A and by the absence of mineral additives in the feeds for that group.

The physiological status of cows (pregnancy, lactation, etc.) influences their hypothalamic-pituitary-adrenal axis activity, and also to establish the relationships between this activity and the alterations in the immune response which may occur in heat-stressed animals.

CONCLUSION

A higher ALP activity was found in the organic herd than in the conventional herd. In both herds, the highest ALP activity was recorded in cows pregnant for more than 140 days.

Results of our study indicate that values of ALP activity could be used as a marker for gestation in cows. However, further studies are necessary in order to determine individual iso-forms of ALP, especially from bones. It would also be important for indirect elimination of increased activity of hepatic ALP using values of gama-glutamyltransferase, which is in close relationship with it.

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