DIFFERENCES IN ALKALINE PHOSPHATASE ACTIVITY IN BLOOD PLASMA OF CATTLE FROM ORGANIC AND CONVENTIONAL STOCKS ACCORDING TO STAGE OF GRAVIDITY

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Abstract

The project was aimed at determining physiological values in activity o ALP (EC 3.1.3.1) in blood plasma of cattle from organic and conventional stocks according to stage of pregnancy. Four blood samples were collected from the two stocks over a period of two years. Blood was collected in the morning and in the afternoon from vena jugularis with one-shot needles into test tubes with heparin. A total of 45 and 43 blood samples were collected in stocks A and B, respectively. The blood serum was obtained by centrifuging blood samples for 10 min at 1500 rpm. To determine ALP [μkat.l^{-1}] activity in blood plasma, Bio-La-Tests (Lachema Brno) and Specol spectrophotometer (Carl Zeiss, Jena) were used. Statistically significant differences in ALP activity between stocks A and B were found ($P < 0.05$). In stock A, statistically significant differences were found between animals in the second 140 days of gestation and non-pregnant animals ($P < 0.01$), and animals after calving. Differences between stocks A and B in animals in the second 140 days of gestation were also found ($P < 0.05$).

Key words: cattle, organic stock, conventional stock, blood plasma, alkaline phosphatase, gravidity

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 (Glenk and Neu, 1990).

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 (Vrzgula *et al.*, 1990).

ALP (EC 3.1.3.1.) belongs to hydrolases that cause hydrolysis of monoesters of phosphoric acid (Jindra *et al.*, 1985; Kořeneková *et al.*, 2002). 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 (Masopust, 1998). ALP is localized mainly in the cellular membrane of hepatocytes (Stockham, 1991; Poráčová *et al.*, 1998).

Physiologically increased levels are found in the growth period, and also during pregnancy after phenobarbital administration. Different ALP reference values in bovine blood plasma have been reported: up to 3.3 μkat.l^{-1} (Ulrich, 1994), from 0.28 to 0.84 μkat.l^{-1} (Vrzgula *et al.*, 1990) and from 0.6 to 3 μkat.l^{-1} (Jelínek *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 (Ulrych 1994). Slanina *et al.* (1992) believe that increased ALP levels very probably indicate myopathy and skeletal disturbances.

MATERIAL AND METHODS

Two stocks were selected for the experiment, an organic stock from a farm in Svojše (Stock A) and a conventional stock from a farm in Zátoň (Stock B) – Tab. 1.

Tab. 1: The characteristic of the stocks

Parameter	Stock A	Stock B
Location	Svojše	Zátoň
Altitude	750 - 1070 metres	800 -850 metres
Character	Organic stock	Conventional stock
Breeds	Czech spotted, limousine, Hereford	Czech spotted, Galloway
Breeding	with bull all year	with bull from May to August
Stabling	outdoor all year	outdoor all year
Feeding	pasture + grazing	pasture + grazing
Average efficiency	no milk production	14.5 litres of milk
Health	very good	very good

In each of the stocks, 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 Stocks A and B, respectively. Blood samples were collected in the morning and in the afternoon from the *vena jugularis* using disposable needles and test tubes with heparin, and were stored at a constant temperature. To obtain serum, blood samples were centrifuged for 10 min at 1500 rpm. To determine blood serum 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 stocks.

The ALP activity levels were then statistically processed using the Student's *t*-test and the Anova – Tukeys test. The data are presented as means with standard deviations. Limit values for each set are also given.

RESULTS

Mean ALP activity values in blood serum of cattle from stocks A and B are given in Tab. 2. A higher mean ALP activity in blood serum was found in the cattle from Stock A, and the difference was statistically significant ($P < 0.05$). Maximum and minimum values and standard deviations found in the two stocks are also given.

Tab. 2: ALP in stock A and in stock B

parameters	stock A	stock B
mean	2,38	1,72
minimum	0,63	0,61
maximum	8,40	3,48
number	45	43
standart deviation	1,70	0,81

ALP activity levels in different stages of the reproduction cycle in cows from stocks A and B are given in Tab. 3. It follows from the table 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 non-pregnant cows.

Tab. 3: ALP activity in dependence on pregnancy stage in stock A

parameters	infertile	first 140 days of gravidity	second 140 days of gravidity	after calving
mean	1,81	2,67	3,52	2,09
minimum	0,63	1,04	1,75	0,85
maximum	4,57	8,40	6,80	5,98
number	15	10	8	12
standart deviation	1,23	2,20	1,58	1,36

A comparison between groups in different stages of reproductive cycle from stock 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. No statistically significant differences were found between the other groups investigated. These facts are documented in

Tab. 4: Statistical differences in ALP in dependence on stage of pregnancy in stock A

stock A	infertile	first 140 days of gravidity	second 140 days of gravidity	after calving
infertile		ns	0,01	ns
first 140 days of gravidity	ns		ns	ns
second 140 days gravidity	0,01	ns		0,05
after calving	ns	ns	0,05	

ALP values found in different stages of the reproductive cycle of stock B cows are given in Tab. 5. It follows 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.

Tab. 5: ALP activity in dependence on pregnancy stage in stock B

parameters	infertile	first 140 days of gravidity	second 140 days of gravidity	after calving
mean	1,43	1,83	2,00	1,57
minimum	0,64	1,19	0,61	0,89
maximum	3,37	3,44	3,93	1,99
number	9	15	9	10
standart deviation	0,89	0,56	1,18	0,41

Statistical evaluation of ALP values in dependence on different stages of the reproductive cycle of stock B cows are given in Tab. 6. In this case, no statistically significant differences between individual groups of cows were found.

Tab. 6: Statistical differences in ALP in dependence on stage of pregnancy in stock A:

stock B	infertile	first 140 days of gravidity	second 140 days of gravidity	after calving
infertile		ns	ns	ns
first 140 days of gravidity	ns		ns	ns
second 140 days gravidity	ns	ns		ns
after calving	ns	ns	ns	

The differences in ALP activity values in blood serum of cattle from stocks A and B are given in Tab. 7. It follows from the data in Tab. 7 that a statistically significant difference between stocks A and B was demonstrated only in the group of cows pregnant for more than 140 days.

Tab.7: Statistical differences in ALP in dependence on stage of pregnancy between stock A and stock B

stock A / stock B	infertile	first 140 days of gravidity	second 140 days of gravidity	after calving
infertile	ns			
first 140 days of gravidity		ns		
second 140 days gravidity			0,05	
after calving				ns

DISCUSSION

A higher level of ALP activity in all groups of cows was found in the organic stock A compared with the conventional stock B. The Student's *t*-test showed that the difference was statistically significant ($P < 0.05$).

The difference may be due to a higher metabolic rate in cows from the organic stock 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 stock compared with the conventional stock.

The highest level of ALP activity among stock A groups 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. Ulrich (1994) and Masopust (1998) consider this situation as normal and thus physiological. Jurajdová and Trcala (1990), however, reported no changes in ALP activity during pregnancy. 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.

Similarly to stock A, the highest level of ALP activity in stock 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 differences were found.The last step was a statistical comparison between peer groups from the two stocks, i.e. non-pregnant cows from stock A were compared with non-pregnant cows from stock B, etc. The only statistical difference found was between cows pregnant for more than 140 days in the two stocks, which might be explained by higher metabolic rates in the ecological stock A and by the absence of mineral additives in the feeds for that group.

CONCLUSION

A higher ALP activity was found in the organic stock than in the conventional stock and the difference was statistically significant ($P < 0.05$).

In both the ecological and conventional stock, the highest ALP activity was found in cows pregnant for more than 140 days, followed by cows in their first 140 days of pregnancy, cows after calving and non-pregnant cows. Internal statistically significant differences were found only in the ecological stock, 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 ($P < 0.01$).

Finally, a comparison between peer groups from the ecological and the conventional stocks was made. A statistically significant difference ($P < 0.05$) was found only in cows pregnant for more than 140 days.

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