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LACTATE DEHYDROGENASE ISOENZYME PATTERN IN TISSUES AND SERUM OF THE CALF

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Abstract

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The aim of this study was to analyze the lactate dehydrogenase (LD) pattern in tissues and serum of calves (Bohemian pied hybrids, with a substantial component of the Holstein breed) and compared with data of similar studies. LD isoenzyme patterns were determined by polyacrylamide gel electrophoresis, data were evaluated by current process and by a sum of vectors method (SV).

In the basic model SV5,LD(1-5), an evaluation of LD patterns as resultant vectors enabled an easy detection of outlying data obtained in this study and those by others. It was found that calf tissues under study differ substantially in their LD patterns. Comparing the angles SV[alpha] of resultant vectors for our samples, we can state the following order: kidney < left atrium ≈ right atrium < serum < lung, spleen, liver << diaphragm. Calf tissues (lung, spleen, liver) differed in SV[c] variable of resultant vector in the following order: lung < spleen < liver. No significant differences between summer and winter serum LD patterns were found in contrast to literature data obtained from cows kept under range conditions. This is necessary to consider when evaluating the results of similar studies.

Polyacrylamide gel electrophoresis, healthy cattle, physiology

After proteosynthesis, H,M polypeptides of lactate dehydrogenase (LD, L-lactate:NAD oxidoreductase, EC 1.1.1.27) combine to form five tetrameric LD isoenzymes: LD1 (H4), LD2 (H3M), LD3 (H2M2), LD4 (HM3) and LD5 (M4). For separation of LD isoenzymes several electrophoretic techniques with different supporting media (cellulose acetate, agar, agarose, polyacrylamide) are currently applied. Eleven electrophoretic methods for quantifying LD isoenzymes in serum are compared in the study of Moses (1988). Tissue LD concentration is approximately 1 000 times higher than LD concentration in serum or plasma, so that a leakage of LD from damaged cells is reflected in serum LD pattern. In medical research, changes of LD pattern in serum has been employed for detection of pathophysiological changes in the organism (Boyd 1982; Maekawa 1988; Pechová 1992; Rodrigue 1995).

LD isoenzyme patterns were currently evaluated using statistical analysis of single LD isoenzymes, ratios of LD isoenzymes and H/M polypeptides until to the sum of vectors method (SV) became available. An application of the SV method makes the evaluation of several quantities possible and it offers a clear and workable information on LD patterns (Šalplachta 1997ab; Šalplachta 1998).

The purpose of the present study was to determine the LD pattern in the serum and tissues of healthy calves and to test its applicability on data from literature.

Materials and Methods

Lactate dehydrogenase and its isoenzymes were determined in the serum and tissues of calves (Bohemian pied hybrids, with a substantial component of Holstein). The determinations of LD and LD isoenzymes were carried out in serum of calves 3-3.5-months-old of both sexes and body weight approximately 100 kg. The animals were conventionally reared on a farm in a loose-housing system.

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Tissue samples were taken from healthy 6-months-old calves immediately after slaughter in a slaugtherhouse (their average body weight was 210 kg, they were fed a milk diet). Pending analysis, the tissues were stored in solid CO_2. They were homogenized by high-speed blender Unipan 309 in a solution of 0.9 % NaCl, 5 mmol TRIS-HCl pH 7.4. The extracts were collected after 30 min, 20 °C, 15 000 × *g* centrifugation and either immediately analysed or stored in solid CO_2.

The total activity of LD in the sample was determined by photometrical method with iodonitrotetrazolium violet (Bio-La-test Lactate dehydrogenase, Lachema, Czech Republic).

To obtain LD isoenzyme patterns, conventional polyacrylamide gel electrophoresis (3 mm inner diameter, 5 cm length of gel) were carried out. Buffer TRIS-HCl (0.1 mol/l) pH 8.6 was in 5.5 % polyacrylamide gel, electrode spaces and deposited sample. Electrophoresis lasted for 70 min with the voltage of 25 V/cm and the temperature was 10 °C. Van der Helm's staining solution modified with NaCl instead of NaCN was used (Dietz and Lubrano 1967) with composition: 0.1 mol/l of sodium lactate, 1.5 mmol/l of NAD, 0.1 mol/l TRIS-HCl pH 8.6, 10 mmol/l of sodium chloride and 5 mmol/l magnesium chloride. The staining solution contained in 10 ml 0.3 mg of phenazinmethosulphate and 2.5 mg nitrobluetetrazolium. After 30 min staining at 30 °C, the gels were transferred into a solution of acetic acid (70 ml/l). Formazane zones were quantified by a Beckman CDS 200 densitometer. All used reagents were of analytical grade and distilled water was used.

The sum of vectors method (SV): LD isoenzyme patterns were converted to the resultant vectors of basic model SV5,LD(1-5) under conditions described in detail elsewhere (Šalplachta 1997). The basic model SV5,LD(1-5) uses a two-dimensional system of rectangular axes x, y in the plane in which LD isoenzymes are represented with constituent vectors starting in the point of intersection of axes. By summation of all constituent vectors every resultant vector is determined so that an end point of resultant vector represents its LD pattern. The end point variables SV5[x;y] of resultant vector were used to create the graphical presentation of the results and for statistic evaluation.

A theoretical vector was calculated for the resultant vector derived from experimental LD pattern in tissue and both vectors have the same orientation (Šalplachta 1998). Theoretical resultant vector represents a theoretical LD pattern resulting from random association of H,M polypeptides. The end point variables SV5[alpha;c] of resultant vector were applied to compare the data presented in this study and those published by other authors (Prasse1969; Keller and Stanbridge 1972; Keller 1974; Lauerman et al. 1978; Jain and Bike 1985; Yasuda et al. 1989; Heinová and Blahovec (1994). Statistical analysis was performed by F-test and Student's *t*-test.

Results

Summer and winter LD isoenzyme pattern in calf serum

Serum samples were taken in August and in January. Mean isoenzyme LD patterns and mean total LD activities in serum are given in Table 1.

Table 1

Isoenzyme L-lactate dehydrogenase patterns and total LD activity in calf serums taken in summer and winter are presented. The end point data of resultant vectors computed in basic model SV5,LD (1-5) are added.

August n = 14	LD 1	LD 2	LD 3	LD 4	LD 5	LDtot	SV[alfa]	SV[c]	SV[x]	SV[y]
Mean	34.2	35.8	22.5	5.8	1.7	7 026	97.8	48.2	-6.4	47.6
s	2.5	1.1	2.1	1.2	0.6	798	4.8	2.9	3.6	3.4
January n = 12										
Mean	33.7	34.8	24.9	4.4	2.2	11 159	99.8	48.3	-8.0	47.4
s	3.3	1.6	2.1	1.4	1.0	1 493	5.7	3.9	4.4	4.4

Relative LD isoenzyme activity is presented; total L-lactate dehydrogenase activity is expressed as [nkat/l]. End point of resultant vector is specified co'ordinate variables SV[x], SV[y] or vector angle SV[alfa] and vector value SV[c]. SV … sum of vectors method; s … standard deviation; n … number of calves.

The resultant vectors of LD isoenzyme patterns were calculated in the basic model SV5,LD(1-5) to demonstrate a distribution of LD patterns in serum. The data (January) are shown in Fig. 1.

The variables SV[x;y] of resultant vectors were tested by two-tailed F-test ($P < 0.05$). We state that there is insufficient evidence to conclude that the variances are not the same. Based on the two-tailed *t*-test ($P < 0.05$), we also state that there is insufficient evidence to conclude

that the means are not the same. It can be stated, that no significant differences between summer and winter serum LD isoenzyme patterns were found.

LD isoenzyme pattern in calf tissues

LD isoenzyme pattern of calf tissues are presented in Table 2, and their resultant vectors are shown in Figs. 1-3, 5 to demonstrate the distribution of LD patterns in tissues. Mean resultant vector is equipped with rectangle and its corner points are defined by the equations: $SV[x] \pm 2s[_x]$; $SV[y] \pm 2s[_y]$. A rectangle area contains an end point of resultant vector whose LD pattern is in the reference interval of a healthy population with 95% probability. Outlying LD patterns were detected. The most differing LD pattern was omitted in some tissue data files and corresponding rectangles were calculated. LD isoenzyme data are presented in Table 2. and rectangles are indicated by a dotted line in Figs. 1-3. As a percentage of rectangle area from all samples, the analogous areas are: right atrium 73%, left atrium 34%, spleen 27%, lung 57%, liver 49%, and diaphragm 64%. Serum and kidney data file were not recalculated. We did not continue the statistic analysis because of the small number of calves in experiment. The evaluation of LD patterns by evaluation of theirs vectors in basic model SV5,LD(1-5), enables an easier detection of outlying LD patterns than up to now used statistic analysis of five LD isoenzymes.

Table 2
The mean LD pattern in tissues of healthy calves. The end points of resultant vectors vere calculated from LD patterns in basic model SV5, LD (1-5). All data are presented as mean ± s.

n = 10	LD 1 [%]	LD 2 [%]	LD 3 [%]	LD 4 [%]	LD 5 [%]	SV [alfa]	SV [c]	SV [x]	SV [y]
Kidney	53.5 ± 2.5	29.6 ± 1.7	11.2 ± 1.3	4 ± 1	1.7 ± 0.6	67.2 ± 2.9	59.6 ± 3.3	23.2 ± 3.4	54.8 ± 2.9
Atrium left	42.1 ± 6.1	39.7 ± 2.6	13 ± 3.6	3.5 ± 1.7	1.6 ± 0.9	81.9 ± 8.4	59.4 ± 6.5	9 ± 8.5	58.2 ± 5.9
Atrium right	39.2 ± 4.1	38.5 ± 1.1	15.9 ± 1.9	4.6 ± 1.2	1.9 ± 0.6	86.1 ± 5.5	54.5 ± 1.0	4.0 ± 5.4	54.1 ± 3.4
Lung	23.5 ± 2.8	27.2 ± 2.5	23 ± 1.9	14.4 ± 1	11.8 ± 2.7	113.2 ± 13.8	20.7 ± 4.7	-7.3 ± 2	19 ± 5.7
Spleen	22.2 ± 2.3	29.3 ± 1.1	24.5 ± 1.6	14.8 ± 0.9	9.2 ± 1	120.9 ± 9.2	25.2 ± 2.1	-12.6 ± 2.7	21.4 ± 3.6
Liver	22.3 ± 3.5	33.2 ± 1.8	27.7 ± 1.4	12.4 ± 2	4.4 ± 1.4	124.3 ± 8.9	36.9 ± 3.8	-20.2 ± 3.6	30.4 ± 5.9
Diaphragm	14.6 ± 2.7	12.7 ± 1.4	16.4 ± 1.9	18.6 ± 1.3	37.7 ± 4.7	310.6 ± 7.3	25.4 ± 6.5	16.3 ± 4.6	-19.2 ± 5.7

n = 9	LD 1 [%]	LD 2 [%]	LD 3 [%]	LD 4 [%]	LD 5 [%]	SV [alfa]	SV [c]	SV [x]	SV [y]
Atrium left	43.4 ± 4.7	40.2 ± 2.2	12.1 ± 2.2	3.1 ± 0.8	1.4 ± 0.4	80.1 ± 6.1	61.3 ± 2.9	10.7 ± 6.7	60 ± 2.3
Atrium right	38.3 ± 3.1	38.7 ± 0.8	16.2 ± 1.6	4.8 ± 1	1.9 ± 0.5	87.2 ± 4.3	53.8 ± 3.1	2.9 ± 4	53.6 ± 3
Lung	24.2 ± 1.6	27.7 ± 2	22.8 ± 1.7	14.2 ± 0.6	11.1 ± 1.5	109.2 ± 5.5	21.8 ± 2.9	-7.1 ± 0.9	20.5 ± 2.5
Spleen	22.9 ± 1.1	29.5 ± 0.9	24 ± 0.5	14.6 ± 0.8	9 ± 0.8	118.3 ± 3.8	25.2 ± 2.1	-11.8 ± 0.9	22.2 ± 2.5
Liver	21.4 ± 2	33.3 ± 1.8	28.1 ± 1	12.7 ± 1.8	4.6 ± 1.2	126.3 ± 6.2	36.5 ± 3.6	-21.2 ± 1.8	29.5 ± 5.2
Diaphragm	14.9 ± 2.6	12.8 ± 1.3	16.8 ± 1.5	18.8 ± 1.1	36.7 ± 3.4	310 ± 7	24.1 ± 5.2	15.2 ± 2.9	-18.5 ± 5.2

Data file with 9 calves arose from data file of 10 calves minus one the most distinct LD pattern. SV … the metod of vectorisation and of summation of biochemical quantity vectors. s … standard deviation. Variables SV [alpha, c] or SV[x, y], specify the end point position of vector. SV [alpha, c] are vector quantities angle and value; SV[x, y] are co'ordinates in axes x, y.

It was found that calf tissues differ significantly in LD patterns. Comparing a ratio (experimental SV[c]/theoretical SV[c]), the following order was created:
diaphragm < lung < spleen < liver < kidney < right atrium ≈ left atrium.

Discussion

A comparison of mean LD patterns obtained in this study with those published by other investigators for serum or tissue has been made by comparison of resultant vectors in basic model SV5,LD(1-5). Data files taken from literature are presented in Table 3 and Figs. 4, 5.

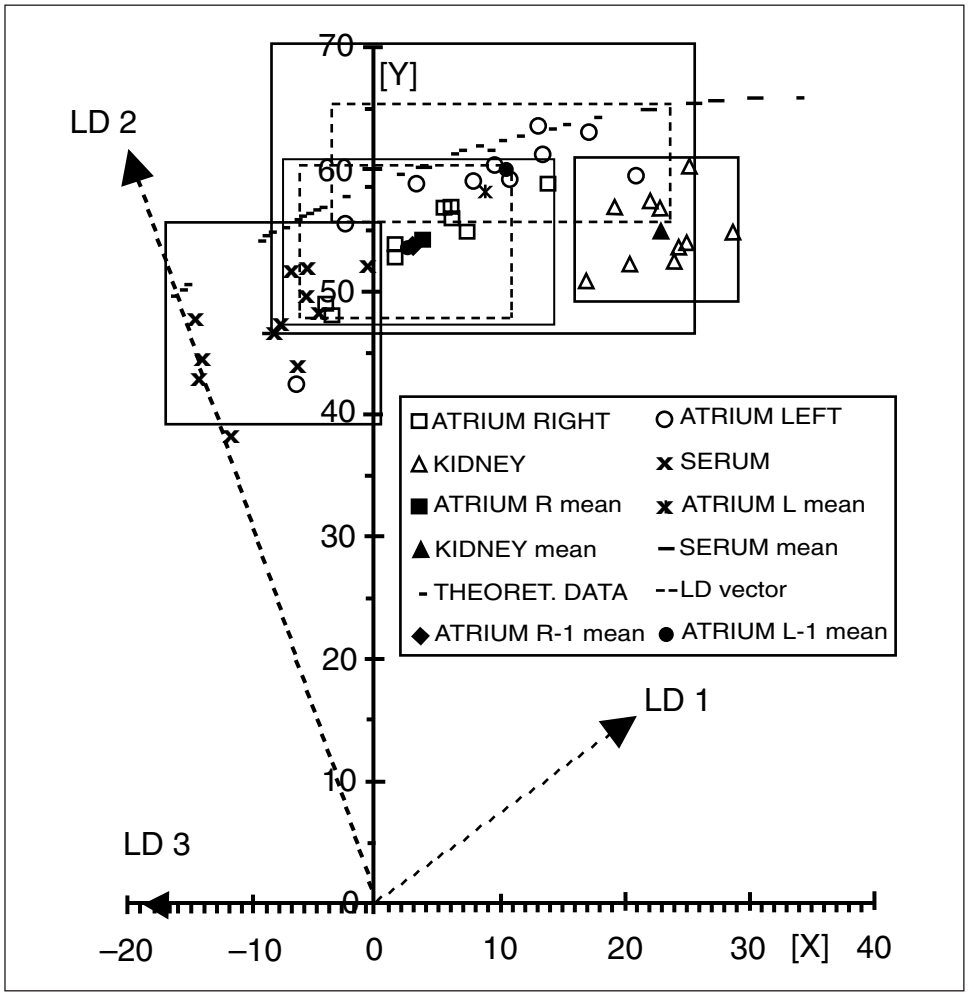

Fig.1. The LD isoenzyme pattern of calf tissues (10 calves) and serum (January, 12 calves) are expressed as the end points of resultant vectors in basic model SV5,LD(1-5).

LD pattern in serum

The analysis of serum data indicates a good concord between the vectors of this study in calves and those in nonpregnant dairy cows, aged 2 to 5 years and 5 steers aged 1 to 2 years (Keller 1974). Moreover, both are very close to data from the study by Prasse (1969). A clear difference has been found between data of the previous three studies and the data from Jain and Bike (1985), Lauerman et al. (1978) and Heinová and Blahovec (1994). The difference cannot be explained by different age of experimental animals. It is more likely that the differences in analytical methods, breed and in management between dairy cattle and feedlot cattle are expressed. The results of study Avallone (1996) indicate the increases of angle SV[alpha] of resultant vector where as vector variable SV[c] is nearly constant at ages ranging from 2 to 10 weeks. Data are not shown in this paper. The study of

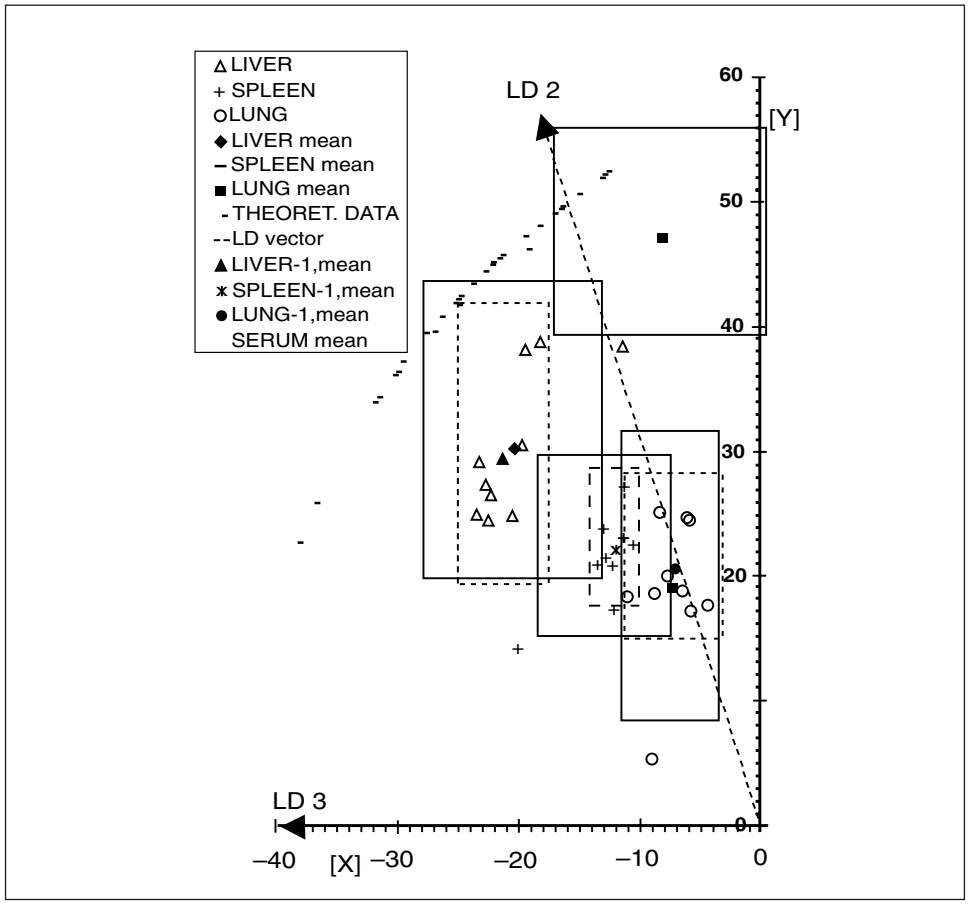

Fig.2. The LD isoenzyme pattern of calf tissues (10 calves) and serum (January, 12 calves) are expressed as the end points of resultant vectors in basic model SV5,LD(1-5).

Prasse (1969) indicates that with advancing age of cattle the angle SV[alpha] of resultant vector decreases whereas the vector variable SV[c] is nearly constant (ages 1 to 6 years). On the other hand, summer/winter seasonal changes in plasma LD pattern of cows under range conditions are characterized by change of vector variable SV[c] whereas the angle SV[alpha] is nearly constant (Jain and Bike 1985).

LD pattern in tissues

The theoretical LD pattern is based on hypothesis LD tetramer is assembled from four randomly selected H,M polypeptides (Emery 1967). A cell population and its heterogenity from which LD was released into serum can be estimated by P_M model distribution (Feldman 1983). We assume that the more the theoretical and experimental vector variable SV[c] differ the broader is the distribution of cell population in LD pattern. However, other mechanisms (non-random association LD tetramers, degradation of isoenzymes, pathological sample, analytical assessment) may be involved in the creation of LD pattern. It was found that calf diaphragm has the most differring, and calf heart has the least differring LD pattern from the theoretical LD pattern. It has been found that every

Table 3
The LD isoenzyme pattern of bovine serum and tissues from the literature: P = Prasse 1969, K = Keller 1972 and 1974, L = Lauerman 1978, J = Jain 1985, Y = Yasuda 1989 and H = Heinová 1994. The end points of resultant vectors were calculated from LD patterns in basic model SV 5, LD (1-5).

Paper/sample	LD 1	LD 2	LD 3	LD 4	LD 5	SV [alpha]	SV [c]	SV [x]	SV [y]
L. serum	36.5	24.7	16.9	12.0	9.1	72.9	29.7	8.7	28.4
J. serum Jan	46.0	27.0	16.5	5.2	3.6	73.3	48.5	13.9	46.4
J. serum Mar	52.1	27.0	13.5	3.2	2.8	67.9	56.4	21.2	52.3
J. serum Jun	43.0	25.0	16.1	7.6	6.3	70.2	41.3	14.0	38.9
J. serum Jul	40.0	24.0	16.5	9.1	9.6	67.3	35.0	13.5	32.3
J. serum Nov	51.0	25.0	14.6	4.9	2.4	67.9	52.6	19.8	48.7
K. serum	36.5	32.7	22.0	5.8	3.0	92.5	45.3	-1.9	45.3
P. serum I.	31.6	29.8	23.8	9.8	4.8	100.7	35.5	-6.6	34.8
P. serum II.	34.1	27.7	21.8	9.7	6.3	91.2	33.6	-0.7	33.6
P. serum III.	37.3	28.4	20.2	9.1	4.9	86.4	37.5	2.4	37.4
H. serum	53.0	35.0	11.0	1.0	0.0	71.9	66.8	20.8	63.5
K. heart	41	38	20	1	0	88.9	59.3	1.1	59.3
K. muscle	4	8	10	22	56	303.7	52.7	29.3	-43.9
K. kidney	72	28	0	0	0	54.3	84.9	49.6	69.0
K. spleen	42	38	16	4	0	85.5	57.2	5.0	57.0
K. brain	49	29	21	1	0	80.4	56.2	9.4	55.4
K. liver	40	39	16	5	0	87.2	55.9	2.8	55.8
Y. liver	31.7	24.8	27.3	12.8	3.3	110.7	30.1	-10.6	28.1

Relative LD isoenzyme activity is presented. SV … sum of vectors metod.
The end point of resultant vector is specified co'ordinate variables SV [x], SV [y], basic method SV 5, LD (1-5).
P. serum … the age of dairy cattle group I. (6-18 mon.), II. 2-5 year, III. 6+ year.

resultant vector with exception of that derived from LD pattern in the kidney (Keller and Stanbridge 1972) is smaller than its theoretical vector. This fact calls for additional investigation.

Comparing the angles SV[alpha] of resultant vectors for calf samples, we found the following order: kidney < left atrium ≈ right atrium < serum < lung, spleen, liver << diaphragm. The lung, spleen, liver differed in SV[c] variable of resultant vectors creating an order lung < spleen < liver. Analogous data from the study of Keller and Stanbridge (1972) gave following order of the angles SV[alpha]: kidney << brain, spleen, liver, heart << muscle. By SV[c] variable comparison, we did not found significant differences between the tissues (brain, spleen, liver, heart) from the study by Keller and Stanbridge (1972). The vector discrepancy of LD patterns in liver, spleen and kidney of this study and that of Keller and Stanbridge (1972) might be explained by different age of the animals, however, other explanation cannot be excluded. The resultant vectors derived from the LD pattern in heart are nearly identical for

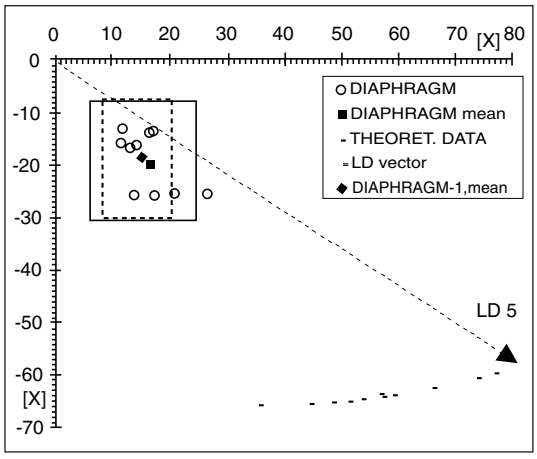

Fig.3. The isoenzyme LD pattern of calf diaphragms (10 calves) are expressed as the end points of resultant vectors in basic model SV5,LD(1-5).

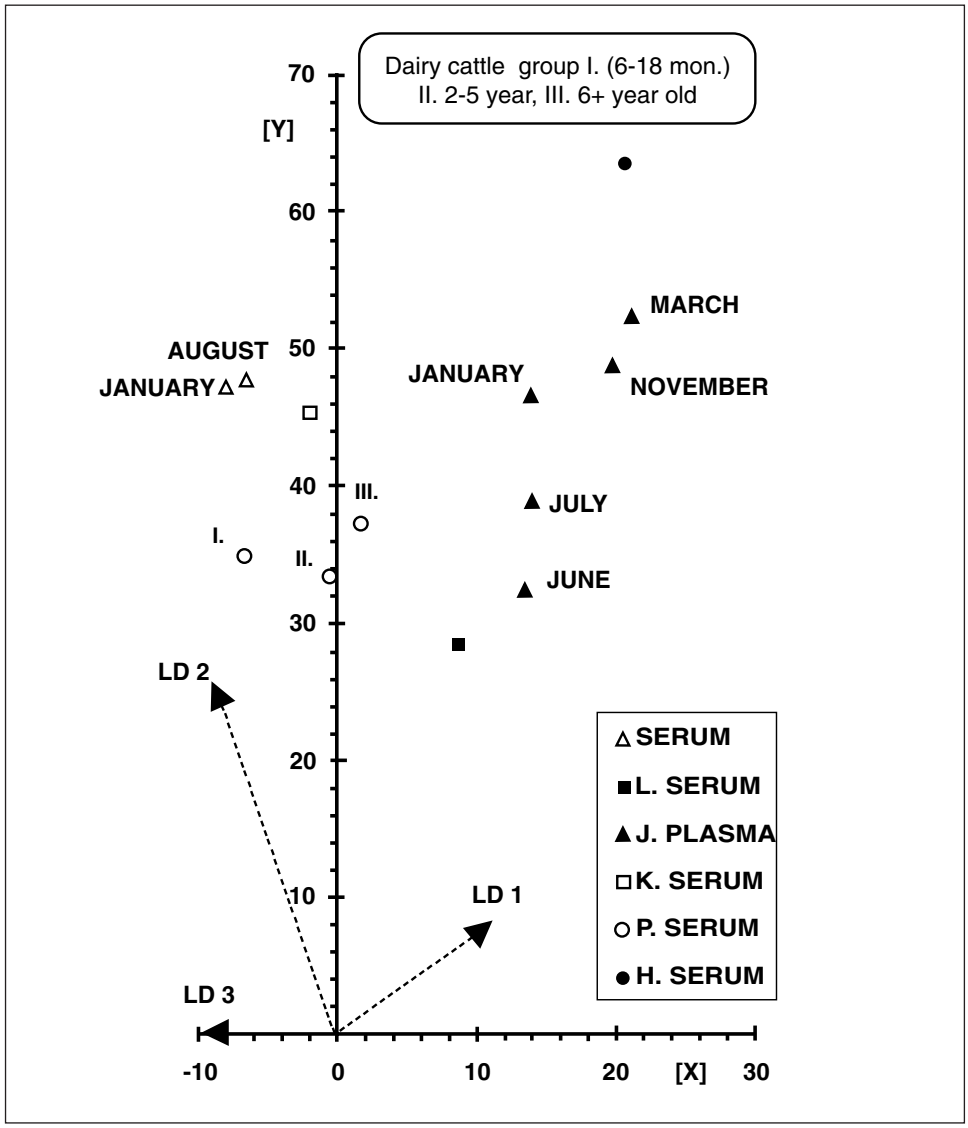

Fig.4. The LD isoenzyme pattern of bovine serum/plasma from this study and literature (P = Prasse 1969, K = Keller 1974, L = Lauerman 1978, J = Jain 1985, H = Heinová 1994) as the end points of resultant vectors in basic model SV5,LD(1-5).

the data from this study and that of Keller (1972). A similar situation was with the vectors of liver LD patterns from this study and from the paper by Yasuda et al. (1989).

In conclusion, the SV method enabled a clearer, more detailed and simpler inspection and evaluation of data files and their comparison than currently used statistical analyses of five LD isoenzymes piece by piece. We can thus easily differentiate calf tissues by their LD patterns. We expect that the presented data and the evaluation method may be successfully used for more detailed evaluation of enzyme patterns in similar studies.

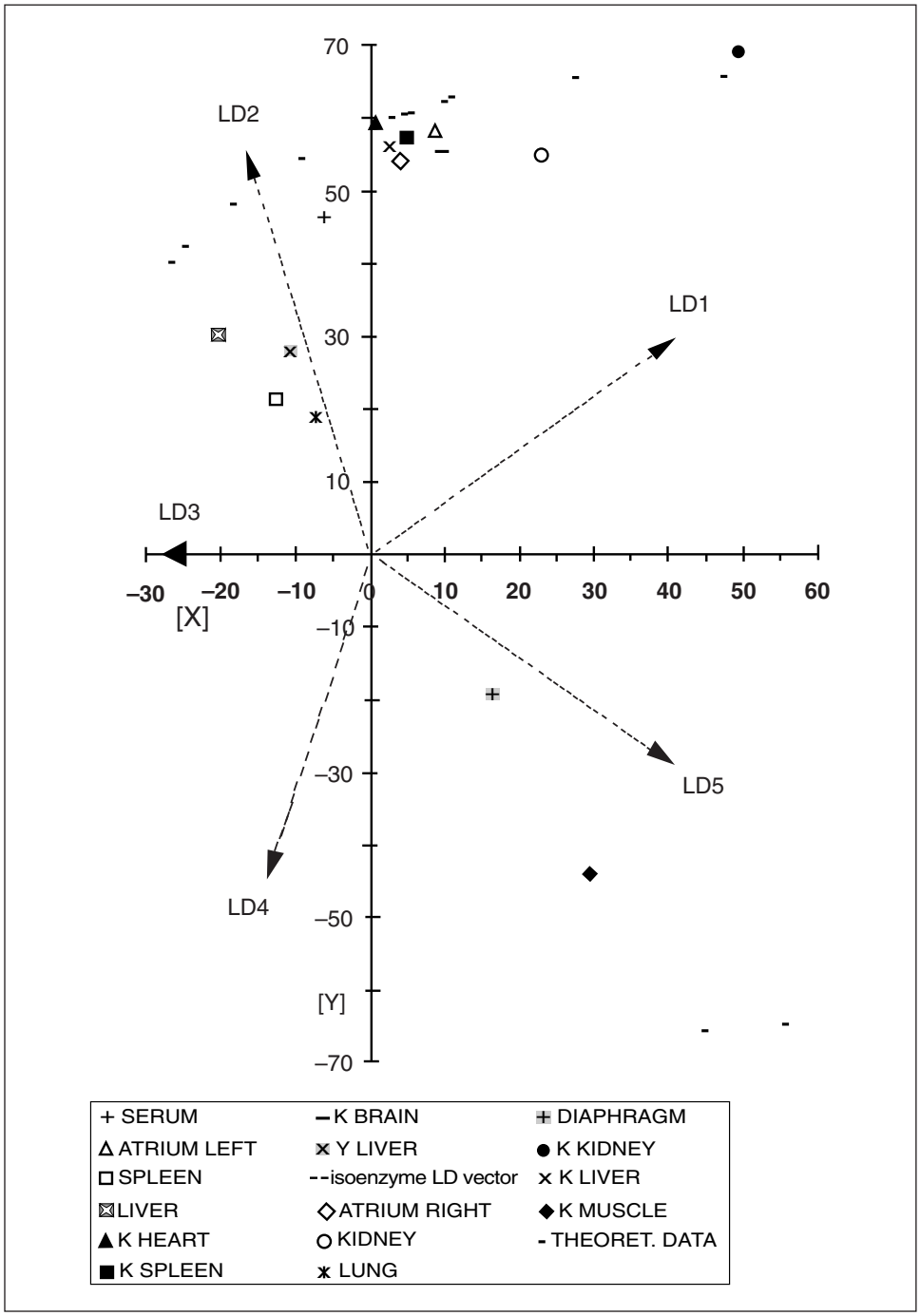

Fig.5. The LD isoenzyme pattern of bovine serum and tissues from this study and literature (K = Keller 1972 and 1974, Y = Yasuda 1989) as the end points of resultant vectors in basic model SV5,LD(1-5).

Isoenzymové složení laktátdehydrogenasy v séru a tkáních telat

Cílem této práce bylo získat hodnoty složení laktátdehydrogenasy z tkání a séra telat (hybrid Český červenostrakatý se značnou složkou Holstein) a jejich srovnání s daty z podobných studií. Isoenzymové složení laktátdehydrogenasy bylo stanoveno metodou polyakrylamidové elektroforézy, data byla vyhodnocena jak běžným postupem, tak metodou součtu vektorů (SV).

Hodnocení LD složení jako výsledného vektoru v základnim modelu SV5,LD(1-5) umožnilo snadnou identifikaci odlehlých hodnot stejně jako snadné srovnání dat z této studie s literárními údaji. Bylo zjištěno, že se složení LD zkoumaných vzorků významně liší. Pro naše vzorky jsme vytvořili po srovnání úhlů SV[alpha] vektorů následující pořadí: ledvina < levé atrium ≈ pravé atrium < sérum < plíce, slezina, játra << bránice. Tkáně telat (plíce, slezina, játra) se liší v hodnotách proměnné SV[c] v pořadí: plíce < slezina < játra. Nebyly zjištěny významné rozdíly v LD složení séra odebraného v létě nebo v zimě na rozdíl od literárních dat (krávy chované na pastvině). Tuto skutečnost je nezbytné brát v úvahu při hodnocení dat z podobných studií.

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