CONCENTRATION OF FREE AMINO ACIDS IN BLOOD PLASMA OF DAIRY COWS WITH DEVELOPING HEPATIC STEATOSIS

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Abstract

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The aim of this experiment was to study the changes in free amino acid (AA) concentration in blood plasma of dairy cows developing hepatic steatosis after parturition and to establish the diagnosis by means of Fischer's index. Ten dry dairy cows of Bohemian Black Pied breed with body condition score 4.5 – 5.0 were subjected to feed restriction at the end of the first week after parturition. They were divided into two groups (S - cows with steatosis, N – cows with no increased deposition of fat in the liver) based on morphological examination of hepatic tissue on day 4 after feed restriction. Blood was sampled in the last week prior to parturition, in weeks 1, 2 (on the day of hepatic biopsy) and 4 after parturition. Plasma free amino acids were determined after preceding plasma deproteination with 0.22 M solution of salicylic acid. Total free amino acids (TAA) in blood plasma, Fischer's index and EAA/NEAA (essential/non-essential amino acids) were not influenced by liver fat. The group of cows suffering from hepatic steatosis showed the following values as compared with controls: TAA (1690.8 ± 537.7 vs. 1732.7 ± 456.7 μmol·l^{-1}), EAA/NEAA (0.67 ± 0.07 vs. 0.65 ± 0.15), Fischer's index (4.73 ± 1.21 vs. 3.80 ± 0.77) on the day of biopsy. Liver impairment made itself felt in high variability of measured values in both groups. The more expressive relationship to hepatic steatosis appeared only in the concentration of methionine that was significantly lower ($p < 0.05$)before delivery (24.27 ± 6.39 vs. 41.24 10.75 μmol·l^{-1}) in group S than in group N, remaining unchanged during the postparturient period (1st week 27.02 ± 5.15 vs. 36.20 ± 27.04; 2nd week 16.46 ± 8.25 vs. 21.84 ± 12.92, 4th week 22.90 ± 4.49 vs. 24.0 ± 1.44 μmol·l^{-1}). The shortage of methionine appears as one of predisposing factors in liver steatosis. The concentration of free amino acids in blood is not a sensitive indicator of liver function impairment, therefore cannot be used in the diagnose of liver steatosis.

Hepatic steatosis, blood plasma, free amino acids, Fischer's index, methionine

Nowadays, the problem of hepatopathy is being intensively studied. The biopsy of hepatic tissue belongs to the most reliable evidence of hepatic steatosis (Kolouch et al. 1991). For the diagnosis of lipomobilization syndrome many variables of metabolic profile in dairy cows were presented (Pechová et al. 1997) but main attention is drawn to the investigation of liver function activity and energy metabolism (West 1997).

Liver function tests have been used for diagnostic purposes in man and animals, e. g. administration of amino acids (tyrosine, lysine) and the rate of their deamination. These data are of diagnostic value only with an 85 % loss of liver parenchyma (Kaneko 1997). Changes of AA contents in blood plasma are a useful diagnostic tool in hepatopathies in man. Important is the so-called Fischer's index which represents molar ratio of BCAA/AAA (BCAA – branched chain amino acid = valine, leucine, isoleucine; AAA – aromatic amino acid = tyrosine, phenylalanine). This ratio is based on different metabolic routes of these AA groups. Aromatic AA are metabolised only in the liver, therefore, in the case of liver impairment they cumulate in blood. On the other hand, AA with branched chain are splitted to yield energy by peripheral muscles and in case of liver impairment their amount decrease

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(Barber and Teasley 1984). Molar ratio of BCAA/AAA correlates with liver impairment and serum concentration of albumin, bilirubin and γ-globulin but does not correlate with hepatic encephalopathy. It is a valuable diagnostic, prognostic and therapeutic aid (McCollough et al. 1981).

Relatively small attention has been devoted to the study of AA spectrum. The AA concentration in blood is influenced by a great range of factors – nutrition, age, pregnancy, oestrus, circadian and seasonal rhythms (Foldager et al. 1980). Falloon et al. (1988) studied the plasma free AA concentration in relation to the metabolic disturbances in cattle. These authors recommend the study of mutual concentration ratio to absolute concentration of simple free AA; Fischer's index (BCAA/AAA) can be used as an indicator of liver impairment, independent of animal species, diet, and/or the cause of liver impairment. These authors set up the value of Fischer's index for cattle 2.1 - 5.2 (with average value 3.4) and for horses 3.5 - 4.5. Scholz et al. (1992) use the decrease of Fischer's index under 4.0 as diagnostic criterion for hepatic failure. Changes in the spectrum of AA has been found as the earlier indicator of postpartum paresis (Falloon et al. 1988). Cattle with calcium concentration under 1.6 $mmol \cdot l^{-1}$ had higher values of alanine, glycine, glutamine, taurine, proline, and lower values of citrulline, isoleucine and valine.

The purpose of this work was to study the plasma free AA in the development of hepatic steatosis during puerperium, to evaluate the changes in substitution of individual AA, and to verify the use of some indices depending on liver impairment stages.

Materials and Methods

The changes of plasma free amino acids were evaluated in context with the development of hepatic steatosis. Based on the evaluation of metabolic tests and that of feed ration a farm with overfed dry dairy cows Bohemian Black Pied breed was chosen. Ten dry dairy cows with body condition score 4.5 – 5.0 were included in the study. Their feed ration was restricted in week 2 after parturition when the cows were given only roughage (hay, corn silage, barley straw) without any concentrate during three days. Hepatic biopsy was performed on day 4 of restriction ratio. According to its results the animals were divided into group S (7 cows) - cows suffering from various stages of hepatic steatosis, and group N (3 cows) with no increased deposition of fat in the liver. Blood samples were taken in the last week prior to parturition and after parturition in weeks 1, 2 (on the day of hepatic biopsy) and 4.

The bioptates of hepatic tissue were processed using a freezing technique and stained with oil red to detect neutral fat. The morphological evaluation and semiquantitative assessment were carried out at the Department of Pathological Morphology of the University of Veterinary and Pharmaceutical Sciences. Plasma free amino acids were determined using the automatic amino acid analyser CHROMASPEK J 180 (Hilger Analytical, UK) after preceding plasma deproteination with a 0.22 M solution of salicylic acid. Twenty plasma free amino acids in the basic two-hour-cycle were determined. They were divided as in other papers in EAA (threonine, lysine, histidine, arginine, valine, methionine, isoleucine, leucine, tryptophane, phenylalanine) and NEAA (aspartate, serine, glutamate, glutamine, proline, glycine, alanine, cysteine, tyrosine, ornithine) (Overton et al. 1998; Fraser et al. 1991). The differences were statistically evaluated by Student's *t*-test.

Results

Concentrations of single free amino acids in blood plasma of dairy cows are presented in Table 1. Significant differences ($p < 0.05$) between N and S groups were only shown for methionine in group S in the last week of pregnancy.

Total free amino acid concentration was low in weeks 1 and 2 after delivery in both groups, and in group S also in the last week of pregnancy with average values ranging from 1690.8 to 1732.7 $\mu mol \cdot l^{-1}$. The average concentration of TAA of the second blood sampling was higher than 2000 $\mu mol \cdot l^{-1}$ (Table 2). The ratio EAA/NEAA was low during the whole period under study, with the lowest value in week 4 postpartum (Table 2). On the other hand, Fischer's index (BCAA/AAA) was high and ranged from 3.56 to 4.87; these results indicated a good liver function as compared to 2.1-5.2 found by Falloon et al. (1988) (Table 2).

The concentration of plasma single free AA fluctuated considerably during the study. In the

Table 1
Concentration of free amino acids [µmol·l^{-1}] in blood plasma (means ± SD) of dairy cows with liver steatosis (S) and with no increased deposition fat in the liver (N)

	Group	Before calving	Week 1 p. p.	Week 2 p. p.	Week 4 p. p.
Asp	N S	19.9 ± 4.7 16.8 ± 4.0	10.6 ± 0.4 18.2 ± 7.8	16.7 ± 10.0 21.1 ± 15.1	23.3 ± 3.9 25.8 ± 6.7
Thr	N S	81.1 ± 40.4 51.3 ± 21.1	44.1 ± 1.6 57.2 ± 13.3	83.5 ± 29.8 68.9 ± 40.9	52.8 ± 9.8 67.1 ± 22.6
Ser	N S	75.9 ± 9.9 70.8 ± 21.6	59.8 ± 19.5 73.9 ± 22.1	83.6 ± 29.9 68.9 ± 40.9	80.0 ± 15.0 85.4 ± 17.4
Glu	N S	79.1 ± 12.2 64.1 ± 15.1	72.5 ± 7.7 66.4 ± 15.1	67.4 ± 18.5 66.2 ± 31.9	68.8 ± 15.3 70.4 ± 13.5
Gln	N S	307.4 ± 77.7 225.9 ± 83.9	173.2 ± 11.7 170.1 ± 86.5	144.3 ± 38.1 139.9 ± 57.6	168.7 ± 18.5 185.8 ± 68.2
Pro	N S	75.9 ± 16.6 70.9 ± 13.3	56.8 ± 24.4 63.8 ± 15.4	71.2 ± 12.9 62.5 ± 13.8	58.0 ± 8.3 56.5 ± 10.4
Gly	N S	244.7 ± 28.2 277.1 ± 70.2	331.1 ± 88.6 380.0 ± 46.4[a]	361.7 ± 165.0 389.5 ± 155.2	480.0 ± 49.9 492.0 ± 81.0
Ala	N S	294.02 ± 66.1 245.93 ± 48.5	208.2 ± 71.4 207.9 ± 53.9	231.3 ± 70.5 195.6 ± 66.6	358.3 ± 51.3 449.1 ± 227.2
Cys	N S	42.1 ± 10.8 43.7 ± 12.2	41.3 ± 11.7 44.8 ± 19.1	44.0 ± 3.7 41.2 ± 14.5	22.5 ± 39.0 34.5 ± 36.0
Val	N S	205.5 ± 86.0 145.3 ± 20.5	161.9 ± 1.7 165.7 ± 43.4	135.4 ± 40.9 152.4 ± 60.0	194.9 ± 15.8 208.7 ± 49.5
Met	N S	41.2 ± 10.8 * 24.3 ± 6.4	36.2 ± 27.0 27.0 ± 5.2	21.8 ± 12.9 16.5 ± 8.3[b]	24.0 ± 1.4 22.9 ± 4.5
Ile	N S	96.4 ± 35.0 74.2 ± 39.0	83.2 ± 1.4 97.0 ± 30.8	73.7 ± 17.9 93.7 ± 47.9	82.5 ± 13.9 87.4 ± 18.5
Leu	N S	109.1 ± 29.9 105.8 ± 15.0	110.4 ± 3.4 116.1 ± 21.5	77.3 ± 17.1 89.3 ± 28.6	105.5 ± 13.6 103.5 ± 13.7
Tyr	N S	49.3 ± 11.0 40.0 ± 7.8	36.8 ± 8.0 31.9 ± 7.1[a]	43.4 ± 23.1 35.0 ± 11.7	40.5 ± 5.2 43.6 ± 6.4
Phe	N S	45.7 ± 8.2 44.7 ± 6.9	64.7 ± 10.8 50.3 ± 6.1	35.5 ± 11.1 36.0 ± 11.1[b]	36.4 ± 6.4 40.1 ± 5.1
His	N S	71.2 ± 7.4 73.5 ± 11.1	89.4 ± 36.0 84.6 ± 21.0	45.0 ± 16.9 56.3 ± 23.5[b]	62.9 ± 4.7 55.4 ± 10.2
Trp	N S	16.0 ± 14.7 10.3 ± 8.6	21.7 ± 1.9 25.1 ± 10.1[a]	27.1 ± 6.8 23.3 ± 8.3	7.2 ± 6.3 3.8 ± 5.1
Orn	N S	35.4 ± 17.7 45.7 ± 18.2	28.1 ± 5.3 27.5 ± 10.4[a]	30.9 ± 1.0 22.5 ± 7.2	73.6 ± 15.1 56.1 ± 6.8
Lys	N S	79.9 ± 29.6 80.9 ± 15.2	70.4 ± 17.5 71.7 ± 19.7	68.1 ± 15.0 66.1 ± 23.5	83.0 ± 21.1 78.9 ± 16.9
Arg	N S	97.1 ± 40.1 83.2 ± 40.7	72.4 ± 19.7 71.1 ± 21.7	82.8 ± 14.5 68.1 ± 18.1	77.9 ± 8.3 75.2 ± 12.4

* $p < 0.05$ (between groups N and S), [a]$p < 0.05$ (before calving and week 1 p. p.), [b]$p < 0.05$ (between weeks 1 and 2)

Table 2
Concentration of TAA, EAA/NEAA and Fischer's index in blood plasma (means ± SD) of dairy cows with liver steatosis (S) and with no increased deposition fat in the liver (N)

	Group	Before calving	Week 1 p. p.	Week 2 p. p.	Week 4 p. p.
TAA [µmol·l^{-1}]	N S	2025 ± 422 1762 ± 334	1772 ± 463 1841 ± 219	1733 ± 457 1691 ± 538	2100 ± 98 2225 ± 366
EAA/NEAA	N S	0.71 ± 0.09 0.67 ± 0.12	0.89 ± 0.05 0.76 ± 0.21	0.65 ± 0.15 0.67 ± 0.07	0.54 ± 0.08 0.52 ± 0.06
FI	N S	4.25 ± 0.78 3.97 ± 0.36	3.56 ± 0.62 4.25 ± 0.54	3.80 ± 0.77 4.73 ± 1.21	4.72 ± 0.71 4.87 ± 1.02

group with bioptically shown liver steatosis, a significant decrease in methionine, phenylalanine and histidine was noticed ($p < 0.05$, between weeks 1 and 2 after parturition); tyrosine, ornithine ($p < 0.05$, between week 1 prior to parturition and week 1 after parturition). Non-significant decrease was found in glutamine, alanine, lysine, and arginine. Significant increase was found in glycine and tryptophan ($p < 0.05$, between week 1 prior to parturition and week 1 after parturition), and a non-significant increase in cysteine and isoleucine were found.

Discussion

Total free amino acids in blood plasma ranged from 1690 to 2500 µmol·l^{-1}. Our results were comparable to the values presented by other authors: Broderick et al. (1974) 1770 µmol·l^{-1} Rogers et al. (1987) 1880 – 2000 µmol·l^{-1} during the observation of similar spectrum of plasma free amino acids.

We have examinated above all Fischer's index (BCAA/AAA), total free amino acids in blood plasma and EAA/NEAA ratio as related to hepatic steatosis. Fischer's index is considered by some authors (Falloon et al. 1988; Azuma et al. 1989) to be a reliable indicator of hepatic steatosis but in our study it was not affected by liver fat, and the average values were even higher in group S than in group N. All obtained values corresponded to those of healthy cows: 2.1 – 5.2 (Falloon et al. 1988), 3.68 – 7.16 (Jagoš et al. 1989). The lowest value of Fischer's index 3.12 was found during the entire experiment. Our results have shown that Fischer's index is not useful as an early indicator of liver steatosis since its evident decrease occurs only when liver damage becomes serious. Our results of Fischer's index do not correspond with the values measured by Scholz et al. (1992), Mudroň et al. (1999), who evaluate the ratio under 4.0 as an indicator of hepatic failure. By this criterion, 28 % cows with and 60 % cows without liver steatosis should have suffered from hepatic failure.

Neither EAA/NEAA nor total plasma free amino acids changed in relation to liver fat. Hepatic steatosis connected with mild impairment of liver parenchyma caused only higher variability of simple free amino acids in blood plasma, which cannot be used for diagnosis of liver steatosis. During the study, in group S we found a concentration of total free amino acids ranging from 958 to 2700 µmol·l^{-1} , and in group N from 1445 to 2446 µmol·l^{-1}. These results approached the values by Rogers et al. (1987) who found 1880 - 2000 µmol·l^{-1} with great differences among individual animals. This fact is probably caused by the health status impairment (liver steatosis) including shortage of digestible crude proteins as followed from the lower values of EAA/NEAA (Oldham 1984). The ratio of EAA/NEAA decreased in both groups during the study.

Among the individual free amino acids in blood plasma we found significantly ($p < 0.05$) lower methionine concentration in blood plasma in cows of group S than in those of group N before parturition, and lower values also during the postparturient period. Methionine belongs to the essential amino acids, and it has sulphur, carboxy- and amino group in its molecule. Methionine is an important donor of CH_3 for the majority of methylation processes. We suppose that due to its metabolic function this amino acid belongs to one of the factors affecting hepatic steatosis in dairy cows. This finding corresponds to earlier data that have shown methionine to be the first limiting amino acid for dairy cows (Bergen 1979). Maiga and Schingoethe (1997) evaluate the lactational response to diets containing fat and increased amounts of bypass proteins. Body condition score and weight gains were unaffected, but for all diets methionine, lysine and phenylalanine were the first three limiting EAA for milk protein synthesis. In our experiment, methionine concentration in blood plasma significantly dropped in cows with liver steatosis after feed restriction. Significant decrease of methionine during starvation was also reported by Ndibualonji et al. (1997). Decrease of methionine concentration was noticed in all of dairy cows that did not suffer from liver steatosis due to energy deficit. In spite of the fact that this decrease was high enough, methionine concentration did not drop below 21 $\mu mol \cdot l^{-1}$ as it was by hepatic steatosis. Ianovych et al. (1998) studied methionine metabolism in tissues of cattle. They found the highest rate of methionine utilisation in protein synthesis in skeletal muscle, in energy metabolism in liver, in the process of gluconeogenesis in skeletal muscles and liver and in lipid synthesis in brain. The close relationship between methionine and metabolic function of liver was also confirmed by Wray-Cahen et al. (1997). Bertics and Grummer (1999) studied effects of supplemental methionine or fat on hepatic triglyceride concentration. Results of these studies indicate that feeding supplemental fat or methionine at levels tested does not prevent or alleviate fatty liver induced by feed restriction. These authors supplemented methionine during and after feed restriction. However, our results indicate that a methionine supplementation is important before feed restriction.

The concentration changes of simple free amino acids in dairy cows during the postpartum period were influenced above all lower feed intake, energy deficit and catabolic processes in the organism. By the evaluation of dynamics of other amino acids our results were not comparable to values reported by Ndibualonji et al. (1997) with higher concentration of 1-methylhistidin, Arg, Leu and lower value Asn, Asp, Glu, Met, and Tyr. We suppose that these differences are caused by a different experimental scheme: our animals were fed roughage whereas those mentioned above did the study during starvation for 3 days only with water supply.

Our results show that the shortage of methionine appears to be one of predisposing factors in the development of liver steatosis. We also found that the concentration of plasma free amino acids cannot serve as a sensitive indicator of liver function impairment; therefore it is of no value in early diagnosis of liver steatosis.

Sledování koncentrace volných aminokyselin v krevní plazmě dojnic při rozvoji steatózy jater

Cílem pokusu bylo sledování změn koncentrace volných aminokyselin v krevní plazmě dojnic v poporodním období při rozvoji steatózy jater a ověření využití Fischerova indexu (BCAA/AAA - poměr aminokyselin s rozvětveným řetězcem a aromatických aminokyselin) při diagnostice steatózy jater. U 10 krav černostrakatého plemene ve velmi dobré až tučné tělesné kondici (BCS 4,5-5) byla provedena restrikce krmné dávky koncem 1.týdne po porodu. Čtvrtý den po zahájení restrikce krmné dávky byla provedena biopsie jater a na základě morfologického vyšetření jaterní tkáně byly dojnice rozděleny do dvou skupin (S - dojnice se steatózou, N - dojnice bez zvýšeného obsahu tuku v játrech). Odběry krve byly provedeny 1 týden před porodem, 1. týden po porodu, 2. týden po porodu (v den biopsie jater) a 4. týden po porodu. V krevní plazmě byly stanoveny volné aminokyseliny

po deproteinaci 0.22 M kyselinou salicylovou na automatickém analyzátoru aminokyselin. Hladina celkových volných aminokyselin (TAA) v krevní plazmě, Fischerův index ani poměr EAA/NEAA nebyly ovlivněny obsahem tuku v játrech. V den biopsie jater byly u skupiny krav se steatózou jater ve srovnání se zdravými zjištěny následující hodnoty: TAA (1690,8 ± 537,7 vs. 1732,7 ± 456,7 $\mu mol \cdot l^{-1}$), EAA/NEAA (0.67 ± 0.07 vs. 0.65 ± 0.15), Fischerův index (4,73 ± 1,21 vs. 3,80 ± 0,77). Narušení funkce jater se projevilo pouze vysokou variabilitou zjištěných hodnot v rámci skupin. Výraznější vztah ke steatóze jater se projevil u hladiny methioninu v krevní plazmě, který byl před porodem (24,27 ± 6,39 vs. 41,24 ± 10,75 $\mu mol \cdot l^{-1}$) signifikantně nižší ve skupině S než ve skupině N a nižší hodnoty přetrvávaly v celém poporodním období (1. týden 27,02 ± 5,15 vs. 36,20 ± 27,04; 2. týden 16,46 ± 8,25 vs. 21,84 ± 12,92, 4. týden 22,90 ± 4,49 vs. 24,0 ± 1,44 $\mu mol \cdot l^{-1}$). Na základě našich výsledků se jeví nedostatek methioninu jako jeden z predispozičních faktorů pro rozvoj jaterní steatózy. Sledování volných AA v krvi však nelze využít v časné diagnostice jaterní steatózy jako citlivého indikátoru narušení funkce jater.

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