BLOOD PLASMA FATTY ACID CONCENTRATIONS IN CATTLE DURING THE TRANSITIONAL FEEDING PERIOD (WINTER – SUMMER)

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Abstract

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Changes of fatty acids levels in the blood plasma of heifers were studied in a locality with annual incidence of nutritional muscle dystrophy. Pinzgau breed heifers divided into 4 groups (6 animals per group) were used in the experiment. Groups I (with i.m. administered Selevit) and II (without treatment) were abruptly turned out to pasture. Groups III and IV were left indoors: group III was abruptly and group IV gradually turned to green feeding.

With regard to changes in proportions of major plasma fatty acids (those of about 5 % and higher) and essential plasma fatty acids of n-6 (linoleic acid) and n-3 series (linolenic acid) as well as their higher metabolites, the differences between the groups of animals during stabled period (1st sampling) and on the 2nd day on the pasture (2nd sampling) were not significant. At the following samplings, significant differences were observed within the groups of animals with increasing proportion of total monounsaturated fatty acids and n-3 polyunsaturated fatty acids (especially that of linolenic acid) and decreasing proportion of n-6 polyunsaturated fatty acids including linoleic acid as well as higher n-6 metabolites (especially dihomogamma-linoleic and arachidonic acids). In contrast to linolenic acid, a decrease of its higher n-3 metabolites was recorded. Although these changes were the most marked in the group II of animals (animals abruptly turned to pasture without previous treatment), and in all groups at the last but one sampling (8th day on pasture), similar tendency was recorded also in other groups.

In conclusion, the results of this study indicate that the observed changes are related to turning the animals to green feeding.

Pinzgau heifers, nutrition, total, mono-, polyunsaturated fatty acids, blood plasma, metabolites

Nutritional stress (deficiencies of Se – Kováč and Sankari 1988; vitamin E during indoor winter feeding - Kováč et al. 1979, Vrzgula et al. 1979), physical stress-unusual exercise (turn on the pasture in the spring time - Kováč et al. 1987), environmental stress (cold and wet weather) and chemical stress (increased polyunsaturated fatty acid intake from young grass, rumen microbial adaptation from winter feeding to the consumption of young leafy grass, reducing of hydrogenation capacity in the rumen, increasing the susceptibility of muscle membranes to peroxidases) play important roles in ethiopathogenesis of nutritional muscular dystrophy in young cattle (Vrzgula and Kováč 1991).

In mammalian tissues there are four families of polyunsaturated fatty acids derived from the parent fatty acids; palmitoleic and oleic acids, which can be synthetized endogenously, and linoleic and linolenic acids that must be obtained from the diet and are known as essential fatty acids. These four precursors are desaturated and chain elongated to form the long chain highly unsaturated fatty acids. The principal products of linoleic acid are arachidonic, with four double bonds (tetraene), and dihomogamma linolenic acids; those of linolenic acid are eicosapentaenoic and docosahexaenoic acids. These polyunsaturated acids derived from essential fatty acids when incorporated into membrane phospholipids can alter membrane fluidity, which determines the permeability of membranes and the behaviour of membrane-bound enzymes and receptors. The dihomogammalinolenic, arachidonic, and eicosapentaenoic acids are also the precursors of eicosanoids which influence many cellular

processes (prostaglandins, prostacyclins, thromboxanes and leukotrienes - Dobroňová and Šajbidor 1992).

When the dietary amounts of linoleic and linolenic acids are inadequate, palmitoleate and oleate are desaturated and chain elongated to give rise to eicosatrienoic acids (triene). An elevated tissue triene/tetraene ratio is, therefore, used as a marker for essential fatty acid deficiency. The essential fatty acid deficiency symptoms include reduced growth rate, scaly dermatitis, impaired reproduction, and susceptibility to infection (Sardesai 1992).

Unsaturated fatty acids, particularly the polyunsaturated fatty acids, form not only a part of the energy supply, but also an essential part of membranes. The unsaturated double bonds of membrane polyunsaturated fatty acids are inherently unstable and readily attacked by peroxides and other forms of active oxygen. This process tends to produce a chain reaction and more free radicals and hydroperoxides are formed. The α-tocopherol acts as a scavenger of free radicals and prevents this runaway reaction. It follows that the greater the level and intake of polyunsaturated fatty acids into the cells, the greater the amount of α-tocopherol that is needed to prevent the reaction. There is a mathematical relationship between the input of polyunsaturated fatty acids and the vitamin E requirement. This varies in different species from 0.5 to 3.0 mg α-tocopherol per each 1 g of dietary polyunsaturated fatty acids (Putnam and Comben 1987).

With regard to previous studies of regional character with annual incidence of nutritional muscular dystrophy (Kováč et al. 1993), the present experiment was aimed at evaluation of blood plasma fatty acids in heifers before and after turning out to pasture.

Materials and Methods

Twenty four Pinzgau breed heifers (mean body mass 240 kg) were divided into four groups. The first group (animals 1-6) was treated once with 25 ml of Selevit (tocopherol acetate 25 mg, sodium selenite 2.2 mg, solubilizers and water to 1 ml) subcutaneously. The second group (animals 7-12) was not treated. After two weeks the first and second groups were abruptly turned out to the pasture, without previous feeding adjustment. The remaining two groups were left indoors. The third group (animals 13-18) was abruptly turned to green feeding. In the fourth group (animals 19-24), the original ration of pelleted feed, hay and straw was gradually enriched with green forage. Heparinized blood samples were collected 15 days before, and on days 2, 4, 8, and 11 after turning the animals to green feeding. For determination of the blood plasma fatty acids after saponification of lipid extracts (Lowenstein 1981), methylesters of fatty acids were prepared (McLaughter and Engel 1979, followed by their determination by gas chromatography (Carlo Erba, Italy). The fatty acids concentrations in the tables are expressed as average values + SD in mg/l and weight %. Comparison of fatty acids concentrations between the groups were performed using the Student s t-test.

Results

With regard to proportions of major plasma fatty acids, and essential plasma fatty acids, significant differences were observed within the groups with increasing proportion of total monounsaturated fatty acids and n-3 polyunsaturated fatty acids (especially that of linolenic acid) and decreasing proportion of n-6 polyunsaturated fatty acids including linoleic acid as well as higher n-6 metabolites (especially dihomogamma-linolenic and arachidonic acids). In contrast to linolenic acid, decrease of its higher n-3 metabolites was recorded. Although these changes were the most marked in the group II, and in all groups at the last but one sampling (8th day on pasture), a similar tendency was recorded also in other groups. The less marked changes of total fatty acids were observed in the fourth group (2 239, 2 273, 2 125, 1 707, 2 615, and 2 658 mg/l of plasma). In this group as well as in the first group the mean highest concentration was observed on the 11th day of green feeding (fourth group - 2 658, first group - 2 335 mg/l of plasma) see Tables 1-4.

Table 1

Average values of fatty acids (weight %) ± standard deviation in the first group

Fatty acids		−15th	2nd	4th	6th	8th	11th days
TFA*	x	2 063	1 770	1 675	1 363	2 614	2 335
	±s	545	513	719	544	347	498
TSFA	x	42.67[a]	43.21	40.22	39.13[c]	42.19	46.16
	±s	1.71	2.40	2.53	0.41	3.45	6.91
TMUFA	x	16.86[a]	22.95[c]	21.41[c]	19.94[c]	21.24[c]	21.14[c]
	±s	1.10	2.79	1.99	1.06	0.75	2.10
Tn-6 PUFA	x	33.97[a]	27.75[c]	29.10[c]	28.64[c]	24.95[c]	21.57[c]
	±s	0.88	3.52	3.10	1.00	2.20	5.35
Tn-3 PUFA	x	4.50[a]	4.01	6.68[b]	9.51[c]	8.32[c]	7.18
	±s	0.73	0.03	1.15	0.47	1.55	3.80
TMn-6 PUFA	x	7.65[a]	5.80	4.92[b]	5.18[b]	3.66[c]	2.85[c]
	±s	1.84	1.19	0.71	0.48	1.75	2.67
TMn-3 PUFA	x	2.41[a]	1.69	1.47[b]	2.29	1.39	1.10
	±s	0.87	0.30	0.35	0.35	0.97	1.40

[a.b] ($P < 0.05$); [a.c] ($P < 0.01$)

TFA* (total fatty acids) (mg/l); TSFA (total saturated fatty acids); TMUFA (total monounsaturated fatty acids); Tn-6 PUFA (total n-6 polyunsaturated fatty acids); Tn-3 PUFA (total n-3 polyunsaturated fatty acids); TMn-6 PUFA (total metabolites of n-6 PUFA) (higher as 18:2 n-6); TMN-3 PUFA (total metabolites of n-3 PUFA (higher than 18:2 n-3)

Table 2

Average values of fatty acids (weight %) ± standard deviation in the second group

TFA*	x	2 188[a]	2 284	1 980	1 387[c]	1 855[c]	1 972
	±s	98	409	204	221	60	459
TSFA	x	41.11[a]	44.20[c]	41.21	42.48	45.07[c]	42.70
	±s	1.52	1.17	4.41	1.72	1.80	6.41
TMUFA	x	16.61[a]	23.65[c]	20.96[c]	21.43[c]	23.41[c]	19.51
	±s	1.25	1.62	2.08	1.01	1.69	2.71
Tn-6 PUFA	x	35.65[a]	26.55[c]	30.56[b]	28.62[c]	24.24[c]	26.82[c]
	±s	1.54	2.35	3.48	2.28	3.90	2.83
Tn-3 PUFA	x	4.69[a]	3.53[b]	5.41	5.49	4.58	7.57
	±s	0.79	0.53	3.29	2.27	1.67	5.25
TMn-6 PUFA	x	8.47[a]	5.04[c]	4.40[c]	3.87[c]	2.05[c]	3.67[c]
	±s	1.27	0.29	1.82	0.53	0.27	2.14
TMn-3 PUFA	x	2.45[a]	1.23[c]	1.25[b]	1.02[c]	0.41[c]	1.24[b]
	±s	0.55	0.15	1.00	0.36	0.12	1.03

[a.b] ($P < 0.05$); [a.c] ($P < 0.01$)

TFA* (total fatty acids) (mg/l); TSFA (total saturated fatty acids); TMUFA (total monounsaturated fatty acids); Tn-6 PUFA (total n-6 polyunsaturated fatty acids); Tn-3 PUFA (total n-3 polyunsaturated fatty acids); TMn-6 PUFA (total metabolites of n-6 PUFA) (higher as 18:2 n-6); TMN-3 PUFA (total metabolites of n-3 PUFA (higher than 18:2 n-3)

Table 3
Average values of fatty acids (weight %) ± standard deviation in the third group

Fatty acids		–15th	2nd	4th	6th	8th	11th days
TFA*	x	2 189[a]	2 370	1 511	1 410[b]	2 261	2 053
	±s	516	737	494	287	840	422
TSFA	x	41.63[a]	46.28[b]	40.74	44.12	46.95[c]	45.90
	±s	1.66	3.29	1.38	3.51	2.50	6.28
TMUFA	x	15.41[a]	20.43[c]	15.28	16.68	16.01	14.27
	±s	0.54	1.21	0.55	3.18	2.27	1.92
Tn-6 PUFA	x	36.47[a]	27.47[c]	34.26[b]	30.03[b]	28.41[c]	30.15[b]
	±s	1.62	3.46	0.34	4.62	3.66	5.27
Tn-3 PUFA	x	4.75[a]	3.69[c]	6.87[c]	6.58	5.99	6.89
	±s	0.29	0.99	0.47	2.15	1.60	2.71
TMn-6 PUFA	x	7.92[a]	4.72[c]	6.41[b]	4.39[c]	2.53[c]	3.87[c]
	±s	0.61	1.69	0.89	2.00	0.79	2.37
TMn-3 PUFA	x	2.51[a]	1.00[c]	1.85[c]	1.25[b]	0.71[c]	1.26[b]
	±s	0.21	0.64	0.41	0.99	0.59	1.18

[a.b] ($P < 0.05$); [a.c] ($P < 0.01$)
TFA* (total fatty acids) (mg/l); TSFA (total saturated fatty acids); TMUFA (total monounsaturated fatty acids); Tn-6 PUFA (total n-6 polyunsaturated fatty acids); Tn-3 PUFA (total n-3 polyunsaturated fatty acids); TMn-6 PUFA (total metabolites of n-6 PUFA) (higher as 18:2 n-6); TMN-3 PUFA (total metabolites of n-3 PUFA (higher than 18:2 n-3)

Table 4
Average values of fatty acids (weight %) ± standard deviation in the fourth group

Fatty acids		–15th	2nd	4th	6th	8th	11th days
TFA*	x	2 239	2 273	2 125	1 707	2 615	2 658
	±s	456	173	419	698	57	256
TSFA	x	40.26[a]	44.05[b]	39.65	40.70	44.54[c]	42.33
	±s	1.76	2.51	3.06	2.13	1.53	3.44
TMUFA	x	15.56[a]	21.50[c]	19.20[c]	18.92[c]	17.89[c]	15.18
	±s	1.38	1.62	1.70	0.46	0.50	1.20
Tn-6 PUFA	x	37.16[a]	28.57[c]	33.10	30.63[c]	28.33[c]	32.27[c]
	±s	2.24	2.27	3.61	1.40	1.71	3.22
Tn-3 PUFA	x	4.98[a]	3.68	5.61	7.39[c]	6.73[c]	8.05[c]
	±s	0.54	1.27	0.86	0.98	0.69	0.66
TMn-6 PUFA	x	8.38[a]	4.70[c]	6.11[c]	5.70[c]	4.15[c]	6.07[b]
	±s	0.59	1.91	0.79	0.40	1.31	1.56
TMn-3 PUFA	x	2.59[a]	1.08[c]	1.74[b]	1.80[c]	1.24[c]	2.38
	±s	0.33	0.76	0.54	0.31	0.45	0.55

[a.b] ($P < 0.05$); [a.c] ($P < 0.01$)
TFA* (total fatty acids) (mg/l); TSFA (total saturated fatty acids); TMUFA (total monounsaturated fatty acids); Tn-6 PUFA (total n-6 polyunsaturated fatty acids); Tn-3 PUFA (total n-3 polyunsaturated fatty acids); TMn-6 PUFA (total metabolites of n-6 PUFA) (higher as 18:2 n-6); TMn-3 PUFA (total metabolites of n-3 PUFA (higher than 18:2 n-3)

Discussion

The unsaturated fatty acids that have a direct biochemical role in mammalian systems vary from monounsaturated fatty acids such as oleic (18:1) to polyunsaturated fatty acids (PUFA) containing a number of double bonds, e.g. linolenic (18:3) and arachidonic acid (22:4). Unsaturated fatty acids have a number of essential roles in mammalian metabolism, through maintaining fluidity of membranes as the primary constituent of sub-cellular membranes and acting as important precursors for prostanoids (McMurray et al. 1983).

In vitamin E and Se- deficiency diseases inherent instability of unsaturated fatty acids leads to the formation of hydroperoxides. The chemical structure of these acids makes them particularly susceptible to degradation by peroxidative mechanisms. The concept of peroxidizability refers to the relative rates at which the unsaturated fatty acids form peroxides and the peroxidizability ratios for oleic, linoleic and linolenic acids are 1:12:25, respectively. Peroxidation can be regarded as a number of distinct steps. Initiation - an initiator is necessary to start the peroxidation process and leads in the first instance to the formation of hydroperoxide. A number of chemical mechanisms have been proposed, involving, among other substances, the toxic metabolites of oxygen, e.g. hydrogen peroxide, superoxide, hydroxyl radical and singlet oxygen. Such initiators could be regarded as endogenous initiators while others such as carbon tetrachloride can be regarded as exogenous. Peroxides can also be formed as the product of the activity of the enzyme lipoxigenase. Propagation - the lipid hydroperoxide, once formed, give rise to further free-radicals and the reaction becomes self-sustaining as the radical will initiate formation of futher hydroperoxides. The process is referred to as autoxidation. Termination - the chain proliferation steps can be interrupted by compounds that arrest the propagation sequence, either by removing peroxides or quenching the free-radicals produced in such reactions. Peroxidation is deleterious to cell structure and function. For example, attack on unsaturated fatty acids within membranes both surrounding and within the cell can affect their fluidity and integrity. The peroxides themselves display biological activity, either through their similarity to prostaglandin precursors, or, alternatively, by degradation of the peroxide giving rise to cytotoxic compounds, e.g. aldehydes (Rice and Kennedy 1988).

The use of fats and oils to increase the energy density of diets and to correct fatty acid deficiency in farm animals, has been established for many years (Smith 1991).

Polyunsaturated fatty acids are precursors of lipid mediators which play a key role in cardiovascular and inflammatory diseases of human population. Dietary sources of essential fatty acids are vegetable oils for either linoleic or alpha-linolenic acids, and sea fish oils for eicosapentaenoic and docosahexaenoic acids. Because of the specificity of the pancreatic lipid hydroxylases, triglyceride fatty acid distribution is an essential parameter in the digestibility of fats. The efficiency of the intestinal uptake depends on the hydrolysis and especially on their micellarization. Digestion of n-3 polyunsaturated fatty acid ethyl ester is recognized to be impaired, but n-3 polyunsaturated fatty acid triglyceride hydrolysis remains a controversial point, and, according to some authors explain differences observed between vegetable and fish oil absorption. Additional studies are required to investigate this intestinal step. In enterocytes, morphological and biochemical absorption processes involve reesterification of long-chain fatty acids and lipoprotein formation. At this level, specific affinity of l- and L- FABPc (cytosolic fatty acid binding proteins) to polyunsaturated fatty acids requires further investigation. A better understanding of the role of these FABPc might bring to light the esterification step, particularly the integration of polyunsaturated fatty acids into phospholipids (Carlier et al. 1991).

On the other hand, regarding the outbreaks of nutritional degenerative myopathy in young cattle the main factor relating to this disease is the high concentration of polyunsaturated fatty acids in young grass. A twofold increase in the linolenic acid in plasma occurred within 48 h of turnout to pasture and threefold increase within 72 h. Over the same period there was a decrease in concentration of linoleic acid (McMurray et al. 1980).

The results of this study justify special husbandry (gradual change to green feeding) and veterinary (treatment with tocopherol acetate and sodium selenite) measures. Particularly in the fourth group, gradually turned to green forage, the less marked changes of investigated parameters were observed, especially that of total fatty acids. Increase of n-3 polyunsaturated fatty acid levels (especially of linolenic acid) and decrease of n-6 polyunsaturated fatty acid including linoleic acid were confirmed.

Sledovanie koncentrácie mastných kyselín v krvnej plazme hovädzieho dobytka počas prechodného obdobia (zima - leto)

V etiopatogenéze viacerých ochorení hospodárskych zvierat významnú úlohu zohrávajú vitamín E, selén a mastné kyseliny.

V práci boli sledované zmeny koncentrácie mastných kyselín v plazme jalovíc pred a počas prechodu na zelené kŕmenie v lokalite s každoročným výskytom nutričnej svalovej dystrofie. Do pokusu boli zaradené 4 skupiny (n=6) Pinzgavských jalovíc. Skupina I (ošetrená Selevitom, i.m.) a II (bez ošetrenia) boli náhle vyhnané na pastvu. Ostatné dve skupiny zostali ustajnené, pričom skupina III prešla náhle a skupina IV postupne na zelené krmivo. Z pohľadu zastúpenia hlavných mastných kyselín (5 % a viac) a esenciálnych mastných kyselín radu n-6 (kyselina linolová) a n-3 (kyselina linolénová) ako aj ich vyšších metabolitov počas obdobia ustajnenia (1. odber) ani na 2. deň po vyhnaní na pastvu neboli medzi skupinami zistené signifikantné rozdiely. Pri ďalších odberoch boli v rámci skupín zistené signifikantné rozdiely v smere zvýšeného zastúpenia celkových mononenasýtených mastných kyselín a n-3 polynenasýtených mastných kyselín (hlavne kyseliny linolénovej) a zníženého podielu n-6 polynenasýtených mastných kyselín vrátene kyseliny linolovej ako aj vyšších n-6 metabolitov (hlavne kyseliny dihomogama-linolovej a arachidonovej). Na rozdiel od kyseliny linolénovej bol zaznamenaný pokles jej vyšších n-3 metabolitov. Napriek tomu, že tieto zmeny boli najvýraznejšie v druhej skupine zvierat (zvieratá náhle vyhnané na pastvu bez predchádzajúceho ošetrenia) a vo všetkých skupinách pri predchádzajúcom odbere, zmeny hladín mastných kyselín vykazovali podobnú tendenciu aj v ostatných skupinách.

Možno predpokladať, že tieto zmeny súvisia s prechodom zvierat na zelené kŕmenie.

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