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EFFECT OF SUPPLEMENTAL RUMEN-PROTECTED LYSINE, METHIONINE OR BOTH ADDED TO DIET OF LACTATING DAIRY COWS ON MILK FATTY ACIDS PROFILE

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Abstract

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The objective of this study was to determine the effect of supplemental lysine (Lys), methionine (Met) or both added to diet of dairy cows in the form of rumen-protected (RP) tablets on changes in milk fatty acids (FA) profile. The trial was carried out on four lactating Holstein cows in the form of Latin square design and was divided into 4 periods of 14 d (10-d preliminary period and a 4-d experimental period). The four treatments were as follows: C – control without amino acids (AA) supplementation, L – supplement of RP Lys, M – supplement of RP Met and ML – supplement of RP Met and Lys. Cows were fed on a diet based on maize silage, lucerne hay and supplemental mixture. Milk yield in ML (34.18 kg/d) was higher than in L or M (32.46 kg and 32.13 kg, respectively, $P < 0.05$) and tended to be higher than in C (33.33 kg/d, $P > 0.05$). Protein yield in ML (1054 g/d) was higher than that found in C, L or M (990, 998 or 968 g/d, respectively, $P < 0.05$). Milk fat content and yield in C and ML was higher in comparison to L and M ($P < 0.05$). Content of short-chain FA (C 4:0–C 12:0) was not affected by the treatment except of L that was lower than in C ($P < 0.05$). Content of medium-chain FA in M was lower compared to C, L or ML ($P < 0.05$). The content of long-chain FA in M was significantly higher than in other groups ($P < 0.05$). The total content of SFA in M was lower than in C or ML ($P < 0.05$) and tended to be lower than in L. Contents of UFA, MUFA and PUFA in M were higher than in C and ML ($P < 0.05$).

fatty acids, rumen protection, Holstein cows, milk, lysine, methionine

Lysine (Lys) and Methionine (Met) have been implicated most often as the most limiting or co-limiting amino acids (AA) for milk protein synthesis when a variety of diets are fed (e. g. Schwab et al., 1992). Production responses of lactating dairy cows to supplemental Lys, Met, or both, fed in a ruminally protected (RP) form or infused to the intestine, have been reported in numerous studies, however, the milk protein as well as fat response to such supplement have been often variable (e. g. Doepel et al., 2004). There is only a few studies refering to the effect of RP AA supplement on fatty acid (FA) profile of milk because the composition of milk fat is a result of complex interactions among diet composition, dry matter intake, rumen fermentation, liver metabolism, body fat mobilization, and mammary absorption and synthesis of FA (Garnsworthy et al., 2006). Results of several studies (e. g. Guinard and Rulquin, 1994; Pisulewski et al., 1996 or Rulquin et al., 2006) suggested that feeding of RP AA may have an impact on *de novo* synthesis of short- and medium-chain FA in the mammary gland. Especially Met plays an important role from this point of view because it is not only required for milk protein synthesis but also is involved in many metabolic pathways including the synthesis of phospholipids, carnitine, creatine, and polyamines (Bequette et al., 1998). In addition, Met serves as a methyl donor for transmethylation reactions in the biosynthesis of li-

pids and other compounds and is involved in lipid transport in the blood (Glascock and Welch, 1974).

The objective of this study was to determine the effect of supplemental Lysine (Lys), Methionine (Met) or both (Met and Lys) added in the form of rumen-protected (RP) tablets to diet of dairy cows on fatty acids (FA) profile of milk.

MATERIAL AND METHODS

The present experiment is an extension of a previous study (Třináctý et al., 2009) in which the effect of supplemental Lys, Met or both added to a diet of dairy cows in the form of RP tablets on milk yield and composition and concentration of plasma AA was studied.

Material and method has been described in Třináctý et al. (2009). Briefly, four high-yielding lactating Holstein cows (2.–5. lactation) with similar milk yield were used in this study. Experiment, in the form of Latin square design, was divided into 4 periods of 14 d (10-d preliminary period and a 4-d experimental period). The four treatments were as follows: C – control without AA supplementation, L – control plus supplement of RP Lys, M – control plus supplement of RP Met and ML – control plus supplement of RP Met and Lys. Cows were fed individually twice daily (07:00 and 17:00 h) *ad libitum* the diet based on maize silage (346 g/kg), lucerne hay (86 g/kg) and supplemental mixture (568 g/kg, containing (in g/kg): barley 350; oats 250; wheat 80; sugar beet chippings 150; flax seed 50; soybean meal 70; sodium chloride (NaCl) 5; dicalcium phosphate (DCP) 15; limestone (CaCO3) 15; sodium bicarbonate (NaHCO3) 1; monosodium phosphate (MSP) 2; magnesium phosphate (MgP) 2; microelements and vitamin mixture 10). The diets were balanced to meet 100% of NEL requirement (Sommer, 1994) and 95% of PDI requirement by reason of a better manifestation of experimental treatment. Based on Rulquin et al. (2001), the diets were found to be deficient in Met (cca 26%) and Lys (cca 5%) that were applied in the form of RP tablets resulting in daily intakes of Met = 18.2 g in M, Lys = 11.7 g in L and that of Met and Lys = 18.2 and 11.7 g, respectively in ML. Tablets were applied during the whole period (14 d) twice a day immediately before feeding by mixing tablets into the part of supplemental mixture.

Sampling and analyses of feed, feed refusals and milk for basal constituent were as described in Třináctý et al. (2009). Milk fat was extracted with diethylether and petrolether (1:1), according to ISO 1211. Alkaline transmethylation of fatty acids present in extracts was carried out according to ISO 5509. Gas chromatographic analysis of the methyl esters was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a programmed 60 m HP-Innowa capillary column (180–240°C) and a FI detector.

Data obtained in the experiment were analysed using GLM procedure of the Statgraphics 7.0 package (Manugistics Inc., and Statistical Graphics Corporation, Rockville, Maryland, USA) according to the following model: $Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl}$ where μ = general mean, T_i = effect of treatment (i = 4), C_j = effect of cow (j = 4), P_k = effect of period (k = 4), D_l = effect of day of sampling (l = 4) and ε_{ijkl} = error term.

RESULTS

Average daily nutrient intake and lactational performance of dairy cows in dependence of experimental treatment is presented in Tab. I. The intake of dry matter (DM) in M and ML was higher compared to C or L ($P < 0.05$). Supplementation of RP Lys resulted in significant increase of LysDI content in L and ML ($P < 0.05$) while addition of RP Met increased content of MetDI in M and ML ($P < 0.05$).

I: *Average daily nutrient intake and lactation performance of dairy cows fed basal diet supplemented with rumen-protected lysine, methionine or both in comparison to unsupplemented diet*

Item	Unit	C[1]	L[1]	M[1]	ML[1]	SE
Nutrient intake						
Dry matter	kg/d	20.83[a]	20.73[a]	21.39[b]	21.64[b]	0.262
LysDI[2]	% PDIE	6.96[a]	7.46[b]	6.93[c]	7.25[d]	0.011
MetDI[2]	% PDIE	1.85[a]	1.84[a]	2.25[b]	2.39[c]	0.013
Yield and composition of milk						
Milk yield	kg/d	33.33[ab]	32.46[a]	32.13[a]	34.18[b]	0.880
4% FCM[3]	kg/d	30.76[a]	27.43[b]	27.32[b]	30.32[a]	1.265
Fat	g/kg	35.9[a]	30.7[b]	31.9[b]	33.7[ab]	2.52
Fat	g/d	1162[a]	963[b]	965[b]	1110[a]	78
Protein	g/kg	29.7[a]	30.8[c]	30.1[ab]	30.7[bc]	0.48
Protein	g/d	990[a]	998[a]	968[a]	1054[b]	30

[a, b] means in the same row followed by the different superscripts differ ($P < 0.05$)

[1] treatments were as follows: C – control, L – control + supplemental Lys (11.7 g/d), M – control + supplemental Met (18.2 g/d), ML – control + supplemental Met and Lys (18.2 and 11.7 g/d, respectively)

[2] digestible AA in the intestine

[3] 4% fat corrected milk

Milk yield in ML was higher than in L or M ($P < 0.05$) and tended to be higher than in C ($P > 0.05$). Milk yield expressed in 4% FCM in C and ML was higher compared to L or M ($P < 0.05$). Supplementation of RP Lys or Met resulted in lower milk fat content and yield in L and M in comparison to C and ML ($P < 0.05$). Content of milk protein in L and ML was higher than in C ($P < 0.05$) and tended to be higher than in M ($P > 0.05$). Due to differences in milk production, milk protein yields in C, L or M were lower than that in ML ($P < 0.05$).

The fatty acid profile of milk fat as affected by the RP Met, Lys or both is shown in Tab. II. Content of short-chain FA (C 4:0–C 12:0) was not affected by the treatment except of L that was lower than in C ($P < 0.05$). This decline was caused by the decreased levels of C 4:0, C 6:0, C 8:0 and C 10:0 in L in comparison to C ($P < 0.05$). Content of C 14:0 and C 16:0 that was lower in M compared to C, L or ML ($P < 0.05$) contributed to overall decrease in total medium-chain FA in M in comparison to C, L or ML ($P < 0.05$). The total content of long-chain FA in M was significantly higher than in other groups ($P < 0.05$). Content of C 18:0 in L was significantly lower than in C, M or ML ($P < 0.05$). The sum of C 18:1 in M was higher than in C and ML ($P < 0.05$) and tended to be higher than in L. Proportion of C 18:2 in M was higher compared to other treatments ($P < 0.05$). Two main isomers of CLA (conjugated linoleic acid) were determined. The content of cis-9, trans-11 CLA isomer in ML was the lowest (0.634 g/100 g) and differed ($P < 0.05$) from those in C and L or in M which was the highest (0.843 g/100 g). Content of trans-10, cis-12 CLA isomer was not affected by the treatment except of that in L that was higher than in C ($P < 0.05$). Proportion of C 18:3 in M and L was higher compared to C ($P < 0.05$). The concentrations of other individual long-chain FA with the chain length greater than 20 carbon atoms were very low and ranged be-

II: *Profile of selected individual milk fatty acids (g/100g) as affected by supplementation of rumen-protected lysine, methionine or both added to diet of lactating dairy cows*

Item	C[1]	L[1]	M[1]	ML[1]	SE
C4:0	1.67 [a]	1.43 [bc]	1.60 [ac]	1.51 [ac]	0.127
C6:0	1.40 [a]	1.11 [b]	1.31 [ac]	1.26 [c]	0.084
C8:0	1.01 [a]	0.85 [b]	0.94 [c]	0.94 [c]	0.151
C10:0	2.65 [a]	2.44 [bc]	2.49 [bc]	2.57 [ac]	0.108
C12:0	3.40 [ab]	3.43 [a]	3.25 [b]	3.40 [ab]	0.116
C14:0	12.04 [a]	12.00 [a]	11.26 [b]	11.93 [a]	0.323
C16:0	32.32 [a]	32.15 [a]	28.89 [b]	31.44 [a]	1.260
C18:0	8.91 [a]	7.72 [b]	9.69 [a]	9.07 [a]	0.608
C 18:1	25.29 [a]	27.09 [bc]	28.56 [b]	26.35 [ac]	1.039
C 18:2	4.33 [a]	4.53 [a]	5.13 [b]	4.09 [a]	0.380
from that:					
C18:2 (9,11)	0.80 a	0.75 a	0.84 a	0.63 b	0.079
C18:2 (10,12)	0.02 [a]	0.03 [b]	0.03 [ab]	0.03 [ab]	0.008
C18:3	0.26 [a]	0.31 [b]	0.31 [bc]	0.28 [ac]	0.020
C20:0	0.14 [a]	0.11 [b]	0.14 [a]	0.14 [a]	0.011
Sums of fatty acids according to chain lenght[2]					
C4:0 – C12:0	10.22 [a]	9.37 [b]	9.66 [ab]	9.93 [ab]	0.397
C13:0 – C16:0	49.58 [a]	49.54 [a]	45.07 [b]	48.86 [a]	1.332
C17:0 – C22:0	39.57 [a]	40.42 [a]	44.58 [b]	41.04 [a]	1.388
Sums of fatty acids according to (un)saturation[2]					
SFA [3]	65.80 [a]	63.76 [ab]	61.85 [b]	64.83 [a]	1.471
UFA [4]	34.20 [a]	36.24 [ab]	38.15 [b]	35.13 [a]	1.460
MUFA [5]	29.15 [a]	30.93 [bc]	32.20 [b]	30.28 [ac]	1.143
PUFA [6]	5.05 [a]	5.31 [a]	5.95 [b]	4.85 [a]	0.394

[a,b] means in the same row followed by the different superscripts differ ($P < 0.05$)

[1] treatments were as follows: C – control, L – control + supplemental Lys (11.7 g/d), M – control + supplemental Met (18.2 g/d), ML – control + supplemental Met and Lys (18.2 and 11.7 g/d, respectively)

[2] including following minor fatty acids: C 11:0, C 13:0, C 14:1-n5, C 15:0, C 16:1-n7, C 17:0, C 20:1-n9, C 20:2-n6, C 21:0, C 20:3-n6, C 20:4-n6, C 20:3-n3, C 20:4-n3, C 22:0, C 20:5-n3, C 22:4-n6, C 24:0, C 22:5-n3, C 22:6-n3

[3] saturated fatty acids

[4] unsaturated fatty acids

[5] monounsaturated fatty acids

[6] polyunsaturated fatty acids

tween physiological ranges typical for this type of diet (not given in Table).

Content of saturated fatty acids (SFA) in M was the lowest and differed significantly from the C and ML ($P < 0.05$). The total content of unsaturated fatty acids (UFA) in M was higher than in C or ML ($P < 0.05$) and tended to be higher than in L ($P > 0.05$). Content of monounsaturated fatty acids (MUFA) in M was higher than those in C and ML ($P < 0.05$) and tended to be higher than in L. Similarly, content of polyunsaturated fatty acids (PUFA) in M was higher compared to C, L or ML ($P < 0.05$).

DISCUSSION

Differences in DM intake found in our experiment are in agreement with e. g. Schwab et al. (1992). On the other hand, in some studies no effect of supplementation with RP Met and Lys on intake of DM and its components was observed (Blum et al., 1999; Robinson et al., 1999). In the present experiment milk yield and 4% FCM in ML was higher than in L or M ($P < 0.05$) but did not differ between ML and C ($P > 0.05$). Similar findings were reported by e. g. Donkin et al. (1989) or Blum et al. (1999). Milk protein content and yield in our study was higher in ML than in C ($P < 0.05$). These findings are in accordance with other studies, e. g. Seymour et al. (1990) or Robinson et al. (1999). Although milk fat concentration in C was higher ($P < 0.05$) than in L or M, none of the means differed from that in ML ($P > 0.05$). This is in agreement with e. g. Donkin et al. (1989).

Published results desribing the effect of RP Lys, Met or both on the FA profile of milk are scarce and inconsistent. In our study, content of total short-chain FA was not affected by the treatment except of L that was lower than C ($P < 0.05$). Our findings are in disagreement with Pisulewski et al. (1996) who reported linearly increased proportion of short-chain FA with graded amounts of Met or with Guinard and Rulquin (1994) who found small but significant increases in the concentrations of short-chain FA in milk with graded amounts of infused Lys. On the other hand, similarly to our study, no effect of RP Met + Lys on short-chain SFA was reported in studies of Canale et al. (1990) or Christensen et al. (1994). Total content of medium-chain FA in M was lower in comparison to C, L or ML ($P < 0.05$). This depression was caused by significant decreases in proportion of C 14:0 and C 16:0 in M compared to other treatments ($P<0.05$). The decline in the content of C 14:0 in M compared to C ($P < 0.05$) found in our study is in disagreement with Pisulewski et al. (1996) who reported that the proportion of C 14:0 continued to increase linearly over the range of Met infusions. On the other hand, our findings that supplementation of RP Met resulted in significant decrease in C 16:0 proportion in M compared to C is in accordance with the above mentioned study. Content of medium chain FA in our study was not influenced by the Lys or Met + Lys supplementation. Similar findings reported also Canale et al. (1990), Guinard and Rulquin (1994) or Christensen et al. (1994). Content of total long-chain FA in M was increased in comparison with treatments C, L or ML ($P < 0.05$) mainly due to differences in stearic acid (C 18:0) content. In our study, proportion of C 18:0 was not affected by the treatment except of that in L that was lower than in other treatments ($P < 0.05$). This is in disgreement with the Guinard and Rulquin (1994) who did not find any effect of Lys infusion on stearic acid content. Furthermore, Pisulewski et al. (1996) who studied effect of graded infusions of Met described increases in C 18:0 over the first four infusions (0 to 18 g/d Met) followed by a decrease (24 g/d Met) indicating the significant quadratic effect of the treatment. Although nonsignificant, there was a tendency to increased proportion of C 18:0 in M in our study. Similar effect of RP Met described also Rulquin et al. (2006). According to Canale et al. (1990) or Christensen et al. (1994) addition of RP Met + Lys did not affect the content of C 18:0 in milk fat. Similar results were also found in our study.

The sum of C 18:1 FA in M was significantly higher than in C. This is in dicrepancy with Pisulewski et al. (1996) who determined linear decrease in C 18:1 with graded levels of dudenal infusions of Met and with Rulquin et al. (2006) who did not find any effect of various forms of RP Met on C 18:1. Proportion of C 18:1 in L was higher compared to C ($P < 0.05$) while sum of C 18:1 in ML was not affected by the treatment ($P > 0.05$). These findings are in accordance with Canale et al. (1990), Guinard and Rulquin (1994) or Christensen et al. (1994). Proportion of C 18:2 was not affected by the treatment except of that in M which was higher compared to other treatments ($P < 0.05$). This is in agreement with Canale et al. (1990), Guinard and Rulquin (1994) or Christensen et al. (1994). On the other hand, according to Pisulewski et al. (1996) content of C 18:2 FA was not influenced by graded levels of duodenally infused Met which is in discrepancy with our findings. The inconsistency of the results may arise from the role of mentioned AA in FA metabolism. FA in milk fat can be divided into different groups, based on the metabolic pathways of their production. Short- and medium-chain FA (C 4:0–C 14:0 and approximately half of C 16:0) are synthesised *de novo* in the mammary gland from two major sources – β-hydroxybutyrate and particularly acetate originating from ruminal fermentation. The remaining C 16:0 and almost all of the long-chain milk FA (C 18:0–C 22:0) originate from circulating blood lipids, after absorption from the small intestine or mobilization from adipose tissue (Craninx et al., 2008). Dietary FA are absorbed as NEFA (nonesterified fatty acids) by the enterocytes to be transported in the plasma. NEFA originating from body reserve mobilization can also be taken up by the mammary gland. Thus, at the various metabolic levels, it is intestinal absorption, lipomobilization and mammary uptake, lipid flows consist of NEFA flows (Glasser et al., 2007). Lipids are transported as lipoproteins, and research indicates that very low density lipoproteins (VLDL) are a major

pathway for lipid transport into the mammary gland (Glascock and Welch, 1974). Met, an important precursor in protein synthesis, serves as a methyl donor for transmethylation reactions in the biosynthesis of lipids and other compounds and is involved in lipid transport in the blood (Lundquist et al., 1985). Furthermore, methionine as a possible limiting AA (together with lysine) for apolipoprotein B synthesis, has been used for stimulating VLDL secretion and milk yield. Based on the results of trial on high yielding dairy cows equipped with catheters in portal and hepatic veins, it was proven (Durand et al., 1992) that perfusion of Met and Lys in a mesenteric vein stimulated the net hepatic output of VLDL during early lactation. If protein or amino acids are inadequate, then lipoprotein synthesis may be decreased resulting in reduced transport of precursors to the mammary gland. Addition of RPAA may have increased lipoprotein synthesis, causing enhanced movement of dietary lipid to the mammary gland (Canale et al., 1990). Thus, according to e. g. Chow et al. (1990), supplying limiting amino acids could improve the ability of liver to synthesize lipoproteins necessary for lipid transport to the mammary gland and other tissues.

CONCLUSION

Under described feeding conditions, supplementation of the diet with rumen-protected Met or Lys alone caused a decrease in milk fat content and yield in comparison to control or supplementation with Met+Lys. Milk fatty acid profile was positively influenced by Met supplementation that resulted in significant increase in unsaturated fatty acids (both, mono- and polyunsaturated) and decrease in saturated fatty acids in milk.

SUMMARY

The objective of this study was to determine the effect of supplemental Lysine (Lys), Methionine (Met) or both (Met and Lys) added in the form of rumen-protected (RP) tablets to diet of dairy cows on fatty acids (FA) profile of milk. The present experiment is an extension of a previous study of Třináctý et al. (2009). Four high-yielding lactating Holstein cows (lactation 2–5) with similar milk yield were used in this study. Experiment, in the form of Latin square design, was divided into 4 periods of 14 d (10-d preliminary period and a 4-d experimental period). The four treatments were as follows: C – control without AA supplementation, L – control plus supplement of RP Lys, M – control plus supplement of RP Met and ML – control plus supplement of RP Met and Lys. Cows were fed individually twice daily (07:00 and 17:00 h) *ad libitum* the diet based on maize silage (346 g/kg), lucerne hay (86 g/kg) and supplemental mixture (568 g/kg). The diets were found to be deficient in Met (cca 26%) and Lys (cca 5%) that were applied in the form of RP tablets resulting in daily intakes of Met = 18.2 g in M, Lys = 11.7 g in L and that of Met and Lys = 18.2 and 11.7 g, respectively in ML. Tablets were applied during the whole period (14 d) twice a day immediately before feeding by mixing tablets into the part of supplemental mixture. As a criteria of response milk FA profiles were determined using a Hewlett-Packard 5890 gas chromatograph. Data were analysed using GLM procedure of the Statgraphics 7.0 package according to the following model: $Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl}$ where μ = general mean, T_i = effect of treatment (i = 4), C_j = effect of cow (j = 4), P_k = effect of period (k = 4), D_l = effect of day of sampling (l = 4) and ε_{ijkl} = error term.

The intake of dry matter (DM) in M (21.39 kg/d) and ML (21.64 kg/d) was higher compared to C or L (20.83 or 20.73 kg/d, respectively, $P < 0.05$). Milk yield in ML (34.18 kg/d) was higher than in L or M (32.46 or 32.13 kg/d, respectively, $P < 0.05$) and tended to be higher than in C (33.33 kg/d, $P > 0.05$). Protein yield in ML (1054 g/d) was higher than that found in C, L or M (990, 998 or 968 g/d, respectively, $P < 0.05$). Milk fat yield in C (1162 g/d) and ML (1110 g/d) was higher in comparison to L and M (963 or 965 g/d, respectively, $P < 0.05$). Content of short-chain FA (C 4:0–C 12:0) was not affected by the treatment except of L that was lower than in C ($P < 0.05$). Content of medium-chain FA in M was lower compared to C, L or ML ($P < 0.05$). The total content of long-chain FA in M was significantly higher than in other groups ($P < 0.05$). Content of C 18:0 in L was significantly lower than in C, M or ML ($P < 0.05$). The sum of C 18:1 in M was higher than in C and ML ($P < 0.05$) and tended to be higher than in L. Proportion of C 18:2 in M was higher compared to other treatments ($P < 0.05$). Proportion of C 18:3 in M and L was higher compared to C ($P < 0.05$). Content of saturated fatty acids (SFA) in M (61.85 g/100g) was the lowest and differed significantly from the C (65.80 g/100g) and ML (64.83 g/100g, $P < 0.05$). The total content of unsaturated fatty acids (UFA) in M (38.15 g/100g) was higher than in C or ML (34.20 or 35.13 g/100g, respectively, $P < 0.05$) and tended to be higher than in L ($P > 0.05$). Content of mono-unsaturated fatty acids (MUFA) in M was higher than those in C and ML ($P < 0.05$). Similarly, content of (poly-unsaturated fatty acids) PUFA in M was higher compared to C, L or ML ($P < 0.05$).

SOUHRN

Vliv doplňkového ruminálně chráněného lysinu, methioninu nebo obou do krmné dávky laktujících dojnic na skladbu mastných kyselin mléka

Cílem práce bylo určit vliv doplňkového lysinu (Lys), methioninu (Met) nebo obou (Met a Lys) přidaných ve formě ruminálně chráněných (RP) tablet do krmné dávky laktujících dojnic na profil mastných kyselin (FA) v mléčném tuku. Uvedený pokus je rozšířením předchozí práce (Třináctý et al., 2009). V pokusu byly použity čtyři vysokoprodukční laktující holštýnské dojnice (2.–5. laktace) s podobnou užitkovostí. Pokus byl proveden ve formě Latinského čtverce a byl rozdělen do čtyř period, každá v délce čtrnáct dní, tj. deset dní přípravné období a čtyři dny experimentální období. Pokusné faktory byly následující: C – kontrola bez doplňku aminokyselin, L – kontrola + doplněk RP Lys, M – kontrola + doplněk RP Met a ML – kontrola + doplněk RP Met a Lys. Dojnice byly krmeny individuálně, dvakrát denně (07:00 a 17:00 h) *ad libitum* krmnou dávkou složenou z kukuřičné siláže (346 g/kg), vojtěškového sena (86 g/kg) a doplňkové krmné směsi (568 g/kg). U této krmné dávky byl zjištěn deficit Met (cca 26 %) a Lys (cca 5 %), který byl uhrazen ve formě RP tablet tak, aby byl denní příjem Met = 18,2 g ve skupině M, Lys = 11,7 g ve skupině L a Met a Lys = 18,2 g, resp. 11,7 g, ve skupině ML. Tablety byly dojnicím podávány během celé periody (tj. čtrnáct dní) dvakrát denně bezprostředně před nakrmením tak, že byly zamíchány do části doplňkové krmné směsi. Jako kritérium odezvy byl sledován profil mastných kyselin v mléce, který byl stanoven na plynovém chromatografu Hewlett-Packard 5890. Získaná data byla analyzována pomocí programu Statgraphics 7.0 podle následujícího modelu: $Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl}$ kde μ = průměr souboru, T_i = vliv pokusného zásahu (i = 4), C_j = vliv dojnice (j = 4), P_k = vliv periody měření (k = 4), D_l = vliv dne měření (l = 4) a ε_{ijkl} = reziduální chyba.
Příjem sušiny u M (21,39 kg/d) a ML (21,64 kg/d) byl vyšší ve srovnání s C nebo L (20,83, resp. 20,73 kg/d, $P < 0,05$). Mléčná užitkovost u ML (34,18 kg/d) byla vyšší než u L nebo M (32,46, resp. 32,13 kg/d, $P < 0,05$) a měla tendence k vyšším hodnotám než u C (33,33 kg/d, $P > 0,05$). Produkce proteinu u ML (1054 g/d) byla vyšší než jaká byla zjištěna ve skupinách C, L a M (990, 998 a 968 g/d, $P < 0,05$). Produkce mléčného tuku u C (1162 g/d) a ML (1110 g/d) byla vyšší ve srovnání s L a M (963 a 965 g/d, $P < 0.05$). Obsah FA s krátkým řetězcem (C 4:0–C 12:0) nebyl pokusným zásahem ovlivněn s výjimkou skupiny L, kde byl nižší než u C ($P < 0,05$). Obsah FA se středně dlouhým řetězcem u skupiny M byl nižší ve srovnání se skupinami C, L a ML ($P < 0,05$). Celkový obsah FA s dlouhým řetězcem u M byl průkazně vyšší než v ostatních skupinách ($P < 0,05$). Obsah C 18:0 u skupiny L byl průkazně nižší než u C, M a ML ($P < 0,05$). Suma C 18:1 u skupiny M byla vyšší než u C nebo ML ($P < 0,05$) a měla tendenci k vyšším hodnotám než u L. Podíl C 18:2 ve skupině M byl vyšší ve srovnání s ostatními skupinami ($P < 0,05$). Podíl C 18:3 u M a L byl vyšší než u skupiny C ($P < 0,05$). Obsah nasycených mastných kyselin (SFA) ve skupině M (61,85 g/100g) byl nejnižší a průkazně se lišil od skupin C (65,80 g/100g) a ML (64,83 g/100g, $P < 0,05$). Celkový podíl nenasycených mastných kyselin (UFA) u M (38,15 g/100g) byl vyšší než u C nebo ML (34,20 resp. 35,13 g/100g, $P < 0,05$) a měl tendenci k vyšším hodnotám než ve skupině L ($P > 0,05$). Obsah mononenasycených mastných kyselin (MUFA) ve skupině M byl vyšší než ve skupinách C a ML ($P < 0,05$). Podobné výsledky byly zjištěny i u vícenenasycených mastných kyselin (PUFA), jejichž obsah byl vyšší u M ve srovnání se skupinami C, L nebo ML ($P < 0,05$).

mastné kyseliny, ruminální ochrana, holštýnské dojnice, mléko, lysin, methionin

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