Transfer of different exogenous fatty acids into milk and muscle in dairy cows

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Abstract— **This study investigated the effect of three different dietary fatty acids (FA) supplements on milk and muscle total fatty acid composition of German Holstein cows (first lactation, 92 days in milk, n=18). Ten weeks feeding of saturated, *n*-3 and *n*-6 enriched fat (3.1% of DM) affected the milk fat yield, fatty acid composition of milk and muscle. Exogenous *n*-3 fatty acid supply (2.7 % linseed oil+ 0.4 % DHA algae of DM) caused significantly higher concentration of *n*-3 PUFA in milk and intramuscular fat. Exogenous *n*-6 FA supplies (2.7 % sunflower oil of DM) and 0.4 % DHA algae increased linoleic acid only in muscle. Feeding plant oil/algae to dairy cows decreased significantly the content of saturated FA in milk. Contrary to this there was a tendency for a higher deposition of SFA in muscle fat.**

Keywords— **fatty acid, muscle, milk, cow.**

I. INTRODUCTION

Milk, meat and their products are significant sources of *n*-3 and *n*-6 PUFA, C18:1*trans*-11, and conjugated linoleic acid isomers (CLA) which have several benefits to human health (Given & Gibbs, 2008; Mann 2005; Benjamin & Spener, 2009). Beside factors like breed, gender, growth or season, the diet composition, and the ruminal microbial metabolism of fatty acids (FA) are effective to change the quality of milk and meat (Raes et al. 2004; Chilliard et al. 2007; Woods & Fearon, 2009). Grass/grass silage/legumes and concentrate supplemented with plant oils, fish oil, linseed, rapeseed or algae increased the *n*-3 fatty acid concentration and the relative amount of C18:1*trans*-11(TVA) and CLA*cis*-9,*trans*-11 (CLA) in beef (Chilliard et al. 2007; Wachira et al. 2002; Warren et al. 2008). Beside the increase of these unsaturated fatty acids, the concentration of saturated fatty acid was decreased in muscle (Dannenberger et al. 2006). It has been reported a significant increase of CLA and TVA in milk fat using soybean oil, sunflower oil, linseed oil or fish oil (Chouinardet et al. 2001; Loor et al. 2005; Harvatine & Bauman 2006).

The aim of the study was to evaluate the effect of different fat supplements (sunflower oil plus algae or linseed oil plus algae supplementation *vs* rumen protected saturated fat) for 10 weeks on the milk production, milk fat, and on fatty acid composition of meat and milk in cows of the first lactation with special focus on *n*-3 fatty acids, CLA and TVA.

A. Material and methods

In total 18 German Holstein cows in their first lactation (92th days in milk, DIM) were included in the feeding experiment. The basal diet consisted of grass silage, maize silage, hay, concentrate, and mineral feed mixture. Three treatments (6 cows per group) were formulated: SAT group with supplementation of rumen-stable fractionated palm fat (3.1 % of the basal diet DM), LINA group with linseed oil (2.7 % DM) and algae rich in DHA (0.4 % DM), and SUNA group with sunflower oil (2.7 % DM) and algae rich in DHA (0.4 % DM) to the same basal ration conforming the total mixed ratio (TMR). Diet nutrient composition differed between treatment groups only by the fatty acid profile of the supplemented fat and was isoenergetic and isonitrogenous calculated. Cows had free access to water. Milk yield was recorded daily. Cows were slaughtered after 10 weeks. Aliquots of the total morning milk were taken at day before slaughter for the lipid analyses and kept at -20 °C. Muscle samples were taken immediately after slaughter (25 minutes) from the right carcass side and stored at -20°C until lipid analyses. Milk and muscle fatty acid profile were analysed according to the method used by Nuernberg et al. (2010) and Duske et al. (2009),

respectively. All data were analysed by the least-squares means method using GLM procedures of SAS.

B. Results

The dietary supplements increased in tendency (not statistically significant) the saturated fatty acid concentration (all single saturated FA as well as the sum of SFA) of intramuscular fat in *longissimus* muscle (Tab. 1). The concentration of the TVA and the sum of all C18:1*trans* isomers were higher in sunflower group compared to SAT and LINA group. Linoleic acid was highly accumulated in the SUNA group. Linolenic acid and the sum of *n*-3 FA were highest deposited in the LINA group. DHA showed the most abundant content in both plant oil/algae fed groups compared to SAT.

Table 1 FA composition (mg/100g) of *longissimus* of German Holstein cows

Fatty acids	SAT LSM	LINA LSM	SUNA LSM
Muscle fat (%)	3.0	4.1	3.5
C12:0	1.4	2.8	2.3
C14:0	60.5	120.1	107.7
C16:0	643.5	1095.6	1062.6
C16:1	97.9	170.8	152.8
C18:0	292.3	417.2	409.6
Σ C18:1*trans*	24.3^{a}	107.9^{b}	119.5^{b}
C18:1*trans*-11	14.0^{a}	35.5^{a,b}	66.4^{b}
C18:1*cis*-9	798.6	1260.7	1075.1
C18:2*n*-6	69.7^{a}	78.2^{a}	98.8^{b}
C18:3*n*-3	15.6^{a}	34.5^{b}	20.6^{a}
C20:4*n*-6	26.4^{a}	20.7^{b}	20.6^{b}
C20:5*n*-3	7.7	9.0	8.2
C22:5*n*-3	14.3^{a}	12.2^{a,b}	10.5^{b}
C22:6*n*-3	1.1^{a}	10.2^{b}	12.0^{b}
CLA*t*10,*c*12	0.6	0.9	0.8
CLA*c*9,*t*11	7.8^{a}	20.9^{b}	16.6
Σ SFA	1030.7	1690.1	1633.0
Σ MUFA	1026.1	1785.7	1593.5
Σ *n*-3 FA	40.5^{a}	68.7^{b}	54.2^{a,b}
Σ *n*-6 FA	111.3	111.1	131.2
n-6/*n*-3 FA	2.8^{a}	1.6^{b}	2.5^{a}

a,b –Different letters indicate significant differences between feeding groups at P≤ 0.05

The significant lowest *n*-6/*n*-3 FA ratio in muscle lipids was calculated in the linseed oil/algae fed cows. Oleic acid was only in tendency (not significant) higher accumulated in muscle from cows fed plant oil/algae diets.

The milk fat content and all single saturated FA as well as the sum of SFA in the milk were significantly reduced by feeding plant oils/algae supplements (Tab. 2). This is contrary to the muscle fatty acid concentration.

Table 2 FA composition (mg/100g) of milk of cows

Fatty acids	SAT LSM	LINA LSM	SUNA LSM
Milk fat (%)	3.8^{a}	2.5^{b}	2.2^{b}
C10:0	89.0^{a}	27.6^{b}	23.7^{b}
C12:0	117.7^{a}	42.3^{b}	39.0^{b}
C14:0	384.8^{a}	186.5^{b}	171.0^{b}
C16:0	1157.2^{a}	451.8^{b}	420.9^{b}
C16:1	56.0	28.7	34.9
C18:0	248.1^{a}	179.1^{b}	172.2^{b}
Σ C18:1*trans*	116.6^{a}	152.1^{a}	214.2^{b}
C18:1*trans*-10	19.3^{a}	21.3^{a}	32.5^{b}
C18:1*trans*-11	45.0^{a}	79.4^{b}	119.4^{c}
C18:1*cis*-9	595.8^{a}	453.0^{b}	428.3^{b}
C18:2*n*-6	62.3^{a}	36.2^{b}	66.1^{a}
C18:3*n*-3	13.9^{a}	19.9^{b}	8.2^{c}
C20:4*n*-6	4.9^{a}	1.6^{b}	1.6^{b}
C20:5*n*-3	1.1^{a}	0.9^{a}	0.7^{b}
C22:5*n*-3	3.0^{a}	1.7^{b}	1.4^{b}
C22:6*n*-3	0.5^{a}	2.5^{b}	2.6^{b}
CLA*t*10,*c*12	0.4^{a}	0.6^{a}	1.2^{b}
CLA*c*9,*t*11	29.5	30.4	27.7
Σ SFA	2070.6^{a}	926.7^{b}	865.5^{b}
Σ MUFA	869.2	731.7	766.3
Σ *n*-3 FA	21.2^{a}	27.3^{b}	15.2^{c}
Σ *n*-6 FA	73.0^{a}	40.1^{b}	70.2^{a}
n-6/*n*-3 FA	3.6^{a}	1.5^{b}	4.6^{c}

a,b,c – Different letters indicate significant differences between feeding groups at P≤ 0.05

In line with muscle the content of the TVA and the sum of all C18:1*trans* isomers were also higher in LINA and SUNA groups compared to SAT group. Feeding linseed and sunflower oil with algae caused a significant reduced production of oleic acid in milk. In milk linseed oil/algae supplement caused an increase of the linolenic acid concentration and the sum of *n*-3 FA whilst SUNA only increased the C18:2*n*-6 but not the sum of *n*-6 FA concentration in milk fat.

C. Discussion

Muscle composition

The effects of dietary fat on muscle lipids in beef cattle and dairy cows have been intensively and separately investigated (Nuernberg 2009; Scollan et al. 2006; Dewhurst et al. 2006; Chilliard et al. 2007). Ten weeks feeding 3.1 % plant oils/algae supplements induced a higher deposition of C18:1*trans* isomers. LINA fed cows accumulated more linolenic acid and *n*-3 FA whilst C18:2*n*-6 concentration in the muscle fat was higher in SUNA group. Sunflower oil/algae feeding induced the highest accumulation of C18:1*trans*-11 (mg/100 g and %). The Δ^9 desaturase converts this fatty acid to CLA*cis*-9,*trans*-11 in different tissues. In the present study there was a correlation between C18:1*trans*-11 concentration and the CLA*cis*-9,*trans*-11 in muscle fat r= 0.64. Restricted grazing with 3 % supplementation of sunflower oil or linseed oil to beef heifers for 158 days increased the proportion of C18:1*trans*-11 and CLA*cis*-9,*trans*-11 in muscle lipids and subcutaneous adipose tissue whereas sunflower oil was more effective (Noci et al. 2007). Saturated fatty acid concentration in muscle was only in tendency higher in plant oil/algae fed cows in the present study. Contrary to cows feeding grazing beef heifers with linseed or sunflower oil decreased the SFA percentage in muscle fat (Noci et al. 2007).

The higher C18:3*n*-3 and DHA concentration, and the lower *n*-6/*n*-3 ratio represent a nutritional improvement of the fatty acid profile in muscle of linseed oil/algae fed dairy cows compared with muscle fat of saturated or sunflower group. Plant oils and algae supplementation did not increased the PUFA concentration in muscle lipids. Noci et al. (2007) reported a higher PUFA/SFA ratio in muscle and subcutaneous fat in beef heifers caused by higher PUFA and decreased SFA percentage. There seems to be differences in lipid metabolism in muscle of heifers and cows.

Milk composition

Supplementation of diet with polyunsaturated plant oils in combination with algae decreased significantly the milk fat content compared to SAT. Shingfield et al. (2009) reported a milk fat decrease (MFD) in tendency in 750 g/d sunflower oil supplementation to diet for dairy cows. Milk fat percentage was also lower in cows fed 5 % linseed oil or 21 % extruded linseed compared to linseed meal or crude linseed (Chilliard et al. 2009). The theories for the dietary milk fat decrease extend from low acetate and butyrate production, increased propionate concentration, enhanced hepatic gluconeogenesis and adipose tissue lipogenesis to an inhibition of the mammary lipid synthesis by specific *trans* fatty acids (Bernard et al., 2008; Bauman et al., 2003). In general factors have been associated to the alteration of the ruminal biohydrogenation and its shift towards to the production of intermediates that cause MFD (Lock et al. 2006). A lower rate of lipogenesis in the mammary gland seems to be a result of the PUFA abundant plant oils to a starch-rich diet (Harvatine et al., 2009). In the present study, all TMR had around 55% of roughage, and the fibre, structural value, and starch intake was similar among diets. However, it was not enough to avoid MFD. Probably, more attention should be focused not only in the amount of roughage, but also on the roughage type as it has been mentioned before by Shingfield et al. (2005). The increase of the proportion of C18:1*trans*-10 (SAT: 0.63 % *vs* 1.26 % in linseed group or 1.9 % in SUNA group) in the milk indicates in the present study the shift of rumen biohydrogenation.

In contrast, other studies have reported an increase of milk fat in goats induced by plant oil supplementation for 28 days (Ollier et al., 2009; Bernard et al., 2009) showing differences in the regulation of mammary lipogenesis between ruminants species. The inclusion of unsaturated fat in dairy cows diets inhibited the *de novo* synthesis of medium-chain fatty acids (Chilliard et al., 2007; Cruz-Hernandez et al., 2007). In line with the lower milk fat content it has been shown a reduced concentration of *de novo* synthesised saturated fatty acids in the milk (C10:0, C12:0, C14:0 and C16:0) by supplementation of 2.7 % plant oil and 0.4 % algae compared to the saturated diet.

Between CLA*cis*-9,*trans*-11 (%) and C18:1*trans*-11 (%) in milk was also a significant correlation calculated (r= 0.6). Only sunflower/algae feeding increased the CLA*trans*-10,*cis*-12 concentration but the proportion of this FA is higher in both plant oil/algae groups (SAT: 0.01 %; LINA: 0.04%; SUNA: 0.07%). Cruz-Hernandez et al. (2007) reported also a

very high percentage of total C18:1*trans* and CLA isomers by feeding 3 % sunflower and 0.5 % fish oil (13.7 and 2.36 %, respectively) which in the present study were also measured at a similar level (12.4 % and 1.67 % only CLA*cis*-9,*trans*-11, CLA*trans*-8,*cis*-10, CLA*trans*-7,*cis*-9, CLA*trans*-10,*cis*-12 by GC).

The exogenous linseed oil/algae supply induced a significantly higher C18:3*n*-3 and DHA concentration and lower C18:2*n*-6 content in milk fat. Loor et al. (2005) reported also a higher linolenic acid yield and lower C18:2*n*-6 yield in milk of Holstein cows fed 3 % linseed oil supplementation to a high concentrate diet. The sunflower oil/algae addition caused a higher C18:2*n*-6 content (mg/100g) compared to linseed oil group but not to SAT. Contrary to the quantitative content, the relative proportion of C18:2*n*-6 in milk fat was significantly increased by feeding sunflower oil/algae. Similar results were measured by Cruz-Hernandez et al. (2007) and Ollier et al. (2009).

II. CONCLUSIONS

There are tissue specific differences in the *de novo* synthesis of SFA between milk and muscle. In tendency the total SFA and oleic acid C18:1*cis*-9 were increased in muscle but decreased in milk fat of cows fed plant oil/algae. Further investigations should be focused on the protein and gene expression of lipogenic enzymes in mammary gland and adipocytes in different tissues.

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REFERENCES

1. Givens DI, Gibbs RA (2008) Proc Nutr Soc, 67 (3):273-280.
2. Mann N J (2005) Lipid Technolology, 17 (4):79-82.
3. Benjamin S, Spener F (2009) Nutr & Metab, 6:11-13.
4. Raes K, DeSmet S, Demeyer D (2004) Anim Feed Sci Technol, 113:199-221.
5. Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M (2007) Europ J Lipid Sci Technol, 109:828-855.
6. Woods VB, Fearon AM (2009) Livestock Science, 126:1-20.
7. Wachira AM, Sinclair LA, Wilkinson RG, Enser M, Wood JD, Fisher AV (2002) Brit J Nutr, 88:697-709.
8. Warren H E, Scollan ND, Enser M, Hughes SI, Wood JD, Richardson RI (2008) Meat Sci, 78:270–278.
9. Dannenberger D, Nuernberg K, Nuernberg G, Ender K (2006) Archives Animal Breeding, 49:315-328.
10. Chouinard PY, Corneau J, Butler WR, Chilliard Y, Drackley J K, Bauman DE (2001) J Dairy Sci, 84:680–690.
11. Loor JJ, Ferlay A, Ollier A, Doreau M, Chilliard Y (2005) Journal Dairy Science, 88:726-740.
12. Harvatine K, Bauman DE (2006) J Nutr, 136:2468-2474.
13. Nuernberg K, Nuernberg G, Dannenberger D (2010) Fleischwirtschaft, 10:99-102.
14. Duske K, Hammon HM, Langhof AK, Bellmann O, Losand B, Nürnberg K, Nürnberg G, Sauerwein H, Seyfert HM, Metges CC (2009) J Dairy Sci, 92:1670-1684.
15. Nuernberg K (2009) In: Ed. Kerry, J.P.; Ledward, D. *Improving the sensory and nutritional quality of fresh meat*. Woodhead Publishing, Cambridge, England, 2009, pp.321-341.
16. Scollan N, Hocquette JF, Nuernberg K, Dannenberger D, Richardson I, Moloney A (2006) Meat Science, 74:17-33.
17. Dewhurst RJ, Shingfield KJ, Lee MRF, Scollan ND (2006) Animal Feed Science and Technology, 131:168-206.
18. Noci F, French P, Monahan FJ, Moloney AP (2007) Journal Animal Science, 85:1062-1073.
19. Shingfield KJ, Ahvenjärvi S, Toivonen V, Vanhatalo A, Huhtanen P, Griinari JM (2009) Brit J Nutr, 99:971-983.
20. Chilliard Y, Martin C, Rouel J, Doreau M (2009) J Dairy Sci, 92:5199-5211.
21. Bernard L, Leroux C, Chillard Y (2008) Advances of Experimental Medicine and Biology, 606:67-108.
22. Bauman DE, Corl BA, Peterson DG (2003) In: *Advances in Conjugated Linoleic Acid Research*. 2, J.-L. Sebedio, W. W. Christie, & R. O. Adlof, ed., AOCS Press, Champaign, IL, pp.146-173.
23. Lock A, Overton T, Harvatine K, Giesy J, Bauman D (2006) Proceedings Cornell Nutrition Conference, pp. 75-85.
24. Harvatine KJ, Boisclair YR, Bauman DE (2009) Animal, 3:40-54.
25. Shingfield KJ, Reynolds CK, Lupoli B, Toivonen V, Yurawecz MP, Delmonte P, Griinari JM, Grandison AS, Beever DE (2005) Animal Science, 80:225-238.
26. Ollier S, Leroux C, de la Foye A, Bernard L, Rouel J, Chillard Y (2009) J Dairy Sci, 92:5544-5560.
27. Bernard L, Leroux C, Faulconnier Y, Durand D, Shingfield KJ, Chilliard Y (2009) J Dairy Res, 76:241-248.
28. Cruz-Hernandez C, Kramer JKG, Kennelly JJ, Glimm DR, Sorensen BM, Okine EK, Goonewardene LA, Weselake RJ (2007) J Dairy Sci, 90:3786-3801.