Lipid Metabolism Gene Expression Profiles Correlated With Fatty Acids In The Milk Of Dairy Cows Fed Unprotected Unsaturated Fatty Acids

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Introduction

Supplementing the total mixed ration (TMR) diets with different unsaturated fatty acids (UFA) may improve milk fat composition for human consumption (Harvatine and Bauman, 2006). The effect of these dietary UFA on the resulting milk fatty acid (FA) composition and expression of genes in the mammary gland involved in lipid metabolism has recently been studied (Bauman et al., 2008; Bionaz and Loor, 2008). However, information on the relationship between lipid metabolism gene networks in the mammary gland tissue and the resultant milk FA composition of grazing dairy cows supplemented with dietary UFA, is lacking. Therefore, the objective of the present study was to correlate the expression of lipid metabolism genes in the mammary gland tissue affected by unprotected dietary unsaturated fatty acids to the resulting milk fatty acids composition in grazing dairy cows, and to classify milk fatty acid groups based on variations in lipid metabolism gene expression patterns.

Material and methods

Animals and diets. Twenty-eight Holstein-Friesian dairy cows were randomly assigned to 4 concentrated UFA-sources based on unprotected rapeseed oil, soybean oil, linseed oil, or a proportional mix of them all for 23 days, after which all cows were switched to a non-UFA-supplemented concentrate for an additional 28 days. The cows were grazing on pasture during the day and were fed the TMR, based on 52% corn silage, 12% grass silage and 36% concentrate, indoors at night. On the last day of both periods, mammary gland biopsies were taken to study genome-wide differences in gene expression on Affymetrix GeneChip® Bovine Genome Arrays, and milk samples were stored at -20ºC until analysis for FA composition by gas chromatography. Microarray data was validated by confirming the altered expression of 5 key genes using quantitative qPCR

Statistical analyses. Milk fatty acid composition and gene expressions were analyzed using a mixed-effects model with repeated measures (SAS Inst. Inc. Cary, NC, release 9.1). The model included UFA-sources, UFA supplementation (experimental periods), and their interaction as fixed effect, and cow within pen as a random effect. Period was considered a repeated factor, and for each analyzed variable, cow was subjected to a compound symmetry

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variance-covariance structure. The same model was performed to analyse gene expression using a mixed-effects ANOVA (MAANOVA package of R, release 1.16). The false discovery rate (FDR) method of 5% (q-value < 0.05) was used as a threshold for significance of differential expression. The Ingenuity Pathways Analysis (IPA; ver. 5.5, Ingenuity Systems, Redwood City, CA) allowed us to identify the genes involved in relevant lipid metabolism canonical pathways, and their biological interaction networks. Further, to classify and visualize milk FA groups based on variations in lipid metabolism gene markers, or gene expression patterns, regularized canonical correlation Analysis (rCCA) from the mixOmics package of R (release 2.7) was applied. The function CIMs (Clustered Image Maps) of R was used to generate color-coded clustered image maps with a dendrogram added to the left side and to the top. In order to test the significance of the correlations described, a linear model was fitted to the expression genes for each fatty acid using the Bioconductor's Linear Model (lmFit).

Results and discussion

Supplementation of dietary UFA decreased (P < 0.001) the production of the short chain fatty acids (SCFA), the 16-carbon FA, the saturated fatty acids (SFA), and the omega-3 and omega-6 FA, and increased the production of *trans*- FA in the milk (Table 1), specifically, the production of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA. Therefore, we suggest that supplementing the TMR diets of dairy cows with dietary UFA may help to improve the health and nutrition quality aspects of dairy milk, and thereby increasing the positive effects on human health when consumed. Applying a statistical cut-off of FDR q-values < 0.05, and FC=1.3, we identified a total of 78 lipid metabolism-related genes differentially expressed in the mammary gland tissue when supplementing grazing dairy cows with UFA compared with when cows were fed with non-UFA-enriched concentrate. Most of them were involved in phospholipids degradation and glycerophospolipid metabolism, FA metabolism, FA biosynthesis, import, trafficking, and droplet secretion. Gene network analysis of this 78 lipid metabolism-related genes identified two major nodes affected by consumption of UFA: SREBP1 and PPARG, which are well-known regulators of the lipid metabolism (Figure 1A). The most prominent result was the ability of hierarchical clustering analysis to classify FA groups by identifying gene expression patterns of lipid metabolism genes. Of particular interest is the finding that FA groups could be classified into two main branches based solely on differences in these gene expression patterns (Figure 1B). One of the branches included all the *trans*-FA, suggesting that *trans*-FA may have potential strong effect on lipid metabolism genes in the mammary gland tissue. Lastly, specific examination of linear regression analyses revealed that 15 of these lipid metabolism genes significantly contributed most to the classification of these FA groups. Of them, *SREBP1* (Sterol regulatory element binding transcription factor 1), *LPIN1* (Lipin 1), *SERPINA1* (Serpin peptidase inhibitor, clade A), *INSIG1* (insulin induced gene 1), and *ACACA* (Acetyl-Coenzyme A carboxylase alpha) suggested a key role in milk FA composition. Such relevant genes could be referred to as marker genes for fatty acid composition, although further functional knowledge will be of great importance to provide new molecular insight into lipid metabolism, and improve quality aspects of dairy milk.

Conclusion

The results of this study clearly show that supplementing grazing dairy cows with different unprotected unsaturated fatty acids reduced the production of short chain fatty acids, C16 and saturated fatty acids in the milk, whereas that of *trans*-fatty acids increased. Dietary unsaturated fatty acid supplementation affected 78 lipid metabolism-related genes involved in phospholipids degradation, glycerophospholipid metabolism, triaglyceride formation, as well as FA metabolism, import and trafficking. A novel finding was that the milk *trans*-FA presented a distinctive correlation profile to the lipid metabolism genes. Moreover, a total of 15 lipid metabolism-related genes significantly correlated to different milk FA groups, but also contributed the most to the classification of the different fatty acid groups, thus, suggesting a significant role in mediating the lipid metabolism in the mammary gland tissue and determining the milk fatty acids composition. Further functional knowledge of these genes will be of great importance in understanding the relationship between lipid metabolism genes and the resulting milk composition and quality.

Table 1: Milk fatty acid production (mg/L) when comparing dairy cows fed with UFA-enriched-concentrate relative to the same cows fed non-UFA-enriched-concentrate

	UFA-concentrate supplementation			
	Non-UFA-enriched concentrate	UFA-enriched concentrate	SEM	*P*- value[a]
Item[b]				UFAL
Short Chain Fatty Acids, mg/L	11,343	8,035	418.5	<0.001
[c]16C, mg/L	13,200	9,780	416.7	<.0001
Long-Chain Fatty Acids, mg/L	15,282	15,412	467.9	0.84
Unsaturated Fatty Acids, mg/L	12,023	12,397	349.6	0.45
Saturated Fatty Acids, mg/L	28,393	21,160	938.6	<.0001
Polyunsaturated Fatty Acids, mg/L	1,393	1,388	43.1	0.93
[d]n-3 Fatty Acids, mg/L	269.3	201.7	9.28	<.0001
[e]n-6 Fatty Acids, mg/L	721.1	652.46	20.01	0.01
Trans-octadecenoic Fatty Acids, mg/L	1,798	2,900	72.5	<0.001
cis-9, *trans*-11-CLA, mg/L	263.9	368.8	18.80	0.0002
trans-10,*cis*-12-CLA[f], mg/L	3.56	6.4	0.46	<0.0001

[a]UFAL= effect of UFA supplementation.
[b]Included n= 28 cows
[c]16C = sum of 16:0 and 16:1,*cis*-9
[d]Sum of n-3 fatty acids (18:3n-3, 20:3n-3, 20:5n-3, and 22:5n-3)
[e]Sum of n-6 fatty acids (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, and 22:2n-6)
[f]CLA = Conjugated Linoleic Acid

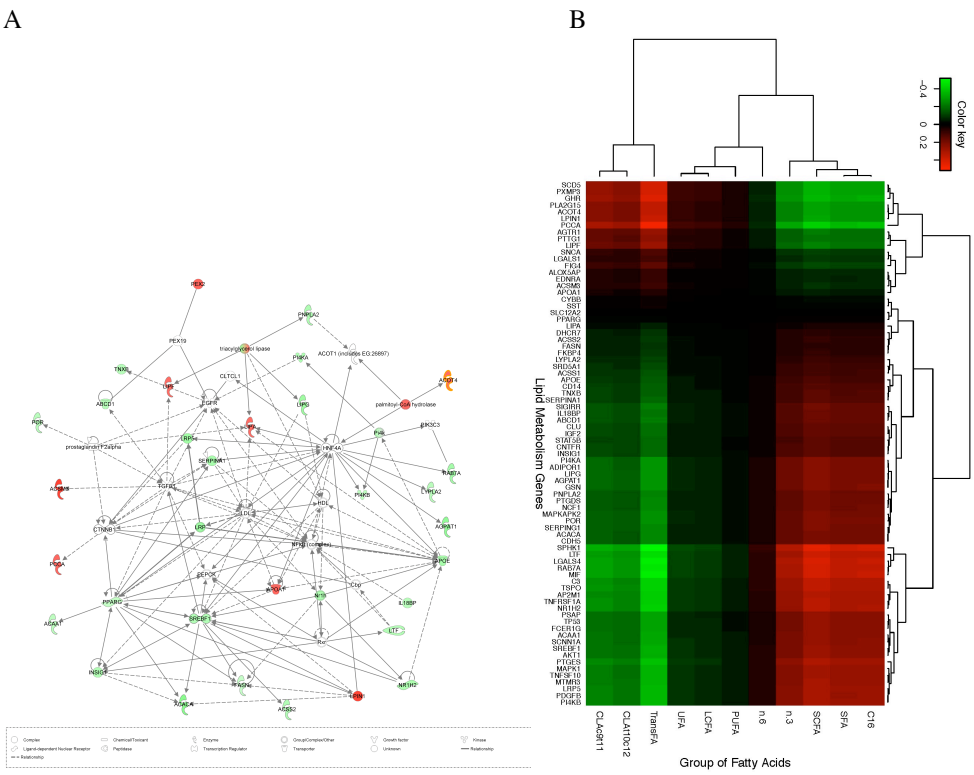

Figure 1A) IPA Network of genes involved in lipid metabolism, with a score of 58 and 30 focus genes. The node colour intensity indicates the expression of genes: red up-regulated, green down-regulated in animals fed with UFA compared with when animals were fed with control diet. B) Heat map of the chosen 78 lipid metabolism genes. The horizontal rows of the map represent genes, whereas the columns represent the different groups of FA. Each pixel represents the expression between each gene and group of fatty acids: the colours depict the coefficient of correlation from green (large negative) to red (large positive).

References

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