Bulletin UASVM, Veterinary Medicine 68(1)/2011
pISSN 1843-5270; eISSN 1843-5378

Effects of Feeding Factors and Breed on Cow Milk Fatty Acid Composition: Recent Data

Anne FERLAY, Frédéric GLASSER, Bruno MARTIN, Donato ANDUEZA, Yves CHILLIARD

INRA, UR1213 Herbivores, F-63122 Saint-Genès Champanelle, France;
anne.ferlay@clermont.inra.fr

Abstract. Manipulating cow milk fatty acid (FA) is of considerable interest to improve the health of consumers. The main targets include reducing the levels of saturated FA and trans FA in milk and increasing those of cis9-18:1, 18:3n-3, and cis9trans11 isomer of conjugated linoleic acid (CLA). The breed has only a minor influence on cow milk FA composition whereas nutrition has major effects. We review recent progress in the effects of nutrition on milk FA composition, namely pasture feeding (phenological stage, botanical diversity, and stocking density) as well as linseed and rapeseed supplementation, in interaction with the nature of forage. Finally, we report the ability of near infrared reflectance spectroscopy (NIRS) to quantify milk concentrations of the main FA groups and individual FA, opening perspectives for large-scale determination of milk FA composition.

Keywords: milk fatty acids, oilseed, forage, grass, dairy cow, breed, NIRS

INTRODUCTION

Bovine milk is composed on average of 4% fat, and 97-98 % of which are triacylglycerols (Jensen, 2002). Milk fat typically contains a high proportion of saturated fatty acids (FA) (70% of total FA), and monounsaturated FA (MUFA, 25.6 %), and small amounts of polyunsaturated FA (PUFA, 3.3 %). The *trans* FA represent approximately 4% (Ferlay *et al.*, 2008, Shingfield *et al.*, 2008). However, these average figures can be largely altered through various breeding and feeding factors.

It has been recognised for many years that diet plays a role as a risk factor for chronic disease in humans. Prospective cohort studies can identify associations between dietary fat type (like saturated FA (SFA)) and cardiovascular disease (CVD) (Givens, 2010). There is evidence that dietary SFA increase the concentrations of serum low-density lipoprotein cholesterol (Givens, 2010), a predictor of CVD risk. In many European countries, milk and dairy products supply in average ca. 40% of all SFA intake. As a result there has been a great deal of interest in manipulating the FA profile of milk fat to respond to consumer' concerns. The main MUFA in milk is oleic acid, followed by *trans* 18:1. Replacement of dietary SFA by oleic acid has been estimated to reduce CVD (Lopez-Huertas, 2010). In contrast, *trans* FA if consumed in excess have been associated with a substantially increased risk of coronary heart disease (Schingfield *et al.*, 2008; Givens, 2010). Nevertheless, recent data seem to indicate that industrial and ruminant *trans* FA have different effects on CVD risk factors (Bassett *et al.*, 2010). Even, within ruminant trans-18:1, it seems that the different isomers could have different effects on CVD risk factors (higher for *trans*10- than *trans*11-18:1) (Roy *et al.*, 2007).

Linoleic (18:2n-6) and α-linolenic (18:3n-3) acids are the main PUFA in milk fat. These FA cannot be synthesized by the body and must be obtained from the diet. These FA can be metabolized to form arachidonic and eicosapentaenoic (EPA) acids, which are precursors for the synthesis of prostaglandins and leukotrienes (Palmquist, 2009). The n-3 FA, and more particularly EPA and docosahexaenoic acid, could reduce the risk of CVD (Mills *et al*., 2011). Milk fat is also the main dietary source of conjugated linoleic acids (CLA). Although there are several isomers, health benefit effects have been mainly attributed to *cis*9,*trans*11-CLA, which has capacities to prevent cancer, hypertension, atherosclerosis and diabetes in animal models (Mills *et al*., 2011).

Milk FA have a dual origin: the long-chain FA (C16 and over) are taken up from plasma lipoproteins (on average 60%), and the short- and medium-chain FA (4:0 to 16:0) are synthesized *de novo* (on average 40%) in the mammary gland from acetate and beta-hydroxybutyrate (Chilliard and Ferlay, 2004). Moreover, the secretory mammary cells present a delta-9 desaturase activity, converting in particular stearic acid into oleic acid and *trans*11-18:1 into *cis*9*trans*11-CLA (Chilliard and Ferlay, 2004). The milk FA composition results from these metabolic pathways and from rumen metabolism (lipolysis, isomerization and biohydrogenation of dietary PUFA) (Chilliard *et al.,* 2007). The cow milk FA composition is linked to intrinsic (stage of lactation, pregnancy, breed or genotype) or extrinsic factors (nutrition, season, temperature). The effects of breed are limited whereas major changes in milk FA composition can be induced by nutrition manipulation, such as feeding pasture, conserved forages, starchy concentrates, or diets supplemented with oilseeds.

Manipulation of milk fat content and its FA composition could become an important target for the dairy industry, which could develop a quality payment for milk, and thus stimulate farmers to adapt their feeding systems correspondingly. The analytical reference methods for milk FA are based on Gas Chromatography after different steps of extraction, saponification and transmethylation. These methods are time-consuming and expensive. Thus, several studies have evaluated the potential of other rapid methods as mid-infrared spectrometry (MIR, Soyeurt *et al.,* 2006) or near infrared reflectance spectroscopy (NIRS, Coppa *et al.*, 2010) to quantify the major FA in milk fat.

This paper reviews recent data on the role of breed, and, feeding factors (interaction between nature of forage, starchy concentrates and oilseeds) on milk FA composition in cow, particularly in relation to oleic acid, 18:3n-3, *cis*9*trans*11-CLA, SFA and *trans* FA. Finally, we examine the ability of NIRS to quantify individual FA concentrations in milk.

EFFECTS OF BREED

The breed differences in milk FA composition are generally minor when compared with the effects of dietary manipulation or variations among individual cows (Palmquist *et al.*, 1993). When the animals were fed the same diet, the milk *cis*9-18:1 was higher for Holstein than for Jersey cows (Drackey *et al*., 2001; White *et al*., 2001; Palladino *et al.*, 2010). Some studies shown a higher content in 16:0 for Jersey than for Holstein cows (Drackey *et al*., 2001, Palladino *et al.*, 2010) but others reported opposite results (White *et al.*, 2001).

Milk from Holstein cows had higher *cis*9*trans*11-CLA than that from Brown Swiss cows (+0.03 g/100g) (Kelsey *et al*., 2003) or Jersey cows (+0.11 g/100g, White *et al*., 2001). Montbéliarde cows had milk containing higher *cis*9*trans*11-CLA (+0.1 - +0.21 g/100g) than Irish Holstein/Friesian, Dutch Holstein/Friesian, and Normande cows (Lawless *et al.*, 1999), in agreement with Ferlay *et al.* (2010). Moreover, the Holstein cows had higher milk percentages of 4:0 and 18:3n-3, and lower percentages of odd-branched chain FA (OBCFA)

than the Montbéliarde cows (Ferlay *et al.,* 2010). Milk fat from Tarentaise cows contained a lower proportion (−3 to 4 g/100 g) of 16:0 and higher proportions of stearic acid than that from Montbéliarde cows (Ferlay *et al.,* 2006). The variation of the delta-9 desaturase activity estimated from specific FA ratios (*e.g. cis*918:1/18:0) could explain partly these breed differences (Arnould and Soyeurt, 2009). Important breeding research programs are in progress in several European countries in order to develop tools helping farmers in the selection of their animals to improve the nutritional quality of the produced milk fat. Overall, these differences are largely lower than the effects of nutrition within a same breed.

EFFECTS OF PASTURE FEEDING

Fresh grass contains 1 to 3 % of FA, of which 50 to 75% is 18:3n-3. A number of studies have compared milk FA composition from grazing cows to milk from cows fed hay or silage-based diets. Grazed grass generally increases levels of milk oleic acid (+8.0 g/100 g of total FA), PUFA, especially 18:3n-3 (+1.0) and *cis*9*trans*11-CLA (+0.6), and decreases saturated medium-chain FA (Chilliard *et al.,* 2007). Some additional factors are reported to explain the important variability of milk FA composition observed for grazing animals, such as the phenological stage of the grass, its botanical composition, and interaction with the grazing management. Young grass has higher content of lipids and 18:3n-3 than mature grass and grazing young grass induced higher levels of this FA (+ 0.3 g/100 g) and *cis*9*trans*11-CLA (+ 0.9 g/100 g) (Ferlay *et al.,* 2006).

Differences in milk FA composition according to the altitude (lowland *vs.* highland *vs.* Alpine) have been also reported (Collomb *et al.,* 2002, Leiber *et al.,* 2004,). Generally, milk from cows grazing mountain pastures had higher concentration of 18:3n-3 and *cis*9-18:1, and lower SFA concentration. Mountain pastures have been characterized by a higher diversity in the botanical composition than in the lowlands (Falchero *et al.*, 2010). Thus, the presence of secondary ingredients as terpenoids or polyphenols could inhibit the biohydrogenation of PUFA, and could explain in part the high content of 18:3n-3 (Chilliard *et al*., 2007). One hypothesis on the high content of oleic acid is that the decrease in temperature or the prolonged walking of the cows on mountain pastures could induce an increased lipomobilization (Leiber *et al.,* 2004).

We have evidenced recently other factors such as level of botanical diversity and stocking density of the animals. We have compared milks from cows grazing 2 different upland grasslands: a highly diversified pasture with a low stocking density and continuous grazing *vs*. a weakly diversified pasture with a higher stocking density and rotational grazing (Coppa *et al.,* 2011). Some differences on milk FA profile have been observed. The milk total *trans* 18:1 content was higher for cows grazing under continuous than those grazing under rotational mode. With the continuous grazing, the milk PUFA content decreased during the season whereas it remained constant with the rotational grazing. These differences could be explained by a combined effect of the phenological stage of the grass and selection of grass by the cows.

EFFECTS OF OILSEED FEEDING

This section is focused on the recent studies on the effects of diets supplemented with linseed and rapeseed on the milk FA composition. Linseed and rapeseed contain a high oil level (40%) with 55% of 18:3n-3 and 60% of *cis*9-18:1, respectively (Glasser *et al*., 2008, Petit, 2010).

Milk fat secretion can be dramatically reduced by high-concentrate/low-fiber diets supplemented with PUFA from oilseeds (Chilliard *et al.*, 2007). This phenomenon is called milk fat depression syndrome (MFD). The biohydrogenation theory is the more commonly accepted and indicates that MFD is due to some intermediates of PUFA biohydrogenation, having an inhibitory effect on *de novo* FA synthesis (Shingfield *et al.*, 2010). The *trans*10*cis*12-CLA is the most studied for its inhibitory effect on mammary lipogenesis, but some other CLA isomers have been identified, and several other *trans* isomers of 18:1, notably *trans*10-18:1, 18:2 or 18:3 produced in the rumen are candidates (Roy *et al.*, 2006; Chilliard *et al.*, 2007; Shingfield *et al.*, 2010).

During short-term studies, feeding up to 15% linseed in diet dry matter (DM) had no effect generally on DM intake. In early lactation, discrepancies among experiments on the effect of whole or processed linseed supplementation on milk yield could result from differences in diet composition and length of experiment. The whole linseed supplementation did not modify milk yield and milk fat content and yield in mid or late lactation (Petit, 2010). Nevertheless, heat treatment of linseeds resulted in variable effects on milk fat concentration, with a possible decrease. One explanation could be the possible increasing rate of oil release from extruded seeds into the rumen compared to whole seeds, which could result in an increased production of *trans* FA in rumen and then a decrease in milk fat content (Chilliard *et al.*, 2009). A decrease in milk fat yield with linseed oil feeding is often reported (Glasser *et al.*, 2008). Generally, feeding diets with whole or crushed or micronized linseed had no effect on the milk protein content in mid lactation (Petit, 2010) whereas a decrease in protein content (0.5 g/kg) was observed with extruded linseed (Brunschwig *et al*., 2010).

Recently, we conducted a study to evaluate the effects of long-term supplementation (2.5 to 3 % of oil in DM) with rapeseed (whole or extruded seeds, or cold-pressed fat-rich meal) or extruded linseed on dairy cow performance over 2 consecutive lactations (including 2 indoor and outdoor periods). During indoor periods, cows were fed a diet based on grass silage and hay, and cows grazed during outdoor periods. During the first year of experimentation, oilseed supplementation had no effect on the milk and fat yields compared to the control diet. Oilseed supplements decreased the milk protein content, without changing protein yield. Whole rapeseed increased the milk fat content during the outdoor period (+5.3 g/kg). Thus, long-term effects of supplementation with oilseeds were similar to those observed during short-term (1 to 3 months) studies (Lerch *et al.*, 2011).

In order to evaluate the general responses of milk FA composition to oilseed feeding a meta-analysis approach has been used (Glasser *et al.*, 2008). Published experiments with linseed and rapeseed lipid supplements were selected, allowing to study the relationships between milk FA variations and supplemental lipids (0.65 and 0.59 kg/d for linseed and rapeseed, respectively). Oilseed supplementation induced a decrease in milk short- and medium-chain FA percentages and simultaneously an increase in percentages of total FA with 18 carbons. The meta-analysis evidences that the form of oilseed plays a role. More precisely, percentages of 6:0 to 14:0 were linearly decreased with increasing linseed as seeds and more largely as oils, and rapeseed (seeds and oils). The decrease in 16:0 percentage was quadratic with increasing doses, in the order linseed seeds ≥ rapeseeds (all forms) ≥ linseed oils. The percentage of the total C18 increased quadratically with supplemental lipids (from 35.4 for unsupplemented diets to more than 50%). Percentages of *cis* and *trans*-18:1 increased linearly with increasing lipids. These increases were higher with oil than with seeds. Concerning the *trans*-18:1, the increase ranked according to linseed oil ≥ rapeseed oil≥ linseed as seed. Total CLA percentage was linearly increased by rapeseed oil, and slightly by linseed. The linseed

increased 18:3n-3 percentage (on average 1.1% of total FA) whereas rapeseed seed increased it slightly.

The milk FA response to oilseed supplementations is time dependent, probably reflecting adaptations of number or activity of ruminal bacteria involved in biohydrogenation, or metabolic adaptations. Indeed, the maximal milk *cis*9*trans*11-CLA and *trans*11-18:1 responses to supplementation were transient, with a maximum observed 4-6 days after the start of supplementation with diets rich in maize silage or starchy concentrates supplemented with sunflower oil (SO), although the response was stable for at least 3 weeks when the diet was rich in hay and supplemented with linseed oil (Roy *et al.,* 2006). The decreases in milk *cis*9*trans*11-CLA and *trans*11-18:1 with diets rich in maize silage and concentrate supplemented with SO were associated with concomitant increases in milk fat *trans*10-18:1 content and a decrease in milk fat content, whereas concentrations of *trans*10-18:1 in milk on the hay diet supplemented with linseed oil remained low throughout the experiment. Temporal effects were also observed for isomers of CLA. The milk fat *trans*11*cis*13-CLA, *trans*11*trans*13-CLA and *trans*12*trans*14-CLA contents were enhanced on the hay diet, while the diets rich in concentrate or maize silage increased *trans*8*cis*10-CLA, *trans*10*cis*12-CLA and *trans*9*cis*11-CLA concentrations (Roy *et al.*, 2006).

The addition of dietary vitamin E in MFD diet has been shown to prevent the *trans*11-to-*trans*10 shift in cows fed a maize silage-based diet supplemented with extruded linseed and linseed oil only if vitamin E was added at the start of oilseed supplementation (Pottier *et al*., 2006). In another experiment, the addition of vitamin E in maize silage diets supplemented with extruded linseed had no effect on milk fat content and only moderate effects on milk concentrations of FA (increase in 16:0, decreases in 18:0 and *trans*6/7/8-18:1). In this experiment, the minor effects of vitamin E may be partly linked to the fact that no MFD occurred with the supplemented diet (Ferlay *et al.,* 2010).

The extent of change in the milk FA concentration is generally proportional to the level of inclusion of oilseeds in the diet (Glasser *et al*., 2008). Detailed studies have been published for extruded linseed in the diet (Brunschwig *et al.,* 2010, Hurtaud *et al.,* 2010, Ferlay *et al.* to be published). The major changes concern *trans* 18:1, and total CLA. These FA concentrations increased linearly with increasing amounts of linseed whereas 18:3n-3 concentration increased slightly, confirming that this FA was highly biohydrogenated in the rumen. In contrast, the milk SFA decreased linearly with increasing amounts of linseed.

The responses of milk FA concentrations to oilseed feeding are also depended on the nature of the forage. Glasser *et al*. (2008) reported higher increase in milk *cis* 18:1 concentration with alfalfa-based diets supplemented with linseed, followed by maize silage, grass hay and finally grass silage. Linseed supplementation of pasture diets did not change milk *cis*9-18:1 content whereas it was enhanced by linseed supplementation of other diets (Brunschwig *et al.,* 2010). Moreover, the decrease in 4:0 to 14:0 concentration was higher with maize silage-based diets than with grazed grass or grass silage (Glasser *et al*., 2008). Concerning the response to rapeseed supplements, the decrease in 10:0 to 14:0 was maximal with grass silage and maize silage and then ranked according to alfalfa ≥ grass hay ≥ pasture. The increase in total 18 FA was maximal with grass silage and maize silage (Glasser *et al.*, 2008).

PREDICTION OF MILK FATTY ACID COMPOSITION BY INFRARED METHODS

Growing consumer demand to be informed on the nutritional quality of foods has prompted dairy producers and transformers to characterize the nutritional composition of

cows' milk, and thus to develop rapid milk FA measurement based on infrared methods. The MIR (Soyeurt *et al.*, 2006, 2011) was successfully used to predict the main FA groups (SFA, MUFA, unsaturated FA and major individual FA) allowing to develop a possible milk payment system. NIRS is also a valuable method (Coppa *et al.*, 2010). To assess this method, we have selected a wide variability of milk FA composition using milk samples derived from different experimental diets (pasture and conserved forages, starchy concentrates, with and without oilseed supplements). The results showed that NIRS can predict the milk FA concentrations, but with a precision varying according to the FA targeted. Predictive equations were good for SFA, MUFA, unsaturated FA, *trans* FA, *trans* and *cis*-18:1, 16:0, and oleic acid, approximate for PUFA, 18:0, *trans*11-18:1 and *cis*9*trans*11-CLA and poor for 18:3n-3 (Coppa et al., 2010). NIRS seems more precise than MIR to predict some minor individual FA having nutritional interest (e.g. *cis*9*trans*11-CLA, *trans*11-, and *trans*9-18:1) but NIRS is less easily applicable for routine analyses because the milk samples were oven-dried before analysis and results can be only obtained 24h after. These infrared methods are promising for large-scale (including on-farm) determination of milk FA composition, even if the quality of prediction are still lower for the FA with the lower concentrations. More research should be done in order to improve the prediction results of these specific FA of nutritional interest (PUFA and 18:3n-3).

CONCLUSIONS

Breed differences are less important than individual variations on milk FA composition. Cow feeding has major effects on milk FA composition. Pasture, compared to winter diets, decreased milk SFA, and increased *cis*9-18:1, *trans*-18:1, 18:3n-3 and *cis*9*trans*11-CLA. The variability of FA composition in milk could be also due to grass phenological stage, botanical diversity and cow stocking density. Oilseed feeding induces changes similar to pasture, but with higher increase in *trans* isomers of 18:1. Linseed increases 18:3n-3, *trans*-18:1, and slightly *cis*9*trans*11-CLA contents whereas rapeseed increases slightly *cis*9*trans*11-CLA and *trans* 18:1 contents. The best accuracy was observed for NIRS prediction of the main FA groups (SFA, MUFA, unsaturated FA, and *trans* FA), and 16:0 and *cis*9-18:1. Given the variability in the FA composition of the tanker milks observed according to the cow nutrition, it seems to be possible to produce milks with an improved FA composition by an appropriate selection of collection rounds arriving at the dairy industry. Further research should consider the various components of the milk quality (nutritional, sensorial, and sanitary) and the factors (animal welfare, environmental impact of farm breeding) contributing to the development of dairy products, especially regarding the consumer expectations.

REFERENCES

1. Arnould, V.M.R., and H. Soyeurt (2009). Genetic variability of milk fatty acids. J. Appl. Genet. 50:29-39.
2. Bassett, C.M.C., A.L. Edel, A.F. Patenaude, R.S. McCullough, D.P. Blackwood, P.Y. Chouinard, P. Pacquin, B. Lamarche and G.N. Pierce (2010). Dietary vaccenic acid has antiatherogenic effects in LDR-/- mice. J. Nutr. 140:18-24.

3. Brunschwig, P., C. Hurtaud, Y. Chilliard, and F. Glasser (2010). L'apport de lin dans la ration des vaches laitières : Effets sur la production, la composition du lait et des produits laitiers, les émissions de méthane et les performances de reproduction. INRA Prod. Anim. 23:307-318.
4. Chilliard, Y., and A. Ferlay (2004). Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Develop. 45:467-492.
5. Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau (2007). Diet, rumen biohydrogenation, cow and goat milk fat nutritional quality: a review. Eur. J. Lipid Sci. Technol. 109:828-855.
6. Chilliard, Y., C. Martin, J. Rouel, and M. Doreau (2009). Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed or linseed oil, and their relationship with methane output. J. Dairy Sci. 92:5199-5211.
7. Collomb, M., U. Bütikofer, R. Sieber, B. Jeangros, and O. Bosset (2002). Composition of fatty acids in cow's milk fat produced in the lowlands, moutains and highlands of Switzerland using high-resolution gas chromatography. Int. Dairy J. 12:649-659.
8. Coppa, M., A. Ferlay, C. Leroux, M. Jestin, Y. Chilliard, B. Martin, and D. Andueza (2010). Prediction of milk fatty acid composition by Near Infrared Reflectance Spectroscopy (NIRS). Int. Dairy J. 20:182-189.
9. Coppa, M., A. Ferlay, F. Monsallier, I. Verdier-Metz, P. Pradel, R. Didienne, and A. Farruggia (2011). Milk fatty acid composition and cheese texture and appearance from cows fed hay or different grazing systems on upland pastures. J. Dairy Sci. 94:1132–1145.
10. Drackley, J.K., A.D. Beaulieu, and J.P. Elliott (2001). Responses of milk fat composition to dietary fat or nonstructural carbohydrates in Holstein and Jersey cows. J. Dairy Sci. 84:1231-1237.
11. Falchero, L., G. Lombardi, A. Gorlier, Lonati M., M Odoardi, and A. Cavallero (2010). Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. Dairy Sci. Technol. 90:657-672.
12. Ferlay, A., B. Martin, Ph. Pradel, J.B. Coulon, and Y. Chilliard (2006). Influence of grass-based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbéliarde cow breeds. J. Dairy Sci. 89:4026-4041.
13. Ferlay, A., C. Agabriel, C. Sibra, C. Journal, B. Martin, and Y. Chilliard (2008). Tanker milk variability of fatty acids according to farm feeding and husbandry practices in a French semi-mountain area. Dairy Sci. Technol. 88:193-215.
14. Ferlay, A., B. Martin, S. Lerch, Ph. Pradel, and Y. Chilliard (2010). Effects of supplementation of maize silage diets with extruded linseed, vitamin E and plant extracts rich in polyphenols, and morning vs. evening milking on milk fatty acid profiles in Holstein and Montbéliarde cows. Animal 4:627-640.
15. Givens, D.I. (2010). Milk and meat in our diet: good or bad for health? Animal 4:1941-1952.
16. Glasser, F., A. Ferlay, and Y. Chilliard (2008). Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J. Dairy Sci. 91:4687-4703.
17. Hurtaud, C., F. Faucon, S. Couvreur, and J.L. Peyraud (2010). Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. J. Dairy Sci. 93:1429-1443.
18. Jensen, R.G. (2002). The composition of bovine milk lipids: January 1995 to December 2000. J Dairy Sci. 85:295-350.

19. Kelsey, J.A., B.A. Corl, R.J. Collier, and D.E. Bauman (2003). The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J. Dairy Sci. 86:2588-2597.
20. Lawless, F., C. Stanton, P. L'Escop, D., R. Devery, P. Dillon, and J.J. Murphy (1999). Influence of breed on bovine milk cis-9,trans-11-conjugated linoleic acid content. Livest. Prod. Sci. 62:43-49.
21. Leiber, F., M.R.L. Scheeder, H.R. Wettstein, and M. Kreuzer (2004). Milk fatty acid profile of cows under the influence of alpine hypoxia and high mountainous forage quality. J. Anim. Feed Sci. 13:693-696.
22. Lerch, S., A. Ferlay, D. Pomiès, B. Martin, J.A.A Pires, and Y. Chilliard (2011). Rapeseed or linseed supplementation of grass-based diets: effects on dairy performance of Holstein cows over two consecutive lactations. J. Dairy Sci. In press.
23. Lopez-Huertas, E. (2010). Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmacol. Res. 61:200-207.
24. Mills, S., R.P. Ross, C. Hill, G.F. Fitzgerald, and C. Stanton (2011). Milk intelligence: mining milk for bioactive substances associated with human health. Int. Dairy Sci. 377-401.
25. Palladino, R.A., F. Buckley, R. Prendiville, J.J. Murphy, J. Callan, and D.A. Kenny (2010). A comparison between Holstein-Friesian and Jersey dairy cows and their F(1) hybrid on milk fatty acid composition under grazing conditions. J. Dairy Sci. 93:2176-84.
26. Palmquist, D.L., A.D. Beaulieu, and D.M. Barbano (1993). Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753-71.
27. Palmquist, D.L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. The Professional Animal Scientist, 25:207-249.
28. Petit, H.V. (2010). Review: feed intake, milk production and milk composition of dairy cows fed flaxseed. Can. J. Anim. Sci. 90: 115-127.
29. Pottier, J., M. Focant, C. Debier, G. De Buysser, C. Goffe, E. Mignolet, E. Froidmont and Y. Larondelle (2006). Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. J. Dairy Sci. 89:685-692.
30. Roy, A., A. Ferlay, K.J. Shingfield, and Y. Chilliard (2006). Examination of the persistency of milk fatty acid composition responses to plant oils in cows fed different basal diets, with particular emphasis on trans-C18:1 fatty acids and isomers of conjugated linoleic acid. Animal Sci. 82:479-492.
31. Roy, A., J.M. Chardigny, D. Bauchart, A. Ferlay, S. Lorenz, D. Durand, D. Gruffat, Y. Faulconnier, J.L. Sébédio, and Y. Chilliard (2007). Butters rich either in trans-10-C18:1 or in trans-11-C18:1 plus cis-9, trans-11 CLA differentially affect plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits. Animal. 1:467-476.
32. Shingfield, K.J., Y. Chilliard, V. Toivonen, P. Kairenius, and D.I. Givens (2008). Trans fatty acids and bioactive lipids in ruminant milk. in: Bioactive components of milk (Ed. Z. Bösze, Springer, USA) Advances in Experimental Medicine and Biology. 606:3-65.
33. Shingfield, K.J., L. Bernard, C. Leroux, and Y. Chilliard (2010). Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.
34. Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler (2006). Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89:3690-3695.

35. Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall, D.P. Berry, M. Coffey, and P. Dardenne (2011). Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci. 94:1657-1667.
36. White, S.L., J.A. Bertrand, M.R. Wade, S.P. Washburn, J.T. Jr Green, and T.C. Jenkins (2001). Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301.