The profile of certain liposoluble components in different types of milk

Anamaria Cozma

"IULIU HAȚIEGANU" UNIVERSITY OF MEDICINE AND PHARMACY CLUJ-NAPOCA

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The profile of certain liposoluble components in different types of milk

PhD Student **Anamaria Paraschiva Cozma**

Scientific supervisors **Prof. Felicia Loghin, PhD**

R.S. Anne Ferlay, PhD, HDR

UMF
UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
IULIU HAȚIEGANU
CLUJ-NAPOCA

To my parents

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ABBREVIATIONS USED IN THE TEXT

AA	Arachidonic acid
ACC	Acetyl-CoA carboxylase
ADF	Acid detergent fibre
ALA	α-linolenic acid
BCS	Body condition score
BW	Body weight
CF	Crude fibre
CLA	Conjugated linoleic acid
CLnA	Conjugated alpha-linolenic acid
CM	Chylomicrons
CP	Crude protein
DAG	Diacylglycerides
DHA	Docosahexaenoic acid
DIM	Days in milk
DM	Dry matter
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl esters
FAS	Fatty acid synthetase
FFA	Free fatty acids
HSO	Hemp seed oil
LA	Linoleic acid
LCFA	Long-chain fatty acids
LPL	Lipoprotein lipase
MAG	Monoacylglycerides
MCFA	Medium-chain fatty acids
MFD	Milk fat depression
MFGM	Milk fat globule membrane
MUFA	Monounsaturated fatty acids
NE	Net energy

NEFA	Nonesterified fatty acids
OBCFA	Odd- and branched chain fatty acids
OM	Organic matter
PDO	Protected designation of origin
PL	Phospholipids
PUFA	Polyunsaturated fatty acids
RBH	Ruminal biohydrogenation
SCC	Somatic cell counts
SCD	Stearoyl-CoA desaturase
SCFA	Short-chain fatty acids
SFA	Saturated fatty acids
SMCFA	Short- and medium-chain fatty acids
TAG	Triacylglycerides
VFA	Volatile fatty acids
VLDL	Very low-density lipoproteins

INTRODUCTION

Changes in dietary patterns during the past decades, including an increased consumption of saturated fats, frequently associated with a sedentary lifestyle, have led to a growing incidence of obesity, type-2 diabetes, cancer, and cardiovascular disease in the human population of Western countries [1]. Therefore, dietary recommendations for cardiovascular health have promoted a reduction in the intake of saturated fatty acids (FA) as well as a reduction in the intake of *trans* FA and cholesterol [2,3]. Nutritional guidelines typically recommended that total fat should not exceed 30% of total energy intake, consumption of saturated FA and *trans* FA should account for less than 10% and 1% of total energy intake, respectively, whereas dietary cholesterol intake should be less than 300 mg per day [4,5]. In this context, milk fat soon became a target of dieticians' criticism, as milk and dairy products provide on average 40% of the overall saturated fat consumption [6]. Additionally, these foods contribute on average to almost 40% of the total *trans* FA intake and range second on contribution to daily cholesterol intake [7,8]. The negative image of milk and dairy products should, however, be weighed by the fact that they represent a valuable source of bioactive liposoluble components, particularly FA, vitamins and β-carotene [9].

Butyric acid (4:0), branched-chain FA, *cis*-9,*trans*-11-conjugated linoleic acid (CLA), and conjugated alpha-linolenic acids (CLnA) in milk fat have been shown to exhibit anticarcinogenic properties in a number of human cell line cultures and animal models [10,11]. Caproic (6:0), caprylic (8:0), and capric (10:0) acids may reduce the risk of developing features of metabolic syndrome, by suppressing fat deposition and preserving insulin sensitivity [12,13]. Oleic acid (*cis*-9-18:1) is considered to be beneficial for cardiovascular health, as it has been reported to lower both plasma total cholesterol, LDL-cholesterol, and triacylglycerol concentrations in human subjects [14]. n-3 FA together with an optimal n-6/n-3 FA ratio have demonstrated potential roles both *in vitro* and *in vivo* in reducing the risk of cardiovascular disease, hypertension, type-2 diabetes, cancer, osteoporosis, rheumatoid arthritis and neurological dysfunctions [10].

With respect to the controversial saturated FA, lauric (12:0), myristic (14:0), and palmitic (16:0) acids are actually considered to be atherogenic only when consumed in excess, whereas stearic acid (18:0) might suppose no cardiovascular risk [5]. Likewise, although epidemiological evidence has associated dietary *trans* FA with heart disease, recent animal studies showed no association between the main isomer of *trans*-18:1 in milk fat (*trans*-11-18:1) and the risk of cardiovascular disease [15]. Similarly for dietary cholesterol, prospective cohort studies have indicated a positive association between cholesterol in diet and cardiovascular risk, whilst observational epidemiologic studies have found either no association or even an inverse association [3]. Moreover, milk fat is not only a source of bioactive liposoluble components, it also serves as an important delivery vehicle for fat-soluble vitamins, including vitamin A [16]. Vitamin A exhibits important functions in the human body such as regulation of cell and tissue growth and differentiation [17].

Finally, all these aspects underline the interest of modulating the fat composition of milk with the overall aim of improving the long-term health of consumers [4]. Milk fat composition is linked to many factors, both intrinsic (animal species, breed, genotype, lactation stage) and extrinsic (environmental) [18,19]. In a given ruminant species, the effects linked to breed or genotype are significant but limited and they can only be achieved over long term [18]. The lactation stage has an important effect on milk FA composition, mainly linked to body fat mobilisation in early lactation, but it only lasts a few weeks each year [20]. Furthermore, changes in feeding have a marked influence on milk fat composition [18]. Nutrition therefore constitutes a natural strategy to rapidly modulate milk FA, vitamin A, and cholesterol composition. The most important changes can be obtained either by changing the forages in the diet of ruminants, pasture in particular, or by supplementing lipids to the diet [18,21,22,23]. Nevertheless, little is known about the effect of cow-calf contact and cow parity on milk fat composition [24,25].

Based on these facts, the present work performs a complex approach of the factors influencing milk fat composition. First, the influence of cow breed, calf presence during milking, cow parity, and season on both milk FA profile and milk lipolytic system is studied. Then, the effect of diet supplementation with hemp seed oil on FA, vitamin A, and cholesterol concentrations and oxidative stability in goat milk is evaluated.

REVIEW OF THE LITERATURE

1. Metabolism of lipids, vitamin A, and cholesterol in dairy ruminants

Milk fat consists predominantly of triacylglycerides (TAG) (> 95% of total milk lipids) containing about 500 individual fatty acids (FA), most of which are present in amounts of <1% of total lipids [26,27]. Only saturated FA (SFA) of chain lengths from 4 to 18 carbon atoms, *cis*-9-16:1, oleic acid (*cis*-9-18:1), *trans*-18:1, and linoleic acid (18:2 n-6) are present in amounts greater than 1% in milk fat [26]. Along with TAG, the lipid composition of milk comprises small amounts of diacylglycerides (DAG), monoacylglycerides (MAG), free fatty acids (FFA), phospholipids (PL), glycolipids and sterols (cholesterol esters, lanosterol, dihydrolanosterol, and 7-dehydrocholesterol). Minor lipids include waxes, carotenoids, liposoluble vitamins (A, D, E, K), and lipoproteins [16].

The lipids in milk occur in the form of globules, comprising a core of TAG and small amounts of cholesteryl esters, FFA and retinol esters, surrounded by a thin membrane [28]. The membrane called the milk fat globule membrane (MFGM) contains primarily phospho- and sphingolipids and membrane-specific proteins [29]. The diameter of the fat globules ranges from <1 to about 10 μm [26]. Some studies reported that the average fat globule size is smaller in goat milk (< 3.5 μm) than in cow milk (~4 μm) [30,31]. This characteristic supports the hypothesis that goat milk fat has a higher digestibility than cow milk fat [30].

1.1. Origins of milk fatty acids

1.1.1. Rumen lipid metabolism

Diets consumed by ruminants generally contain between 20 and 40 g lipid/kg dry matter (DM), with a high proportion of polyunsaturated FA (PUFA) [32]. The predominant PUFA in ruminant diets are linoleic acid (LA, 18:2 n-6 or *cis*-9,*cis*-12-18:2) and linolenic acid (ALA, 18:3 n-3 or *cis*-9,*cis*-12,*cis*-15-18:3), derived from forages, cereals, and oilseeds. Moreover, some oilseeds provide monounsaturated FA (MUFA) (mainly *cis*-9-18:1 from rapeseed oil), whereas marine products (fish oil, algae) provide long-chain PUFA (mainly 20:5 n-3 (eicosapentaenoic acid, EPA) and 22:6 n-3 (docosahexaenoic acid, DHA)) [18].

On entering the rumen, hydrolysis of the ester linkages found in TAG, PL, and glycolipids is the initial transformation dietary lipids undergo [33]. Following this lipolysis carried out by microbial and plant lipases, nonesterified FA (NEFA) are released into the rumen and adsorbed onto feed particles and hydrogenated or incorporated directly into bacterial lipids [21,28]. The second major step in dietary lipids metabolism is the ruminal biohydrogenation (RBH) of unsaturated NEFA [33].

For most diets, RBH averages 80% for LA and 92% for ALA [34]. The major pathways of RBH have been established as a result of numerous *in vitro* and *in vivo* studies. Metabolism of LA and ALA starts with the isomerisation of the *cis*-12 double bond and the formation of a conjugated 18:2 or 18:3 FA, respectively. Conjugated products are further hydrogenated into *trans*-11-18:1 and then into 18:0 as the final end product [35]. The final hydrogenation step is considered to be rate-limiting and therefore *trans*-18:1 intermediates can be accumulated and then flow out of the rumen [32]. RBH of dietary PUFA results in the formation of numerous FA intermediates that following formation in the rumen can be incorporated into milk fat [28]. The occurrence of a wide range of isomers of *trans*-18:1, 18:2, and 18:3 FA containing one or more *trans* double bonds suggests that the metabolic pathways of RBH are much complex than previously thought. More recent *in vitro* and *in vivo* studies have provided additional data regarding possible biochemical pathways accounting for the formation of specific intermediates during the metabolism of LA (Fig. 1) and ALA (Fig. 2) [32].

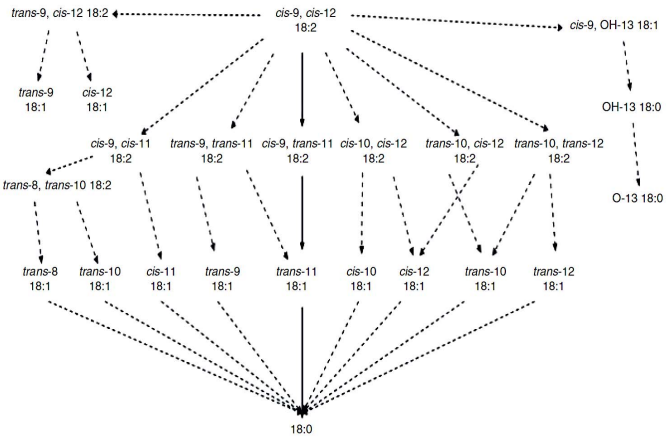

Fig.1. Putative pathways describing linoleic acid (LA, *cis*-9,*cis*-12-18:2) metabolism in the rumen. Arrows with solid lines highlight the major biohydrogenation pathway, whereas arrows with dashed lines describe the formation of minor fatty acid metabolites [32]

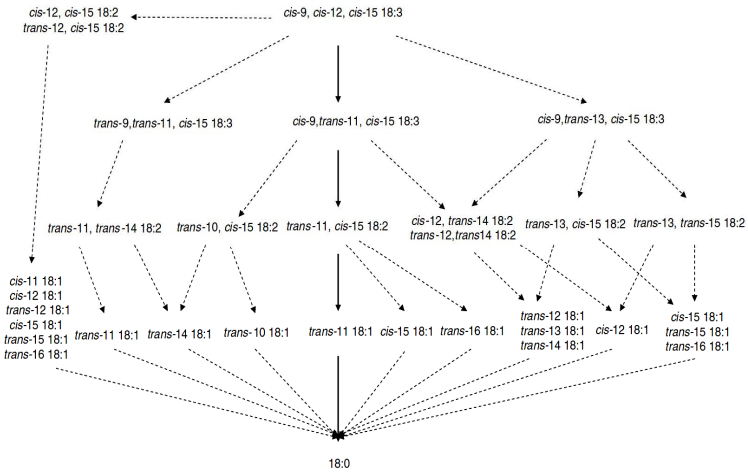

Fig.2. Putative pathways describing linolenic acid (ALA, *cis*-9,*cis*-12,*cis*-15-18:3) metabolism in the rumen. Arrows with solid lines highlight the major biohydrogenation pathway, whereas arrows with dashed lines describe the formation of minor fatty acid metabolites [32]

Regarding the RBH of oleic acid (*cis*-9-18:1), this FA is often shown to form directly stearic acid (18:0) [36]. However, more recent *in vitro* studies reported that *cis*-9-18:1 metabolism results in the formation of hydroxystearic (10-OH 18:0) and ketostearic (10-O 18:0) acids and multiple *trans*-18:1 intermediates with double bond positions from C6 to C16 [37,38]. *Cis-9*-18:1 RBH typically varies between 58% and 87% [32]. Moreover, RBH also occurs on 20- and 22-carbon FA having more than 3 double bonds, such as EPA and DHA in fish oil and marine algae. The RBH of these FA is extensive, but generally they do not become fully saturated [39]. Incubation of EPA (*cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17-20:5) and DHA (*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19-22:6) in cultures of mixed ruminal microorganisms caused the disappearance of these two FA as well as the accumulation of *trans*-18:1 [40]. If consistent with pathways for LA and ALA RBH, the initial isomerisation of EPA and DHA should produce isomers with five and six double bonds, including at least one *trans* double bond. Isomerisation should be followed by hydrogenation to isomers with four and five double bonds [32,36]. However, more research is required to elucidate the biochemical pathways of EPA and DHA rumen metabolism.

1.1.1.1. Microorganisms involved in rumen biohydrogenation

RBH involves only some species of rumen microorganisms, carrying out this process as a means of protection against the toxic effects of PUFA on microbial growth [41,42]. Several studies have shown that within the rumen microbial population, bacteria are mainly responsible for RBH when compared to protozoa and anaerobic fungi [36]. LA and ALA metabolism involves two groups of ruminal bacteria: Group A,

which hydrogenates PUFA to *trans*-18:1 FA, and Group B, which hydrogenates *trans*-18:1 FA to 18:0 [35]. Nevertheless, more recent studies have reported that cellulolytic bacteria from *Butyrivibrio* group are of principal importance in RBH. *Butyrivibrio fibrisolvens* was identified to produce *cis*-9,*trans*-11-18:2 and *trans*-11-18:1 from LA, whilst is does not form 18:0 [36]. To the present, the rumen bacteria identified as having the capacity to produce 18:0 are *Butyrivibrio hungatei* and *Clostridium proteoclasticum*, reclassified as *Butyrivibrio proteoclasticus* [41,43].

The contribution of protozoa to RBH has been suggested to be due to the activity of ingested or associated bacteria [36]. However, recent data indicate that ruminal protozoal cells contain proportionally more *cis*-9,*trans*-11-18:2 and *trans*-11-18:1 than ruminal bacteria. The most likely explanation is that protozoa do not form these FA, but play an important role in the uptake/protection of the intermediates of bacterial RBH [44]. Moreover, an *in vitro* study demonstrated that rumen fungi have the ability to biohydrogenate LA, with *Orpinomyces* fungus being the most active. RBH is slower in fungi than in bacteria and has *trans*-11-18:1 as the end product [45].

1.1.1.2. Effect of diet on rumen biohydrogenation

Diet composition (nature of forage, forage:concentrate ratio), the level and type of lipids in diet, and interactions between these factors have an important influence on the predominant RBH pathways resulting in changes in the profile of FA available for absorption and incorporation into milk fat [28]. In this regard, the extent of RBH is mainly dependent on the percentage of concentrate in the diet. When concentrate exceeds 70%, RBH is strongly reduced [34]. This phenomenon is probably due to a decrease in rumen pH, which normally ranges between 6.0 and 6.7 [41]. At a pH < 6, rumen lipolysis has been reported to be low [46]. Low rumen pH has also been shown to have a negative effect on microbial growth, especially on the growth of cellulolytic bacteria [47]. It is well known that cellulolytic bacteria, the main ruminal biohydrogenating bacteria, are sensitive to acidic condition (pH < 6) in the rumen [48].

Besides diet composition, the nature of dietary FA is a major factor in the variation of RBH. The addition of plant oils or oilseeds rich in LA and ALA to ruminant diet is reported to lead to incomplete metabolism of dietary PUFA into 18:0, with the accumulation of *trans*-18:1 intermediates [18]. Supplementation of diet with fish oil rich in EPA and DHA is also reported to inhibit the complete RBH of 18 PUFA, causing an increase in the ruminal outflow of *trans*-18:1 at the duodenum [49]. This effect may involve alterations in total ruminal bacteria and *Butyrivibrio* populations, probably related to the toxicity of PUFA on rumen bacteria [50]. Fish oil has been shown to be a more potent inhibitor of the hydrogenation of *trans*-18:1 intermediates to 18:0 in the rumen than plant oils and oilseeds [28].

However, differences in milk FA composition responses when lipid supplement are fed also occur as a consequence of the composition of the basal diet [4]. In this

respect, previous studies provided evidence that low-forage/high-concentrate diets supplemented with plant oils rich in PUFA are characterized by a shift in RBH towards *trans*-10-18:1 at the expense of *trans*-11-18:1 [51]. Likewise, on high-concentrate diets with marine lipids supply, *trans*-10-18:1 has often been reported to replace *trans*-11-18:1 as the major *trans* FA in milk fat [52].

1.1.2. Mammary lipogenesis

Milk FA originate from two sources: the uptake from circulation of preformed FA (ca. 60%) and *de novo* synthesis within the mammary gland (ca. 40%) [39]. Precursors for *de novo* FA synthesis are acetate and butyrate, volatile FA (VFA) produced during microbial fermentation of cellulose and hemicellulose in the rumen [21,39]. Butyrate is converted to β-hydroxybutyrate in the rumen wall [26]. Acetate and β-hydroxybutyrate are used by mammary gland for the synthesis of 4:0 to 12:0 FA, most of myristic acid (14:0) (ca. 95%) and about a half of palmitic acid (16:0) in milk fat [53]. The remaining 16:0 and all of the long-chain FA (LCFA) derive from mammary uptake of circulating TAG-rich lipoproteins (very low-density lipoproteins (VLDL) and chylomicrons (CM)), and plasma albumin bound NEFA that arise from intestinal absorption of lipids and body fat mobilization [32,54]. Mammary lipoprotein lipase (LPL) allows TAG hydrolysis and NEFA uptake by the mammary gland [20].

Milk fat contains also odd- and branched-chain FA (OBCFA), which are largely synthesized *de novo* by rumen bacteria [11]. Odd-chain FA are synthesized through elongation of propionate or valerate. Branched-chain FA are formed from their precursors: the branched-chain amino acids (valine, leucine and isoleucine) and the branched-short-chain carboxylic acids (isobutyric, isovaleric and 2-methyl butyric acids) [55].

De novo synthesis of milk FA involves two key enzymes: acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS) [20]. Acetate and β-hydroxybutyrate contribute equally to the initial four carbon unit. Acetate is converted to acetyl-CoA and used to extend the chain length of synthesized FA via the malonyl-CoA pathway, whereas β-hydroxybutyrate is incorporated directly following activation to butyryl-CoA [32]. ACC catalyses the formation of malonyl-CoA from acetate, and FAS catalyses condensation cycles of malonyl-CoA with either acetyl-CoA or butyryl-CoA [39]. LCFA containing 16 or more carbon atoms are known to lower mammary FA synthesis in bovine or caprine mammary epithelial cells *in vitro* due to direct inhibitory effects on ACC. The inhibitory effects have shown to be more potent when FA contain a longer carbon chain and/or have a higher degree of unsaturation [56]. This phenomenon explains the decrease in the concentrations of medium-chain FA (MCFA) in milk fat following an increase in the supply of LCFA to the mammary gland either from the diet, or from body fat mobilisation [39].

In ruminants, FA in milk fat that are taken up from circulation are derived mostly from the digestive absorption of dietary and microbial FA [57]. When reaching the intestine, these FA are usually in the unesterified form. They are absorbed in the duodenum, esterified in the enterocyte, and used in conjunction with PL and cholesterol esters in the assembly of VLDL and CM that pass into the peripheral blood [58]. Before esterification, 18:0 can be desaturated to *cis*-9-18:1 within the enterocyte, but only to a limited extent [59]. The remainder of the circulating FA originates from the mobilisation of body fat reserves, which typically accounts for less than 10% of milk FA [52]. Nevertheless, the contribution from mobilized FA increases when cows are in early lactation and/or in negative energy balance [54]. As 18:0 and *cis*-9-18:1 are the main FA stored in ruminant adipose tissue, body fat mobilisation induces a sharp increase in these FA concentrations in milk [60].

LCFA entering the mammary secretory cells can be desaturated, whereas preformed FA cannot undergo elongation (e.g. 16:0 to 18:0) within the mammary gland [20]. Mammary secretory cells contain the stearoyl-CoA desaturase (SCD) complex, also known as Δ-9 desaturase, an enzyme that acts by adding a *cis* double bond between carbon atoms 9 and 10 of the FA chain [28]. Δ-9 desaturase activity in the ruminant mammary gland is assumed to occur as a mechanism to ensure the liquidity of milk for efficient utilization by the offspring [61]. In this respect, the mammary gland converts 18:0 into *cis*-9-18:1 and contributes to 60% to 80% of all the oleic acid secreted in milk [53,62]. Likewise, the activity of Δ-9 desaturase is estimated to contribute to 90% of *cis*-9-14:1 and 50% of *cis*-9-16:1 in milk fat [63]. Other FA shorter than 18 carbon chain length, such as 10:0, 12:0, 14:0, 15:0, and 17:0, can also be used as substrates for Δ-9 desaturase [32]. Moreover, it is estimated that 25% of the vaccenic acid (*trans*-11-18:1) formed in the rumen is desaturated in the mammary gland to rumenic acid (*cis*-9,*trans*-11-18:2), the main isomer of conjugated linoleic acid (CLA) in milk [64]. Endogenous synthesis in the mammary gland from *trans*-11-18:1 is responsible for 70% to 95% of the milk *cis*-9,*trans*-11-18:2 [53].

The above mentioned metabolic pathways (*de novo* synthesis, uptake from circulation and desaturation) allow the formation of a pool of FA further used to form TAG through glycerol esterification [20]. The central carbon atom of TAG (sn-2) shows chirality, resulting in an asymmetrical TAG molecule, if two different FA are in the primary positions (sn-1 and sn-3) of the molecule [31]. The distribution of FA within TAG synthesis is not random. 8:0, 10:0, 12:0, and 14:0 FA are preferentially esterified at sn-2 position, 18:0 is preferentially esterified at sn-1 position, whereas the distribution of 16:0 between sn-1 and sn-2 positions is equal. Short-chain FA (SCFA) (4:0 and 6:0) and *cis*-9-18:1 are more abundant in the sn-3 position of TAG [26]. FA asymmetrical distribution on the glycerol molecule, as for example the preferential esterification of SCFA and oleic acid on the sn-3 position, influences the physical

properties of milk fat. It decreases milk fat melting point at or below the body temperature of the cow (39°C), thus ensuring its fluidity [61].

1.1.3. Milk fat depression

Feeding cow diets containing rations rich in readily digestible carbohydrates and poor in fibrous components and supplemented with plant oils, or marine oils can result in a decrease in milk fat content and yield but also in changes in milk FA composition. This phenomenon is commonly referred to as milk fat depression (MFD) [57]. Decreases in milk fat secretion during diet-induced MFD often occur within a few days, and in more severe cases milk fat yield can be lowered by up to 50% [52].

In contrast to cow, MFD is not frequently reported in goat, even when diets providing large amounts of starch and supplemented with plant oils or marine oils are fed [60]. Moreover, diets causing MFD in cow usually increase milk fat secretion in goat [18]. The differences between bovine and caprine regarding the impact of diet composition on milk fat synthesis appear to be attributed to differences between these two ruminant species in rumen lipid metabolism and mammary gland sensitivity towards components with anti-lipogenic activity [32,65].

Several theories have been proposed to explain the causes of diet-induced MFD. Owing to the importance as a substrate for *de novo* FA synthesis in the mammary gland, one theory attributes diet-induced MFD to a decrease in acetate and butyrate supply [57]. In this respect, previous studies reported that continuous ruminal infusions of acetate and butyrate increase milk fat concentration and yield [66]. However, these data indicate only that increases in acetate and butyrate supply stimulate mammary lipogenesis, which does not necessarily prove that MFD is caused by a deficit of precursors for the mammary FA synthesis. Diets causing MFD, such as high-concentrate diets, frequently induce variations in molar proportions of VFA in the rumen, but they also induce alterations in ruminal outflow of RBH intermediates and end products [67,68]. Since *trans* FA as RBH intermediates are shown to exert anti-lipogenic effects, discrimination between the contribution of decreases in acetate and butyrate supply and increases in ruminal *trans* FA outflow in the occurrence of diet-induced MFD is difficult [52].

The second theory proposed to explain MFD is the glucogenic-insulin theory, which supports that increased rumen production of propionate and enhanced circulating glucose levels cause an increase in circulating insulin concentrations. Elevated insulin secretion induces further a deficit of precursors for mammary synthesis of milk fat, as it stimulates the partitioning of FA towards adipose tissue rather than mammary gland [54]. One approach to examine the glucogenic-insulin theory has involved providing exogenous propionate and glucose through continuous intraruminal infusions. This experiment reported a decrease in milk fat concentration and yield, which was attributed to an increase in insulin secretion [66]. Nevertheless,

results from studies using hyperinsulinemic-euglycemic clamps do not support the glucogenic-insulin theory of diet-induced MFD and indicate that decreases in milk fat content previously observed with propionate and glucose infusions are most likely due to the capacity of insulin to inhibit lipolysis, therefore limiting the availability of preformed FA mobilised from body fat stores [69].

The most recent theory trying to elucidate the mechanisms underlying diet-induced MFD is the biohydrogenation theory, which appears to offer a more plausible explanation for MFD over a wide range of diets. The biohydrogenation theory states that mammary synthesis of milk fat is inhibited directly by specific *trans* FA formed during RBH of dietary PUFA [54]. This theory is supported by studies showing that *trans*-10,*cis*-12-CLA, an intermediate in LA rumen metabolism, is a potent inhibitor of milk fat synthesis in lactating cows [70]. Further research has established that abomasal infusion of *trans*-10,*cis*-12-CLA decreases milk fat synthesis in the lactating cow in a dose-dependent curvilinear manner [71].

Moreover, several studies reported that diet-induced MFD can also occur in the absence of or after relatively small increases in milk fat *trans*-10,*cis*-12 CLA content, suggesting that other intermediates of RBH may also inhibit milk fat synthesis [52]. In this regard, diet-induced MFD is consistently associated with increases in milk fat *trans*-10-18:1 concentration [51,72]. However, abomasal infusion of *trans*-10-18:1 in lactating cows was found to increase the concentration of this *trans* FA in milk, whereas it had no effect on milk fat secretion, offering therefore little support for the anti-lipogenic effect of *trans*-10-18:1 [73]. Furthermore, post-ruminal infusion experiments provided evidence that *cis*-10,*trans*-12-CLA and *trans*-9,*cis*-11-CLA inhibit milk fat synthesis, but the collective ruminal outflow of these intermediates in LA rumen metabolism does not fully explain the milk fat decreases observed during MFD [74,75].

Nevertheless, to the present, the biohydrogenation theory is considered to be the most robust of all the theories trying to explain diet-induced MFD [32]. Yet, further studies are required to identify and characterise the anti-lipogenic effect of different RBH intermediates, as well as mechanisms other than direct inhibition that could be involved in diet-induced MFD [52]. An example of such a mechanism would be the decrease in the availability of 18:0 for mammary *cis*-9-18:1 synthesis reported to occur when PUFA-rich lipid supplements, particularly fish oil, inhibit the hydrogenation of *trans*-18:1 isomer. Reduced 18:0 and associated increases in *trans*-18:1 have been suggested to inhibit milk fat secretion due to incapacity of the mammary gland to maintain an adequate milk fat fluidity [76].

1.2. Origins of milk vitamin A

Vitamin A in milk has multiple origins. It may be derived from ruminant diet, more precisely from forages, concentrates (cereals, oilseeds) and/or mineral-vitamin supplements [21]. The distribution of a mineral-vitamin supplement is recommended as it has been demonstrated that ruminants requirements for vitamin A are in general not entirely covered by diet, except for pasture diet [77]. Ruminants are also able to synthesize vitamin A from precursors in diet. Thus, vitamin A is formed in the enterocytes by enzymatic hydrolysis of various isomers of β-carotene. The isomer all-*trans* is the major form, because it has the highest concentration in food and it is the best enzyme substrate [21]. Moreover, studies on lactating dairy cows indicated that β-carotene conversion into vitamin A may also occur in the mammary gland [78].

1.3. Origins of milk cholesterol

Despite the extensive knowledge in humans, cholesterol metabolism in ruminants is nowadays still poorly documented. Milk cholesterol may be derived from mammary *de novo* synthesis from acetate, but the amount of milk cholesterol synthesised in the mammary gland has been estimated to represent only 20% of the total [79,80]. Nevertheless, studies conducted to investigate the origin of milk cholesterol in ruminants suggested that this liposoluble component in milk is derived principally from the uptake of serum cholesterol. Furthermore, serum cholesterol originates mainly from synthesis within the liver [8,79].

2. Factors affecting milk fatty acid, vitamin A, and cholesterol composition

Milk fat is the most variable component of milk, depending on intrinsic (animal species, breed, genotype, pregnancy and lactation stages) or extrinsic (environmental) factors [18]. This section reviews some of these factors affecting FA, vitamin A, and cholesterol concentrations in milk from cow and goat.

2.1. Breed

Within a given ruminant species, the differences in milk FA composition linked to breed are significant but restricted and they can only be achieved over long terms [18]. Moreover, breed differences in milk FA composition are generally minor when compared with the effects of dietary modulation or variations among individual animals [81].

When the animals were fed the same diet, milk fat from Jersey cows contained less *cis*-9-18:1 than milk fat from Holstein cows [82,83,84]. Several studies reported higher content of 16:0 for Jersey than for Holstein cows, whereas others indicated opposite results [82,83,84]. *Cis*-9,*trans*-11-CLA milk fat concentration was higher in Holstein cows than in Jersey cows (+0.11 g/100g FA) and Brown Swiss cows (+0.03 g/100g FA) [25,84]. Milk from Montbéliarde cows had higher *cis*-9,*trans*-11-CLA (+0.1 - +0.21 g/100g FA) than that from Irish Holstein/Friesian, Dutch Holstein/Friesian, and Normande cows [85]. Milk fat from Holstein cows was also poorer in OBCFA, but richer in 4:0 and 18:3n-3 than that from Montbéliarde cows [86]. Moreover, the Tarentaise cows had higher milk percentages of 18:0 (+1.86 g/100g FA), and lower percentages of 16:0 (-3.41 g/100g FA) than the Montbéliarde cows [81]. Additionally, some data regarding differences in milk FA composition linked to breed are reported also for dairy goats. A study on two goat breeds from Sindh, Pakistan, showed lower milk concentrations of total SFA (-5.3 g/100 g FA) and higher milk concentrations of *cis*-9,*trans*-11-CLA (+0.12 g/100g FA) for Kamori goats than for Pateri goats [87]. It has been suggested that breed differences in milk FA composition could be due in part to the variation of the Δ-9 desaturase activity estimated from specific FA ratios [88].

Regarding vitamin A, the concentration of this liposoluble component in milk does not show marked variations among dairy breeds. In this respect, studies in dairy cows reported similar milk retinol concentrations for Holstein, Montbéliarde and

Tarentaise breeds. Nevertheless, slightly higher retinol concentrations in the milk fat of Holstein cows (11.8 μg/g fat) than of Jersey cows (8.0 μg/g fat) were observed, with Brown Swiss cows (9.5 μg/g fat) having intermediate concentrations [78].

For milk cholesterol concentration, the differences observed between dairy breeds are minor or even absent. Thus, no significant differences in milk cholesterol concentration were found between Whites Thari cows (10.2-16.9 mg/100 mL) and Red Sindhi cows (13.2-19.68 mg/100 mL), whereas the average cholesterol concentration in milk from Black and White Schleswig-Holsteins cows (246 mg/100 g fat) was reported to be slightly higher than in milk from Angler cows (231 mg/100 g fat) [23,89].

2.2. Species

Milk fat varies widely according to species, as shown by important differences between cow and goat milk fat (Table I). First, goat milk has a higher fat content than cow milk [90]. Goat milk contains higher amounts of vitamin A than cow milk, with goats converting generally all β-carotene into vitamin A in the milk [30,78]. Compared to cow milk, cholesterol content in goat milk is higher [78]. Nevertheless, compared to cow milk fat, the main characteristic of goat milk fat is the higher content in the metabolically valuable 6:0, 8:0, 10:0, 12:0, and n-3 FA [90,91].

Table I. Fatty acid, vitamin A, and cholesterol concentrations in milk from cow and goat

	Cow milk	***Goat milk***
Fat[a] (%)	3.42	5.23
FA[a] (g/100 g total FA)		
4:0	3.84	1.27
6:0	2.28	3.28
8:0	1.69	3.68
10:0	3.36	11.07
12:0	3.83	4.45
14:0	11.24	9.92
cis-9-14:1	0.49	0.14
16:0	32.24	25.64
cis-9-16:1	1.53	0.99
18:0	11.06	9.92
trans-9-18:1	1.63	0.37
cis-9-18:1	21.72	23.80
18:2 n-6	2.41	2.72
cis-9,*trans*-11-CLA	0.40	0.36
trans-10,*cis*-12-CLA	0.05	0.07
18:3 n-3	0.25	0.53
Total SFA	71.24	70.42
Total MUFA	25.56	25.67
Total PUFA	3.20	4.08
Total n-6	2.53	2.81
Total n-3	0.25	0.51
Vitamin A[b] (IU/100g milk)	126	185
Cholesterol[b] (mg/100 g fat)	300	342

[a] Sanz Ceballos et al. [90].
[b] Park et al. [30].

2.3. Stage of lactation

The effect of lactation stage on milk FA composition is marked and mainly linked to body fat mobilisation in early lactation [18]. At initiation of lactation, ruminants are in negative energy balance, causing mobilisation of FA from adipose tissue and incorporation of these FA into milk fat [19]. Since the main FA stored in adipose tissue are 18:0 and *cis*-9-18:1, body lipid mobilisation in early lactation induces a sharp increase in these FA concentrations in milk [60]. Thus, milk from the first week of lactation can contain up to 50% more 18:0 and *cis*-9-18:1 than milk from mid-lactation [19]. Nevertheless, the lactation stage effect on milk FA composition is transient, lasting only a few weeks (6 to 8 weeks) each year [20].

Changes in milk vitamin A and cholesterol concentrations in relation to the stage of lactation are poorly documented. Retinol has been reported to have much higher concentrations in colostrum than in milk, but these concentrations decrease rapidly during the first week after parturition [78]. Moreover, a study in dairy cows indicated only a slight variation in milk retinol concentration during the first 24 weeks of lactation [92]. Immediately after parturition, milk was reported to contain also a high concentration of cholesterol (600 mg/g fat) which then showed a rapid decline during the first ten days post partum [23]. Nevertheless, milk cholesterol concentration was shown to increase with the progress of lactation stage in dairy cows, from 3.74 mg/g fat at stage I (6-60 days of lactation) to 4.35 mg/g fat at stage II (61-210 days of lactation), and then to 4.66 mg/g fat at stage III (between day 211 and end of lactation) [93].

2.4. Diet

Changes in feeding have a marked influence on ruminant milk fat composition [18]. Therefore, diet constitutes a natural strategy to rapidly modulate milk FA, vitamin A, and cholesterol composition. The most important changes can be obtained either by changing the forages in the diets of ruminants, pasture in particular, or by supplementing lipids to the diet [18,21,22].

2.4.1. Influence of nature of forage

Despite having a relative low content of lipids, forages represent often the major source of unsaturated FA in ruminant diet [4]. Fresh grass is a rich source of 18:3 n-3 and, compared to mixed winter diets, results in increased milk fat concentrations of 18:0 (+2 g/100 g FA), *cis*-9-18:1 (+8 g/100 g FA), 18:3 n-3 (+1 g/100 g FA), and *cis*-9,*trans*-11-CLA (+0.6 g/100 g FA), and decreased 10:0-16:0 concentrations (-13 g/100 g FA) [18]. Furthermore, grass conservation through hay making or ensiling leads to decreases in 18:3 n-3 concentrations, with hay having lower 18:3 n-3 concentrations than grass silage [4,94]. Nevertheless, milk from hay diets can often be richer in 18:3 n-3 than milk from silage diets, due to higher transfer efficiency from diet to milk with hay than with grass silage [95]. In contrast to grass, concentrates and soybean meal are rich

in 18:2 n-6 [94]. Milk FA composition varies widely according to the range of concentrate in the diet. In a pasture-based diet, increasing the concentrate from 3 to 35% resulted in increased levels of milk 4:0-14:0, *trans*-18:1 isomers (except *trans*-11-18:1), and 18:2 n-6, and decreased *cis*-9-18:1, *trans*-11-18:1, *cis*-9,*trans*-11-CLA, and 18:3 n-3 contents [96,97]. By contrast, when the concentrate exceeded 60% in a pasture-based diet, an increase in milk fat concentrations of all *trans*-18:1 isomers (especially *trans*-10-18:1), *cis*-9,*trans*-11-CLA, and 18:2 n-6, and a decrease in 14:0, 16:0, and 18:0 were observed [98]. Similar studies conducted in goats showed that changes in milk FA linked to type of forage and forage/concentrate ratio are consistent with the results reported in cows [18].

Vitamin A in milk derives mainly from ruminant diet, the nature of forage having therefore an important influence also on milk vitamin A concentration [17,21]. Morever, since a part o milk vitamin A is synthesized from β-carotene, an association between dietary β-carotene and the concentration of retinol in mik has been suggested [78]. Fresh grass is one of the richest sources of β-carotene (ca. 360 mg/kg DM) [21]. Nevertheless, β-carotene content of grass depends on grass stage of development and decreases during drying and preservation due to β-carotene UV-sensitivity [78,99]. In a study in dairy cows grazing on a middle mountain prairie composed of low diversified grass, found in a leafy stage, milk concentrations reached 7-8 μg/g fat for β-carotene and retinol [99]. In contrast, β-carotene and retinol concentrations in milk were reported to be lower (2.5-2.8 μg/g fat) for diets based on grass silage, hay or maize silage, which are poorer in β-carotene [21]. Likewise, concentrates are typically poor sources of carotenoides [78]. In agreement with the aforementioned data, the average milk fat concentrations of retinol and β-carotene were reported to be 1.2- and 1.6-fold higher, respectively, when milk from dairy cows was produced during the grazing vs. the winter feeding period [100]. Similarly, in dairy goats, retinol concentration in milk was higher during the grazing period (650 μg/100 g DM) than during the indoor feeding period (499 μg/100 g DM) [101].

With respect to milk cholesterol, although it is mainly synthesised through processes independent of the ruminant diet, feed chemical composition is shown to affect the concentration of this liposoluble component in milk [102]. In this respect, cholesterol concentration was reported to be higher in milk from cows fed fresh grass (261 mg/100 g fat) compared to milk from cows fed hay (236 mg/100 g fat) [103].

2.4.2. Influence of diet supplementation with lipids

Over the last decades, dietary lipid supplementation has been used to increase energy intake and/or modify milk FA composition in ruminants [18]. Supplementation of cow and goat diets with vegetable oils rich in either LA (e.g., sunflower or soybean oils) or ALA (e.g., linseed or rapeseed oils) proved to be an effective mean to enhance the *cis*-9,*trans*-11-CLA content of milk fat as well as to decrease milk fat saturated FA, particularly 12:0, 14:0, and 16:0 [104,105,106]. Furthermore, increases in LA or ALA in

milk following vegetable oils supplementation are small or absent, as these PUFA are largely hydrogenated in the rumen [60,106,107]. In this respect, it has been assumed that giving lipids in the form of oilseeds or rumen-protected oils rather than free oils would limit RBH of PUFA by restricting microbial access to lipids [20,26]. Nevertheless, in goats fed a low forage diet, supplemented with either free oil or whole crude oilseeds, from either sunflower or linseed, PUFA were more significantly increased by free oil than by oilseeds [60]. This result was attributed to a slower release of lipids from seeds, thus increasing their RBH [20]. With regard to rumen-protected lipids, encapsulation of plant oils in a formaldehyde-treated casein layer proved to be one of the most effective protection processes in achieving ruminal protection of PUFA [108]. Thus, feeding protected canola/soybean oilseed (70/30 w/w) and protected soybean oilseed/linseed oil (70/30 w/w) to dairy cows at pasture increased the concentration of ALA in milk fat from <1% to 2.49% and 8.45%, respectively [109]. Although effective, such a dietary practice has its limitations, because it is expensive and it uses the controversial formaldehyde [20].

Likewise, diet supplementation with marine lipids, rich in LCFA of the n-3 series, is considered a good nutritional strategy for enhancing *cis*-9,*trans*-11-CLA, 20:5 n-3, and 22:6 n-3 in milk fat of ruminants [110]. When equally added to the ration, marine oils seem more effective than plant oils at increasing milk *cis*-9,*trans*-11-CLA content, as a result of the potent inhibitory effect of long chain FA on the ruminal reduction of *trans*-18:1 to 18:0 [20,111]. Despite the fact that marine oils are rich in 20:5 n-3 and 22:6 n-3, the transfer rates of these FA from diet to milk are low and typically account for 3-4% in cows and 4-5% in goats [60,91]. Low transferts from diet to milk could be caused by the extensive RBH of these FA and by their preferential incorporation into plasma PL and cholesterol esters [18].

Supplementation of ruminant diet with lipids has been shown to alter also milk vitamin A and cholesterol concentrations. In dairy cows, supplementation of diet for 21 days with different lipid sources (300g/d of fish oil, 500g/d of Opal linseed, 500g/d of Szafir linseed, 150 g/d of fish oil or 250 g/d of Opal linseed, 150 g/d of fish oil and 250 g/d Szafir linseed) increased milk α-retinol concentration in all dietary treatments by 23 to 183% [112]. Likewise, 28 days of dietary supplementation with linseed (200 g/d) in dairy cows, caused an important increase in milk vitamin A concentration (+0.147 mg/L) as well as a decrease in milk cholesterol concentration (-0.205 g/100 g fat) [22]. Moreover, cholesterol concentration in milk of cows fed a total mixed ration supplemented with linseed (21 g/d) for seven weeks was reported to be 32% lower than in milk of controls [113]. Similarly, gradual addition of 275 g or 550 g rapeseed oil or corresponding quantities of wholemeal from rapeseed decreased milk cholesterol concentration by 8-13% [23].

3. Milk liposoluble components and their effects on human health

Milk and dairy products provide on average 40% of the overall saturated fat consumption in Europe, which makes them, since decades, the preferential target of dieticians' criticisms due to negative image of saturated FA [6,20]. However, it should be considered that milk fat is also an important source of energy, fat-soluble nutrients and health-promoting bioactive lipids in human diet [16]. A brief overview of the effects of milk liposoluble components on human health is given below.

3.1. Milk fat digestion

Understanding the digestion process of dietary milk fat is the first step in the study on the impact of milk liposoluble components on health. When consumed in human diet, the digestion of milk fat begins in the stomach with lipolysis catalysed by lingual or gastric lipase [114]. These lipases preferentially hydrolyse TAG at sn-3 position ca. twice as fast as at sn-1 position, resulting in the release of mostly SCFA and sn-1,2-DAG [115]. The released SCFA pass through the stomach mucosa, enter the portal vein, and are transported to the liver where they undergo oxidation [26]. The LCFA are reintegrated into the fat globules in the stomach or in the duodenum [31]. TAG digestion occurs 25 to 40% in the stomach [26].

Moreover, the major products of TAG gastric lipolysis act as emulsifying agents and facilitate therefore the intestinal phase of digestion performed by pancreatic lipase [114]. Pancreatic lipase is the primary enzyme involved in fat digestion, which hydrolyses TAG in their primary positions, with a slight preference for sn-1 position, and releases sn-2-MAG, sn-2,3-DAG and FFA [115]. Dietary cholesterol esters are completely hydrolysed by pancreatic cholesterol esters hydrolase yielding to FFA and free cholesterol, activated pancreatic phospholipase A2 hydrolyzes PL into 1-lysophospholipids and FFA, whereas dietary vitamin A present under the form of retinyl esters is hydrolysed by pancreatic and intestinal enzymes releasing retinol [116,117].

Ionized FFA, sn-2-MAG, cholesterol and retinol enter into bile micelles, to form with PL mixed micelles, which help apolar lipids to be absorbed through the microvillous membrane [114]. In the enterocytes, absorbed lipids are re-esterified in the

smooth endoplasmic reticulum, then packaged with TAG, PL and apolipoproteins into CM, and finally secreted to the lymph to be transported into the general blood circulation. In the peripheral tissues, CM are cleaved by LPL off-loading TAG and providing CM remnants [118]. CM remnants interchange first components with other plasma lipoproteins, being afterwards delivered to the liver [114].

3.2. Milk fatty acids and their health effects

3.2.1. Butyric acid

Milk fat is the only dietary source of butyrate, containing between 7.5 and 13.0 mol/100 mol of butyric acid (4:0) [16,119,120]. Butyrate is reported to exert anticarcinogenic effects, by inhibiting cell proliferation and activating apoptosis in several human cancer cell lines [28,121,122]. The main mechanism underlying butyrate anticarcinogenic effects is hyperacetylation of histones and increase of the accessibility of transcription factors to DNA via inhibition of histone deacetylase, making therefore butyrate able to modulate the expression of oncogenes and suppressor genes [16,123].

Since butyrate is formed in the human colon from bacterial fermentation of dietary fibers and serves as the principal source of energy for the colonic mucosa, numerous animal and *in vitro* studies have highlighted the role of butyrate in reducing the risk of colon cancer [124,125,126,127,128]. Moreover, several *in vitro* and *in vivo* studies indicate that dietary 4:0 may have a role in the prevention of breast and liver cancer [129,130,131].

Butyrate has also anti-inflammatory properties, mainly by inhibition of nuclear factor κB (NFκB) activation in colonic cells, which may be due to inhibition of histone deacetylase [132,133]. This effect of butyrate explains its role in the treatment of Crohn's disease and ulcerative colitis, diseases characterised by a dysregulation of NFκB activity [134,135].

Nevertheless, considering that ingested milk butyrate released in the duodenum by lipases is absorbed, processed, and then transported via blood circulation to the liver where most is metabolised, it is difficult to estimate the amount of 4:0 from milk to which tissues are exposed [10]. In this respect, German and Dillard [16] stated that certain tissues can be exposed to millimolar concentrations of 4:0 following consumption of milk fat.

3.2.2. Caproic, caprylic, and capric acids

The milk fat from goats is richer in caproic (6:0), caprylic (8:0), and capric (10:0) acids than the milk fat from cows [136]. Within the human body, the low molecular weight of these MCFA facilitates the action of pancreatic lipase, making hydrolysis faster and more complete than that of LCFA, whereas their reduced chain length determines a more rapid absorbtion and metabolisation, providing quick energy

[137,138]. Due to these properties, 6:0, 8:0, and 10:0 are used in the treatment of several clinical disorders, such as pancreatic insufficiency, deficit of biliary salts, malabsorption syndrome, intestinal resection, malnutrition, and premature infant feeding [9,136,139,140,141].

Likewise, 6:0, 8:0, and 10:0 may exert an important role in the prevention and treatment of metabolic syndrome, due to their ability to suppress fat deposition and preserve insulin sensitivity [12,142,143,144,145,146]. Moreover, recent studies showed that 8:0 and 10:0 could be used as an efficient therapeutic approach for patients with epilepsy [147,148]. Studies to date reported also the antitumor properties of 8:0 [14,149].

3.2.3. Lauric, miristic, palmitic, and stearic acids

For many decades, dietary guidelines have recommended to limit the intake of saturated fats, since these have been frequently associated with increased risk of coronary heart disease due to their capacity to increase serum total and LDL-cholesterol [16,28,150,151,152,153,154]. Nevertheless, it should be mentioned that SFA are not all equally hypercholesterolemic. Intakes of lauric (12:0), myristic (14:0), and palmitic (16:0) acids in human diet have been associated with increases in serum LDL-cholesterol, while stearic acid (18:0) has been shown to be neutral or even beneficial [28,155]. Certain studies indicate that lauric acid (12:0) and 14:0 are more atherogenic than 16:0, while others indicate that 14:0 and 16:0 exert stronger effects on plasma cholesterol than 12:0 [28].

In this context, it is not surprising that milk and dairy products, providing between 20% and 60% of total SFA intake, have been a target of dieticians' criticism for many years [6,7,18,156]. However, studies to date provide no convincing evidence that milk fat is detrimental to cardiovascular health. Several studies have reported a lack of association between intake of milk and risk of coronary heart disease [157,158]. A prospective case-control study has shown that cardiovascular risk factors were inversely associated with milk fat consumption [159]. Another case-control study indicated that intake of dairy fat or some other component of milk products may even protect patients at increased risk from having a first myocardial infarction and that the causal effects may rely on other factors than serum cholesterol [160]. The lack of a positive association, respectively the negative association reported between milk fat intake and cardiovascular disease risk may be attributed to a balance established between positive and negative cardiovascular effects of nutrients and other bioactive components provided by milk and dairy products [161].

3.2.4. Odd- and branched-chain fatty acids

Among odd-chain FA, 15:0 and 17:0 are of nutritional interest, as it has been shown that their content in serum and subcutaneous adipose tissue can be used as a biomarker of milk fat intake in human diet [159,162]. Furthermore, these biomarkers can be useful in establishing links between dairy consumption and diseases occurrence, as

reported by Warensjö et al. [163] who observed a lower risk of developing a first myocardial infarction associated with serum milk fat biomarkers 15:0 and 17:0.

Moreover, several studies have demonstrated that certain branched-chain FA exert anticarcinogenic effects. Iso 15:0 purified from soy fermentation products induced cell death through apoptosis activation in human T-cell non-Hodgkin's lymphoma, prostate, and liver cancer cell lines [164,165]. Wongtangtintharn et al. [166] suggested incorporation of iso 15:0 into glycerolipids of cancer cells as the process inducing apoptosis via caspase-independent death pathway. Furthermore, Wongtangtintharn et al. [167] reported for both anteiso and iso FA the capacity to inhibit tumor growth in human breast cancer cell lines, with iso 16:0 having the highest antitumoral activity and iso 15:0 having a cytotoxicity comparable to that of CLA. The mechanism underlying branched-chain FA anticarcinogenic effects is the reduction of FA synthesis in tumor cells by direct inhibition of FAS and reduction of precursors supply [167].

3.2.5. Oleic acid

Oleic acid (*cis*-9-18:1) is the only unsaturated FA with the highest concentration in milk, of about 8 g/l whole milk [9]. Therefore, milk and dairy products are indicated to have an important contribution to the dietary intake of oleic acid [14].

Cis-9-18:1 is considered to be beneficial for cardiovascular health, as high-MUFA diets have been shown to lower plasma total cholesterol, LDL-cholesterol and TAG concentrations and the substitution of SFA with MUFA has been shown to reduce cardiovascular risk by improving both plasma lipid profile and insulin sensitivity [168,169,170]. These health effects are confirmed also for dairy *cis*-9-18:1 by a clinical trial which reported that daily consumption of 500 mL milk enriched with *cis*-9-18:1, n-3 FA, folic acid, and vitamin E, for 3 months, decreased serum TAG, total cholesterol, LDL-cholesterol, and glucose in patients with metabolic syndrome [171].

3.2.6. *Trans* fatty acids

Trans FA are defined as unsaturated FA with at least one nonconjugated double bond in the *trans* configuration [172]. *Trans* FA present in human diet originate from two sources: the industrial source, in which *trans* FA are mainly derived from partially hydrogenated vegetable oils (*trans* FA *i*), and the natural source, in which *trans* FA are provided by ruminant-derived food products (*trans* FA *r*) [173].

In a study conducted across 14 European countries, *trans* FA *r* from milk and dairy products were found to contribute on average to almost 40% of the total *trans* FA intake [7]. By contrast, in both developed and developing countries, *trans* FA *i* were reported as the major source of *trans* FA in human diet [174]. For both dietary sources, *trans*-18:1 FA are quantitatively the predominant *trans* FA. Typically, *trans*-11-18:1 is the major isomer in ruminant fat, whereas 9, -10, -11, and -12 are the main isomers of *trans*-18:1 in partially hydrogenated oils [28].

In human subjects, *trans* FA adversely affect serum lipid profiles (including increases in LDL-cholesterol and TAG levels, and decreases in HDL-cholesterol levels), systemic inflammation, and endothelial function [174,175]. These potent effects of *trans* FA on a number of cardiovascular risk factors are consistent with the strong association observed between high intakes of *trans* FA and risk of coronary heart disease [28]. Furthermore, extensive research was conducted in a attempt to distinguish between the effects of *trans* FA *r* and *trans* FA *i*.

In this respect, several clinical trials have shown that an intake of *trans* FA representing ≥3% of daily energy, increased LDL-cholesterol more markedly when *trans* FA were provided as *trans* FA *r* than as *trans* FA *i*. In contrast, the total cholesterol:HDL-cholesterol ratio increased equally regardless of the source of *trans* FA [176,177]. Even though *trans* FA *r* and *trans* FA *i* showed similar negative effects on cholesterol homeostasis in the above mentioned clinical trials, it should be taken into consideration that the doses tested exceeded the current estimates for intake of *trans* FA from natural sources in human diet [178]. Moreover, results from animal studies suggest a cardio-protective role of *trans* FA *r* [179]. Consuming a *trans*-9-18:1-rich hydrogenated vegetable shortening stimulated atherosclerosis, whereas a *trans*-11-18:1-rich butter protected against atherosclerosis in LDLr−/− mice [15]. Likewise, a study in rabbits fed a proatherogenic diet, showed that a butter rich in *trans*-10-18:1, an abundant isomer in industrial fats, increased VLDL-cholesterol, LDL-cholesterol, total cholesterol, non-HDL:HDL ratio, and aortic lipid deposition compared to a butter rich in *trans*-11-18:1 and *cis*-9,*trans*-11-CLA [180].

Finally, the intake of some *trans* isomers of 18:2 (e.g. *cis*-9,*trans*-12-18:2, *trans*-9,*cis*-12-18:2, and *trans*-9,*trans*-12-18:2) has been associated to an increased risk of myocardial infarction. However, more research is required to discriminate between industrial and ruminant isomer profiles of fat [18,28].

3.2.7. Conjugated linoleic acid (CLA)

Milk fat is the richest natural common source of conjugated linoleic acid (CLA), with ca. 70% of CLA in human diet coming from milk and dairy products [181,182]. The generic term CLA refers to a group of geometric and positional isomers of linoleic acid with a conjugated double bond located from positions 6-8 to 13-15 of the carbon chain and occuring both in *cis* and *trans* configurations [28,183]. Out the 32 known isomers of CLA, biological effects have been attributed to mainly two of its isomers: *cis*-9,*trans*-11 and *trans*-10,*cis*-12 [21,184]. *Cis*-9,*trans*-11 is the most abundant isomer, comprising 80 to 90% of total CLA in food, whereas *trans*-10,*cis*-12 comprises only 3-5% of total CLA [185].

Following a wide range of *in vitro* and *in vivo* studies, CLA isomers have demonstrated numerous health benefits. CLA, primarily through the isomer *cis*-9,*trans*-11, has been shown to have anticarcinogenic effects in breast, prostate, gastric,

colorectal, liver, lung, and skin cancer [186,187,188,189,190,191,192,193,194,195]. CLA anticarcinogenic activity might be due to anti-proliferative and pro-apoptotic effects observed on several types of human tumor cells [196]. Moreover, CLA has been reported to decrease eicosanoids levels, inhibit angiogenesis and DNA synthesis, all being processes involved in carcinogenesis [197,198,199,200].

Numerous animal and human studies have shown also other positive effects of CLA in relation to human health. CLA has been reported to attenuate cardiovascular risk by reducing atherosclerotic lesions, lowering TAG, total cholesterol, LDL-cholesterol and increasing HDL-cholesterol, respectively [183,201,202,203]. CLA, mainly through the isomer *trans*-10,*cis*-12, has been reported to improve body composition, by lowering body weight, subcutaneous fat mass and increasing lean body mass, respectively [204,205,206,207]. *Trans*-10,*cis*-12-CLA isomer has also been shown to improve blood pressure in obesity-related hypertension [208,209,210]. Moreover, CLA has demonstrated the ability to improve immune function and bone health [211,212,213,214,215,216].

3.2.8. Conjugated alpha-linolenic acid (CLnA)

Milk fat contains small amounts of conjugated alpha-linolenic acid (CLnA) isomers, formed during the biohydrogenation process of ALA [217,218,219]. In several studies, CLnA isomers have demonstrated potent bioactivity [10,220]. *Cis*-9,*trans*-11,*cis*-13-CLnA, *cis*-9,*trans*-11,*trans*-13-CLnA, *trans*-9,*trans*-11,*cis*-13-CLnA, and *cis*-9,*trans*-11,*cis*-15-CLnA have been shown anticarcinogenic properties *in vitro* [220,221,222,223]. Animal studies have also reported hypolipidemic effects for *cis*-9,*trans*-11,*trans*-13-CLnA and anti-adipogenic effects for *cis*-9,*trans*-11,*cis*-13-CLnA [224,225]. Moreover, *cis*-9,*trans*-11,*cis*-13-CLnA has been associated with immunomodulatory properties *in vivo* [226].

3.2.9. n-3 and n-6 fatty acids

LA (18:2 n-6) and ALA (18:3 n-3) are the main PUFA in milk accounting to about 2 g/litre whole milk [10,14]. When obtained from ruminants fed an appropriate diet, milk can be one of the main sources of n-3 FA in the human diet [14]. Moreover, milk is characterised by a beneficial n-6/n-3 FA ratio, with a value as low as 2.1:1 reported in milk from Iceland [227,228]. The recomandation for the n-6/n-3 FA ratio is to not exceed 4:1, in the context in which Western diets are estimated to have currently a ratio of 15/1-16.7/1 [229].

LA and ALA are defined as essential FA, because they are required for survival of humans, but they cannot be synthesised endogenously by humans and have to be obtained from the diet [230,231]. LA and ALA can be coverted by desaturation and elongation processes into AA (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3), which further become substrates for the synthesis of eicosanoids, compounds with important local functions related to human health [14,232].

Evidence from observational studies and clinical trials support the role of n-3 FA together with an optimal n-6/n-3 FA ratio, in the prevention and management of several diseases [231,233]. n-3 FA have been shown to be beneficial on cardiovascular health, by improving blood lipid profile, decreasing blood pressure and reducing platelet aggregation [234,235,236]. n-3 FA have also shown positive effects on patients with obesity, osteoporosis, rheumatoid arthritis and cancer [237,238,239,240,241,242,243].

Likewise, the n-3 FA have been reported to exhibit a beneficial role in the treatment of neurological disfunctions such as depression, psychosis, attention deficit hyperactivity disorder (ADHD) and very mild Alzheimer's disease [244,245,246,247,248]. Moreover, EPA and DHA supplementation in pregnant and breastfeeding women has been indicated to improve the cognitive and visual functions in the baby and to possibly lower the risk of postpartum depression in the mother [249,250,251,252].

3.2.10. Antimicrobial fatty acids

Milk FA have been shown to exhibit antimicrobial properties. 8:0 has been reported to exert antibacterial effects *in vivo* against the foodborne pathogens *Salmonella* Enteritidis and *Campylobacter jejuni* [253,254,255]. 8:0, 10:0, and monocaprin (the monoglyceride of 10:0) have been shown to possess virucidal activity *in vitro* against enveloped viruses such as herpes simplex virus, respiratory syncytial virus, and visna virus [256]. Monocaprin has also been found to exert microbicidal activity *in vitro* against human immunodeficiency virus, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Helicobacter* sp., and *Candida albicans* [257,258,259]. Likewise, 10:0 and 12:0 were reported to be effective bactericidal agents *in vitro* and *in vivo* against *Campylobacter jejuni* and *Listeria monocytogenes* [260].

Moreover, 12:0, *cis*-9-14:1, 18:2 n-6, and 20:4 n-6 as FFA derived from bovine whey cream have been shown to inhibit the germination of *Candida albicans in vitro* [261]. Similarly, 10:0, *cis*-9-12:1, iso-13:0, *cis*-9-14:1, and 18:3n-6 derived from bovine whey cream have demonstrated antifungal activity *in vitro* against *Aspergillus fumigatus* and *Candida albicans* [262].

3.3. Milk vitamin A and its health effects

Vitamin A is an essential liposoluble nutrient which cannot be synthesised in the human body and must be obtained through diet [17]. Milk is a rich source of vitamin A, with one liter of milk covering 25% of the recommended daily intake of β-carotene (provitamin A) and vitamin A [263,264]. Milk fat also serves as an important delivery medium for vitamin A as it has been shown that milk fat is a food matrix which enhances the absorption of liposoluble nutrients [9,16,265]. In this respect, vitamin E provided in milk has demonstrated a higher bioavailability than vitamin E from food supplements [266].

Vitamin A exhibits important functions in the human body including regulation of cell growth and differentiation [17]. Vitamin A is essential for both male and female reproduction as well as a proper embryonic development [267,268]. Vitamin A deficiency may lead to foetal resorption, stillbirth and malformations [117]. Moreover, vitamin A plays a major role in vision so that an insufficient intake of vitamin A can cause severe visual deficiencies such as night blindness or xerophthalmia [269,270]. Vitamin A is also important for the normal functioning of the immune system [117].

3.4. Milk cholesterol and its health effects

In humans, cholesterol is both synthesised endogenously and obtained from dietary sources [264]. With a concentration of about 3 mg cholesterol per g milk fat, milk and dairy products are considered to have a considerable contribution to the cholesterol intake in the human diet [271,272]. In this respect, recent studies indicated that milk and dairy products range second after eggs on contribution to daily cholesterol intake in modern diet, especially among infants [8,273].

Dietary cholesterol is of nutritional interest, as it has been reported to determine hypercholesterolemia. An intake of 100 mg cholesterol has been shown to increase serum cholesterol level by 4–5 mg/dl [89]. Likewise, several prospective cohort studies have indicated a positive association between dietary cholesterol and the risk of cardiovascular disease [3,274]. Nevertheless, this subject is controversial, as there are also numerous epidemiological and clinical data reporting that for the general population, dietary cholesterol has no significant influence on cardiovascular risk [275]. In this regard, it has been shown that hypercholesterolemia is not always a result of an unhealthy diet, but it may be the consequence of an impaired endogenous synthesis of cholesterol [264]. Moreover, it is important to mention that cholesterol exhibits also positive roles in the human body: it serves as a functional component of the cell membrane and it constitutes a precursor for steroid hormones, bile acids and vitamin D_3 [264,271].

PERSONAL CONTRIBUTIONS

1. Aims

There is considerable interest in altering the fat composition of milk with the overall aim of improving the long-term health of consumers [4]. Important targets include enhancing the concentrations of *cis*-9-18:1, n-3 fatty acids (FA), decreasing medium-chain saturated FA, *trans* FA, and cholesterol to reduce cardiovascular disease risk, as well as increasing the content of *cis*-9,*trans*-11-CLA [3,10,14,28]. There is also interest in enhancing the amount of vitamin A in milk, due to the important role of this liposoluble component in the regulation of cell growth and differentiation [17].

In this respect, over the past decades, the modulation of milk fat composition has been a great challenge for scientists working on ruminants [18]. Milk fat composition is mainly linked to nutritional factors and also factors linked to animal (species, breed, genotype, lactation stage, parity) [19]. A number of studies has focused on breed effect on milk FA composition using Holstein, Jersey, Montbéliarde, and Normande breeds [25,81,84,86]. However, few studies were performed on the Salers breed, a dual-purpose breed originating from the Auvergne region of France and oriented mainly towards beef production [276]. Moreover, to our knowledge, no previous work has been published on the comparison between Holstein and Salers cows.

Characteristic for Salers cows is their very time-consuming process of milking, referred to as "traditional", that requires the presence of the calf to stimulate milk ejection [24]. The available literature indicates that in Salers cows, a reduction in cow-calf contact has a negative effect on the fat content of milked milk [24]. Nevertheless, maintaining calf contact during milking considerably increases the milking time, so suppressing the contact would simplify the milking routine. Therefore, it is important to analyse whether calf presence (vs. calf removal) influences milk characteristics, notably milk FA profile because this topic is not documented in the literature. Likewise, few studies have been published on the effect of cow-calf contact during milking on the milk lipolytic system. Milk fat lipolysis is the hydrolysis of fat globule triglycerides into free FA by milk lipoprotein lipase and endogenous lipases [81]. The development of a rancid flavour in cold stored milk is due to free FA [60]. Reports have shown that breed has a major influence on the milk lipolytic system in dairy goats, but little information is available for dairy cows [60,81].

Moreover, previous studies indicate that milk FA profile is strongly influenced by the nature of forage (preserved vs. grazed grass) in ruminant diet [18]. Cow diet could also have an effect on milk lipolytic system. It has been shown that grass-based diets have an influence on the milk lipolytic system in midlactating Tarentaise and Montbéliarde cows, but this factor is not documented in dairy Salers cows [81]. In lactating cows, previous studies indicated also a minor effect of parity on milk FA composition [25]. Ultimately, little is known about the influence of parity on milk lipolytic system.

Important changes in milk fat composition can also be obtained by supplementing plant lipids to the diet [18,22]. For both cows and goats, supplementation of diet with vegetable oils can modify milk FA profile by decreasing saturated FA and increasing polyunsaturated FA [20]. Likewise, adding plant lipids to cow diets can increase milk vitamin A content and decrease milk cholesterol content [22]. Although various lipid sources have been added to ruminant diet to modify milk fat composition, to our knowledge, no previous study has used hemp seed oil. Hemp seed oil is obtained from industrial hemp, a traditional plant in Romanian agriculture, and is characterized by a high content of 18:2 n-6 and 18:3 n-3 (59.8% and 18.2% of total FA, respectively). Furthermore, increasing the level of polyunsaturated FA in milk by feeding plant lipids to ruminants could decrease the oxidative stability of milk, due to high susceptibility of these FA to oxidation [21]. In order to analyse the oxidation status of milk, the concentration of malondialdehyde in milk can be determined [277]. Finally, to our best knowledge, no study has been conducted to evaluate the effect of diet supplementation with vegetable oils on the vitamin A and cholesterol concentrations and oxidative stability in goat milk.

Given all these aspects, the first study included in the present work had the main objective to determine the influence of calf presence during milking and cow breed on FA profile and lipolytic system in bulk milk from Prim'Holstein and Salers cows. The research continued with a second study mainly aimed to evaluate the effects of calf presence, cow parity and season on FA composition and lipolytic system in individual milk samples from Salers cows. The work was completed by a third study with the objective to analyse the influence of a diet supplemented with hemp seed oil on liposoluble components (FA, vitamin A, and cholesterol) concentrations and oxidative stability in milk from Carpathian goats.

2. Study 1. Influence of calf presence during milking on fatty acid profile and lipolytic system of milk in Prim'Holstein and Salers cow breeds

2.1. Introduction

Milk fat content and its fatty acid (FA) profile are important components of milk quality, influencing its transformation into cheese as well as the sensorial and nutritional properties of milk and milk products [18,278].

Diet plays a major role if compared to animal-related factors (species, genotype and breed) in determining variations of the FA composition of milk fat [18]. Few experiments have been conducted comparing Holstein and Jersey breeds [25,81,84]. In the present experiment, two cow breeds were selected: Prim'Holstein (PH), the main French cow breed used for milk production, and Salers (S), a dual-purpose breed originating from the northern half of the Massif Central in the Auvergne region of France. Over the past 30 years, Salers breeding has mainly been oriented towards beef production. Currently, the S cows are reared mainly in suckling livestock systems, and no more than 2% of the French population of S cows is still milked. Even though they are marginal in the production of Auvergne milk, the image of S cows is used to promote some local Auvergne PDO (Protected Designation of Origin) cheese varieties, such as Cantal, Salers or Saint-Nectaire cheeses. With approximately 80 farmers located in the Puy de Dôme and Cantal departments of Auvergne, the Salers dairy system is sparse [279], and the S cows have been replaced by more specialised PH or Montbéliarde cows. The decrease in the number of S cows is related to their low production level (2400 vs. 9000 kg/lactation for Prim'Holstein) [279] and to the very time-consuming process of milking, referred to as "traditional", that requires the presence of the calf to stimulate milk ejection [24]. A range of studies has focused on breed effect using Holstein, Jersey, Montbéliarde and Normande breeds [25,84,86], but few studies were performed on the Salers breed. Moreover, to our knowledge, no previous study has compared the Holstein and Salers breeds.

The available literature indicates that in *Bos taurus* × *Bos indicus* cows, as in S cows, a reduction in cow-calf contact has a negative effect on milk yield and on the fat content of milked milk [24,280]. Nevertheless, maintaining calf contact during milking

considerably increases the time that the farmer must work, so suppressing the contact would simplify the milking routine. Therefore, it is important to analyse whether calf presence vs. calf removal influences milk characteristics, notably cheese-making, milk protein and fat contents, as well as milk FA profile because this topic is not documented in the literature. Likewise, few studies have been published on the effect of cow-calf contact during milking on the milk lipolytic system. Milk fat lipolysis is the hydrolysis of fat globule triglycerides into free FA (FFA) by milk lipoprotein lipase and endogenous lipases [81]. The development of a rancid flavour in cold stored milk is due to FFA, especially to free butyric acid [60]. Reports have shown that breed has a major influence on the milk lipolytic system in dairy goats, but little information is available for dairy cows [60].

2.2. Aims

The objective of this study was to determine the influence of both calf presence and cow breed on milk yield and composition, FA profile and milk lipolytic system.

2.3. Materials and methods

2.3.1. Cows and diets

The study was conducted between October 2009 and June 2010 at the experimental farm of Marcenat - "Institut National de la Recherche Agronomique" (INRA) - located in a mountainous (1135-1215 m above sea level) region of Auvergne (Cantal, France). The duration of the study was 6 weeks from December 2009 14th. A total of thirty-six PH (38±11.6 days in milk - DIM – on December 2009 15th, body weight - BW - 558.2 kg on average during the lactation period, the interval between the extreme dates of parturitions was 36 days) and S (40.3±9.4 DIM, BW 613.8 kg on average, the interval between the extreme dates of parturitions was 36 days) primiparous lactating cows were selected before parturition according to estimated calving date, their potential milk yield (as defined as the best milk yield during an entire lactation of the mother plus the half of the best milk yield of the paternal grandmother recorded by the Recording Milk Organisation), BW and body condition score and divided into four groups: one group of PH cows milked in the presence of the calf (PHCP, n = 6), one group of PH cows milked in the absence of the calf (PHCA, n = 6), one group of S cows milked in the presence of the calf (SCP, n = 9) and one group of S cows milked in the absence of the calf (SCA, n = 15). Among the initial fifteen SCA cows, six cows dried up before DIM 21 and were excluded from the study so that the final number of the SCA group was 9.

The cows were housed in five pens and the calves in a separate pen located in the same barn. Immediately after the parturition, the cows were machine-milked twice daily at 6:00 and 15:30 h. For the cows milked in the presence of the calf, the mother-

calf pair remained together from 24 to 48 h after calving, with cows being milked at the usual hours. At the end of the second milking, the young returned to the calf pen, and the dam was placed into the cow pen. For these cows, milking included a phase of calf suckling before milking (for about 1 min) and then the calf was placed in a pen in front of his mother during milking. Physical contact was allowed, and at the end of milking, the mother-calf pair was placed into a pen, where suckling was allowed until complete emptying of the udder. For the cows milked in the absence of the calf, the newborn calves were separated from their mother immediately after parturition, without any sniffing, licking or suckling.

The total amount of milked milk was recorded individually at each milking by using a continuous milk weighing system. On two consecutive days every week, before suckling and after udder-emptying, calves from both the SCP and the PHCP groups were weighed to estimate the amount of suckled milk according to the method described by Le Neindre [281].

Before calving, the cows were fed collectively with concentrate and 5 kg dry matter grassland hay (regrowth) after morning milking, while grassland hay was offered *ad libitum* in the afternoon. The hay was composed of 87% grasses (essentially 83% *Dactylis glomerata*, 2.2% *Elytrigia repens*, and 1.2% ryegrass), and 12.7% forbs. The regrowth hay was composed of grasses (26% *Poa pratensis*, 19% *Dactylis glomerata*, 16% *Alopecurus pratensis* and 14% *Agrostis capillaris*), and 25% of forbs.

After calving, the animals received the concentrate according to their daily milk production during the morning and afternoon milkings, whereas grassland hay was offered *ad libitum*. The intake amounts were measured on average from 2 pens per group. The feedstuffs distributed per pen were weighted daily. Any refusals were collected and weighed daily (4 days each week) to calculate the net intake of each group. The nutritive value was 4.97 MJ of Net Energy (NE), 80.3 g PDIE and 75 g PDIN for the first hay and 4.97 MJ of NE, 105 g PDIE, 89 g PDIN for the regrowth hay. The control concentrate contained 26.4% barley, 20% wheat bran, 15% triticale, 10% rootlets, 5% wheat, 13.6% rapeseed meal, 4.8% sunflower meal, 2% molasses, 1.5% rapeseeds and 1.75% minerals and premix. The nutritive value of the concentrate was 6.96 MJ of NE, 2.4 % fat, 7.9% crude fiber, 15% crude protein, 116 g PDIE and 115 g PDIN and 32.2% starch. During the experimental period, the PH and S cows were fed on average 8.8 kg and 2.6 kg of concentrate, respectively. The forage to concentrate ratio in the diet was 52 and 23% for PH and S groups, respectively. The diets were formulated to cover 100% of the cow energy and protein requirements [282].

Before December 15th, calves were fed only mother milk. After this date, calves were fed progressively in addition to mother milk 500 g concentrate (dry matter basis) formulated to heifer (6% crude fiber and 17% crude proteins). From April 15th they

received 1 kg concentrate (dry matter basis). Hay was offered *ad libitum*. They were not weaned during the experimental period.

Experimental procedures were approved by the INRA Animal Care and Use Committee and conducted according to French recommendations for the use of experimental animals, including animal welfare and appropriate conditions [283].

2.3.2. Sampling, measurement and analyses

The bulk morning milk was pooled with the previous evening's milk, which had been stored at 4°C, for each group, and thus the bulk milks were collected in four separate tanks corresponding to each experimental group on seven dates (December 15th, 17th and 21th 2009, and January 6th, 11th, 13th and 20th 2010). A set of sub-samples (50 mL) was preserved in tubes with bronopol-B2 (Trillaud, Surgères, France) and stored at 4˚C until analysis for fat, protein, lactose, and somatic cell counts using infrared spectrophotometry (Galilait, Theix, France) according to standard procedures [284]. The somatic cell counts data was log-transformed in order to achieve normality.

A second set of milk sub-samples (3 mL) was stored at -20˚C until the end of the experiment before lyophilisation (Thermovac TM-20, Froilabo, Ozoir-La-Ferrière, France), for analysis of the FA profile. The FA in the lyophilised milk samples were methylated directly according to Ferlay et al. [86] with some modifications. Briefly, 2 mL 0.5 mol/L sodium methanolate and 1 mL hexane were mixed with the lyophilised milk at 50°C for 15 min, followed by the addition of 1 mL 12 N HCl 5% in methanol (v/v) at 50°C for 15 min. The FA methyl esters (FAME) were washed with a saturated K_2CO_3 solution and recovered with 1.5 mL hexane. The FAME were injected (0.6 µL) by auto-sampler into a gas chromatograph equipped with a flame ionisation detector (Agilent Technologies 7890A, Wilmington, USA). The FAME from all the samples were separated on a 100 m × 0.25mm i.d. fused-silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was maintained at 255°C and the detector temperature at 260°C. The initial oven temperature was held at 70°C for 1 min, increased by 5°C/min to 100°C (held for 2 min), and then increased by 10°C/min to 175°C (held for 42 min), and 5°C/min to a final temperature of 225°C (held for 15 min). The carrier gas was hydrogen, and constant pressure (158.6 kPa) was maintained during analysis. A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain FA (4:0 to 10:0). Identification of FAME was accomplished by comparison to a standard mixture purchased from Nu-Chek-Prep, Inc (Elysian, MN 56028 USA). Mixtures of *cis*/*trans* (9-12) isomers of linoleic acid methyl ester and *cis* and *trans* (9-11) and (10-12) isomers of CLA methyl esters purchased from Sigma-Aldrich Corporation (38297 Saint Quentin Fallavier, France) were used to complete the identification.

A third set of sub-samples was used to evaluate milk lipolysis by determining the initial FFA content and the FFA content after 24 h of cold storage (4˚C). Immediately after sampling, 18-mL samples of milk were taken, heated at 60˚C for 30 min to destroy lipase activity and stored at 4˚C until analysed for initial FFA content (lipolysis at t_0). A second 18-mL sample was stored at 4˚C for 24 h, then heated and stored at 4˚C until analysed for FFA content (lipolysis at t_{24}). The milk lipolytic system was evaluated by the copper soap method [285].

2.3.3. Statistical analyses

Data from individual milks were analysed by variance analysis using the GLM procedure [286], with breed (B), treatment (T, presence or absence of the calf during milking) and interaction between breed and treatment (B × T) as main effects.

Data for bulk milk composition, FA profile and lipolytic system were analysed by the mixed models procedure of SAS software [286] with breed (B), treatment (T) and interaction between breed and treatment (B × T) as fixed effects, and cheesemaking dates as random effect. Treatment differences were determined based on *t*-tests and declared significant at $P \leq 0.05$. Trends toward significance were considered at $0.05 < P \leq 0.10$.

2.4. Results

2.4.1. Milk yield and composition

Milk yield, milk fat, protein and lactose contents and somatic cell counts are shown in Table II. During the experimental period, the average milk yield was 26.9 L/d and 11.5 L/d for PH and S cows, respectively. The average amount of suckled milk was 4.2 and 5.2 L/calf/day for PH and S cows, respectively. The milked milk represented 84 and 64% of milk yield for PH and S cows, respectively. The milk yield of the SCP group was higher than that of the SCA group, whereas it did not differ between PHCP and PHCA groups ($P < 0.05$).

The SCA group had higher (significant interaction, $P < 0.05$) milk fat content than the SCP group, whereas no difference ($P < 0.05$) was observed for the PH groups. The milk protein content was higher for the S than for the PH groups ($P < 0.01$). The milk protein content was decreased by the presence of the calf ($P < 0.05$). Concerning lactose, a significant breed × treatment effect was found ($P < 0.001$). In the presence of the calf, the lactose tended to decrease (-2%) and to increase (+ 2%) for the PH and S cows, respectively. The presence of the calf only decreased the somatic cell counts in milk from PH cows (significant interaction, $P < 0.01$).

Table II. Influence of cow breed, calf presence during milking, and their interaction on milk yield and composition and milk lipolytic system

	PH		S		SEM[f]	Significance (*P*-value)[g]		
	CP	CA	CP	CA		B	T	B × T
Milked milk[d] (L/d)	22.9[a]	26.6[a]	9.1[b]	8.7[b]	2.89	***	ns	+
Suckled milk[d] (L/d)	4.2	-	5.2	-	0.78	*		
Total milk yield[d] (L/d)	27.1[a]	26.6[a]	14.3[b]	8.7[c]	2.87	***	**	*
Fat content[e] (g/kg)	31.81[a]	33.31[a]	26.83[b]	35.30[a]	1.67	ns	***	*
Protein content[e] (g/kg)	30.08	30.84	31.58	35.04	0.95	**	*	ns
Lactose content[e] (g/kg)	52.03[b]	53.27[a]	53.10[a]	52.50[b]	0.17	ns	+	***
Somatic cell counts[e] (log/mL)	4.49[b]	5.04[a]	4.93[a]	4.90[a]	0.11	ns	*	**
Lipolysis at t_0[e] (%)	0.79	0.66	0.84	0.83	0.04	**	+	ns
Lipolysis at t_{24}[e] (%)	0.98[a]	0.75[b]	0.97[a]	0.93[a]	0.04	*	**	*
Lipolysis$_{\Delta 24h}$[e] (%)	0.19[a]	0.09[b]	0.13[c]	0.10[bc]	0.03	ns	***	*

PH = Prim'Holstein; S = Salers; CP = calf present during milking; CA = calf absent during milking.
B = breed (PH or S); T = treatment (CP or CA); B × T = breed × treatment interaction.
LipolysisΔ24h = evolution of milk lipolysis after 24 h (Lipolysis at t_0 - Lipolysis at t_{24}).
SEM = Standard Error of the Mean; ns = not significant.
[a-c] Within row means with different letters differ at $P < 0.05$.
[d] Individual milks.
[e] Bulk milks. The number of samples were 7, 7, 7, and 7 for PHCP, PHCA, SCP and SCA groups, respectively.
[f] For individual milks, root SEM is presented.
[g] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

2.4.2. Milk fatty acid profile

The milk FA composition is presented in Tables III and IV.

The milk fat of PH cows had higher percentages of individual saturated FA (SFA) (4:0 ($P < 0.01$), 6:0 ($P < 0.001$), 7:0 ($P < 0.01$), 8:0 ($P < 0.001$), 9:0 ($P < 0.001$), 10:0 ($P < 0.01$), 11:0 ($P < 0.001$), 12:0 ($P < 0.05$), anteiso 17:0 ($P < 0.05$)), total monounsaturated FA (MUFA) ($P < 0.10$), and individual MUFA (*trans*-11-16:1 ($P < 0.01$), *cis*-9-16:1 ($P < 0.05$), *cis*-11-20:1 ($P < 0.001$), *trans*-5-18:1 ($P < 0.05$), *trans*-6, 8-18:1 ($P < 0.01$), *trans*-10-18:1 ($P < 0.001$), *trans*-12-18:1 ($P < 0.001$), *trans*-13-18:1 ($P < 0.001$), *cis*-9-18:1 ($P < 0.10$), *cis*-11-18:1 ($P < 0.001$), *cis*-12-18:1 ($P < 0.001$), *cis*-13-18:1 ($P < 0.001$), *cis*-14-18:1 ($P < 0.001$), total *cis*-18:1 ($P < 0.05$), and 18:2 n-6 ($P < 0.001$) than that of S cows. In contrast, PH cows had lower milk fat concentrations of 14:0 ($P < 0.10$), 15:0 ($P < 0.001$), anteiso 15:0 ($P < 0.001$), iso 16:0 ($P < 0.001$), 17:0 ($P < 0.001$), iso 17:0 ($P < 0.001$), *cis*-9,*cis*-11-CLA ($P < 0.001$), *trans*-9,*trans*-11-CLA ($P < 0.05$), 20:0 ($P < 0.001$), 20:2 n-6 ($P < 0.10$), 22:0 ($P < 0.001$), 20:5 n-3 ($P < 0.001$), and 22:5 n-3 ($P < 0.05$), if compared to S cows.

Calf presence during milking increased *cis*-9-14:1 ($P < 0.001$), 16:0 ($P < 0.01$), *cis*-9-16:1 ($P < 0.001$), 17:0 ($P < 0.10$), and anteiso 17:0 ($P < 0.001$) concentrations, whereas it decreased the concentrations of 8:0 ($P < 0.10$), anteiso 15:0 ($P < 0.01$), 18:0 ($P < 0.05$), several MUFA (*trans*-10-18:1 ($P < 0.10$), *trans*-13-18:1 ($P < 0.01$), *cis*-12-18:1 ($P < 0.05$), 18:2 n-6 ($P < 0.001$), 20:0 ($P < 0.05$), 20:4 n-6 ($P < 0.001$), 22:5 n-3 ($P < 0.01$), and total polyunsaturated FA (PUFA) ($P < 0.001$).

Several significant interactions between cow breed and calf treatment were also observed (Tables III and IV). The presence of the calf decreased milk concentrations of *cis*-14-18:1 ($P < 0.10$) and 18:3 n-3 ($P < 0.10$) (Fig. 3) more markedly for S than for PH cows. In contrast, the presence of the calf decreased the milk concentration of 20:3 n-6 ($P < 0.001$) more markedly for PH than for S cows. The milk fat 15:0 ($P < 0.001$), *cis*-9,*trans*-11-CLA ($P < 0.001$) (Fig. 4), total CLA ($P < 0.001$) (Fig. 5), total odd- and branched-chain FA (OBCFA) ($P < 0.01$) concentrations were decreased with S cows in the presence of the calf, whereas they were increased with PH cows. In contrast, iso 18:0 concentration did not change in the presence of the calf with PH cows and increased with S cows. In the case of iso 13:0 ($P < 0.05$), iso 15:0 ($P < 0.001$), iso 14:0 ($P < 0.01$), *trans*-9-18:1 ($P < 0.01$), *trans*-11-18:1 ($P < 0.05$) (Fig. 6), *trans*-12-18:1 ($P < 0.10$), total *trans*-18:1 ($P < 0.05$) (Fig. 7), *trans*-11,*cis*-15-18:2 ($P < 0.05$) (Fig. 8), and total *trans* FA ($P < 0.05$), the presence of the calf produced a decrease in their concentrations only with S cows. In a similar manner, the presence of the calf increased 5:0 ($P < 0.05$) and decreased *cis*-9-12:1 ($P < 0.10$) and 13:0 ($P < 0.10$) concentrations with PH cows, but had no influence on these FA concentrations with S cows.

Table III. Influence of cow breed, calf presence during milking, and their interaction on milk FA composition

	PH		S		SEM	Significance (*P*-value)[e]		
	CP	CA	CP	CA		B	T	B × T
Fatty acid (g/100 g of FA)[f]								
4:0	2.89	2.86	2.77	2.74	0.07	**	ns	ns
5:0	0.029[a]	0.023[b]	0.019[c]	0.020[b]	0.001	***	ns	*
6:0	2.30	2.30	2.14	2.13	0.02	***	ns	ns
7:0	0.02	0.02	0.02	0.02	0.001	**	ns	ns
8:0	1.41	1.45	1.28	1.30	0.02	***	+	ns
9:0	0.03	0.03	0.02	0.02	0.002	***	ns	ns
10:0	3.07	3.21	2.86	2.93	0.10	**	ns	ns
10:1	0.26	0.25	0.25	0.24	0.01	+	ns	ns
11:0	0.05	0.05	0.04	0.04	0.003	***	ns	ns
12:0	3.34	3.50	3.21	3.28	0.14	*	ns	ns
cis-9-12:1	0.06	0.08	0.07	0.07	0.008	ns	+	+
13:0	0.06	0.08	0.07	0.07	0.008	ns	+	+
iso 13:0	0.03[c]	0.03[c]	0.04[b]	0.05[a]	0.001	***	**	*
anteiso 13:0	0.01	0.01	0.02	0.02	0.003	ns	ns	ns
14:0	11.12	11.12	11.44	11.32	0.35	+	ns	ns
iso 14:0	0.11[c]	0.12[c]	0.20[b]	0.23[a]	0.004	***	***	**
cis-9-14:1	0.83	0.74	0.80	0.72	0.05	ns	***	ns
iso 15:0	0.25[c]	0.25[c]	0.37[b]	0.41[a]	0.01	***	***	***
anteiso 15:0	0.49	0.51	0.70	0.73	0.02	***	**	ns
15:0	1.07[c]	1.04[c]	1.34[b]	1.41[a]	0.03	***	ns	***
16:0	27.27	26.48	27.52	26.38	0.58	ns	**	ns
iso 16:0	0.27	0.27	0.45	0.49	0.01	***	+	ns
trans-11-16:1	0.20	0.20	0.18	0.19	0.006	**	ns	ns
cis-9-16:1	1.30	1.17	1.23	1.15	0.02	*	***	ns
cis-11-16:1	0.04[a]	0.04[a]	0.04[a]	0.03[b]	0.002	***	**	*
17:0	0.71	0.70	0.80	0.78	0.01	***	+	ns
iso 17 :0	0.36	0.37	0.41	0.42	0.01	***	ns	ns
anteiso 17:0	1.30	1.17	1.23	1.15	0.02	*	***	ns
18:0	10.34	10.91	10.44	10.58	0.43	ns	*	ns
iso 18:0	0.06[b]	0.07[b]	0.08[a]	0.07[b]	0.003	*	ns	*
cis-9,*trans*-13-18:2	0.14	0.15	0.13	0.14	0.008	ns	ns	ns
trans-11,*cis*-15-18:2	0.08[c]	0.07[c]	0.11[b]	0.13[a]	0.007	***	ns	*
20:0	0.17	0.17	0.20	0.21	0.006	***	*	ns
cis-11-20:1	0.07	0.07	0.05	0.06	0.004	***	ns	ns

Table III. Influence of cow breed, calf presence during milking, and their interaction on milk FA composition (continued)

	PH		S		SEM	Significance (*P*-value)[e]		
	CP	CA	CP	CA		B	T	B × T
Fatty acid (g/100 g of FA)[f]								
cis-9,*trans*-11-CLA	0.57[c]	0.53[d]	0.86[b]	0.90[a]	0.03	***	ns	***
cis-9,*cis*-11-CLA	0.05	0.04	0.07	0.07	0.006	***	ns	ns
trans-11,*trans*-13-CLA	0.03	0.03	0.03	0.02	0.003	ns	ns	ns
trans-9,*trans*-11-CLA	0.03	0.02	0.03	0.03	0.003	*	ns	ns
18:2 n-6	2.00	2.13	1.43	1.54	0.09	***	***	ns
18:3 n-3	0.82	0.85	0.91	1.00	0.02	***	***	+
20:2 n-6	0.03	0.04	0.04	0.04	0.003	+	ns	ns
22:0	0.07	0.06	0.10	0.10	0.004	***	ns	ns
20:3 n-6	0.07	0.09	0.07	0.08	0.003	ns	***	+
20:4 n-6	0.11	0.12	0.12	0.12	0.003	ns	***	ns
20:5 n-3	0.07	0.07	0.08	0.08	0.003	***	ns	ns
22:5 n-3	0.13	0.16	0.16	0.17	0.01	*	**	ns
22:6 n-3	0.02	0.02	0.02	0.02	0.002	ns	ns	ns
Total SFA	64.19	64.26	64.57	63.63	0.79	ns	ns	ns
Total OBCFA	4.88[c]	4.77[d]	5.82[b]	5.94[a]	0.10	***	ns	**
Total MUFA	27.60	27.45	26.46	27.03	0.72	+	ns	ns
Total *trans* FA	4.36[c]	4.31[c]	4.77[b]	5.04[a]	0.22	***	+	*
Total PUFA	4.38	4.55	4.29	4.60	0.13	ns	***	ns
Total CLA	0.68[c]	0.62[d]	0.99[b]	1.03[a]	0.03	***	ns	***
Short-chain FA	13.41	13.72	12.62	12.73	0.25	***	ns	ns
Medium-chain FA	45.09	44.08	46.68	45.42	1.07	**	*	ns
Long-chain FA	41.92	42.71	40.53	41.77	1.45	*	+	ns
Desaturase ratio								
cis-9-14:1/14 :0	0.07	0.06	0.07	0.06	0.003	*	***	ns
cis-9-16:1/16 :0	0.02	0.02	0.02	0.02	0.001	ns	ns	+
cis-9-18:1/18:0	2.02	1.91	1.91	1.93	0.04	+	*	ns
cis-9,*trans*-11-CLA/*cis*-9-18:1	0.027[a]	0.025[b]	0.043[c]	0.044[c]	0.001	***	ns	**

PH = Prim'Holstein; S = Salers; CP = calf present during milking; CA = calf absent during milking; B = breed (PH or S); T = treatment (CP or CA); B × T = breed × treatment interaction; CLA = conjugated linoleic acid; SFA = saturated FA; OBCFA = odd- and branched-chain FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; short-chain FA = from 4:0 to 12:0; medium-chain FA = from 13:0 to *cis*-9-17:1; long-chain FA = from 18:0 to 22:6 n-3; SEM = Standard Error of the Mean; ns = not significant.

[a-d] Within row means with different letters differ at $P < 0.05$.

[e] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

[f] The number of samples were 7, 7, 7, and 7 for PHCP, PHCA, SCP and SCA groups, respectively.

Table IV. Influence of cow breed, calf presence during milking, and their interaction on concentrations of *trans* and *cis* isomers of 18:1 in milk fat

	PH		S		SEM	Significance (*P*-value)[c]		
	CP	CA	CP	CA		B	T	B × T
Fatty acid (g/100 g of FA)[e]								
trans-4-18:1	0.02	0.02	0.02	0.01	0.002	ns	ns	ns
trans-5-18:1	0.02	0.02	0.01	0.01	0.002	*	ns	ns
trans-6,8-18:1	0.25	0.25	0.23	0.24	0.02	**	ns	ns
trans-9-18:1	0.21[a]	0.21[a]	0.19[b]	0.21[a]	0.009	*	+	**
trans-10-18:1	0.30	0.31	0.18	0.20	0.02	***	+	ns
trans-11-18:1	1.40[b]	1.42[b]	1.89[a]	1.96[a]	0.11	***	ns	*
trans-12-18:1	0.25	0.25	0.18	0.21	0.02	***	*	+
trans-13-18:1	0.46	0.49	0.33	0.39	0.02	***	**	ns
Sum of *trans*-18:1	2.97[b]	2.98[b]	3.05[b]	3.26[a]	0.18	***	*	*
cis-9-18:1	20.86	20.82	19.90	20.30	0.61	+	ns	ns
cis-11-18:1	0.74	0.75	0.58	0.60	0.02	***	ns	ns
cis-12-18:1	0.16	0.17	0.09	0.12	0.02	***	*	ns
cis-13-18:1	0.09	0.08	0.05	0.06	0.006	***	ns	ns
cis-14-18:1	0.25	0.26	0.21	0.24	0.01	***	***	+
Sum of *cis*-18:1	22.11	22.10	20.84	21.32	0.65	*	ns	ns

PH = Prim'Holstein; S = Salers; CP = calf present during milking; CA = calf absent during milking.
B = breed (PH or S); T = treatment (CP or CA); B × T = breed × treatment interaction.
SEM = Standard Error of the Mean; ns = not significant.
[a-b] Within row means with different letters differ at $P < 0.05$.
[c] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
[e] The number of samples were 7, 7, 7, and 7 for PHCP, PHCA, SCP and SCA groups, respectively.

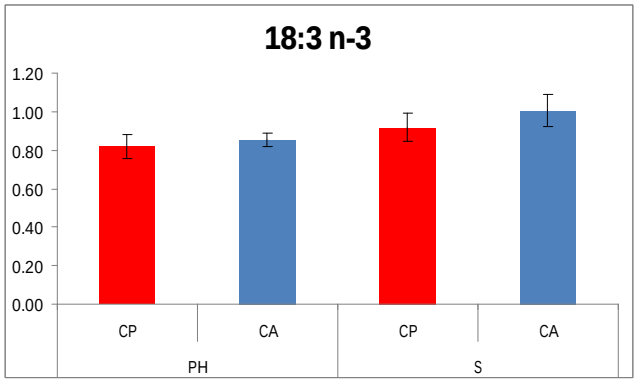

Fig.3. Concentration of **18:3 n-3** in milk fat from Prim'Holstein (PH) and Salers (S) cows, milked in the presence (CP) or the absence (CA) of the calf (breed × treatment interaction, $P < 0.10$)

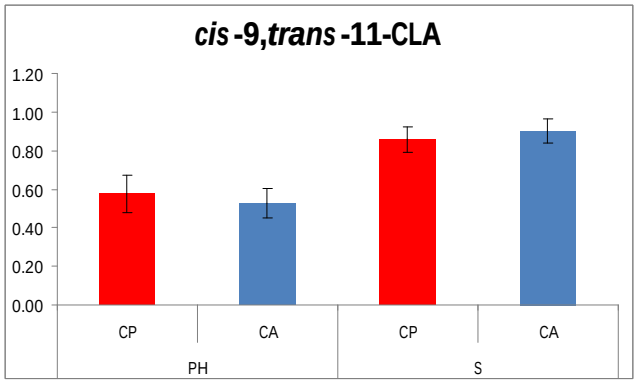

Fig.4. Concentration of ***cis*-9,*trans*-11-CLA** in milk fat from Prim'Holstein (PH) and Salers (S) cows, milked in the presence (CP) or the absence (CA) of the calf (breed × treatment interaction, $P < 0.001$)

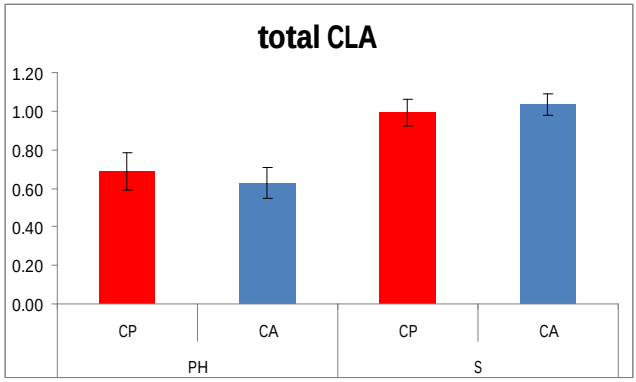

Fig.5. Concentration of **total CLA** in milk fat from Prim'Holstein (PH) and Salers (S) cows, milked in the presence (CP) or the absence (CA) of the calf (breed × treatment interaction, $P < 0.001$)

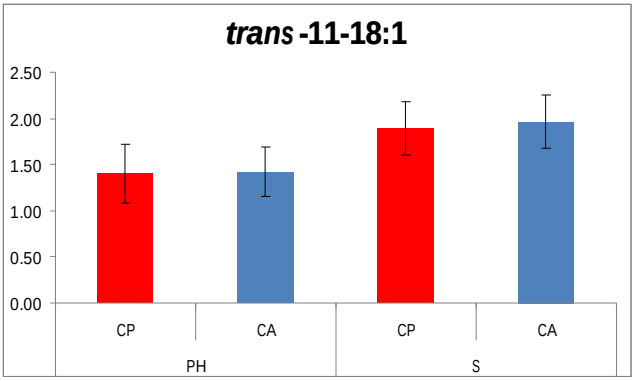

Fig.6. Concentration of ***trans*-11-18:1** in milk fat from Prim'Holstein (PH) and Salers (S) cows, milked in the presence (CP) or the absence (CA) of the calf (breed × treatment interaction, $P < 0.05$)

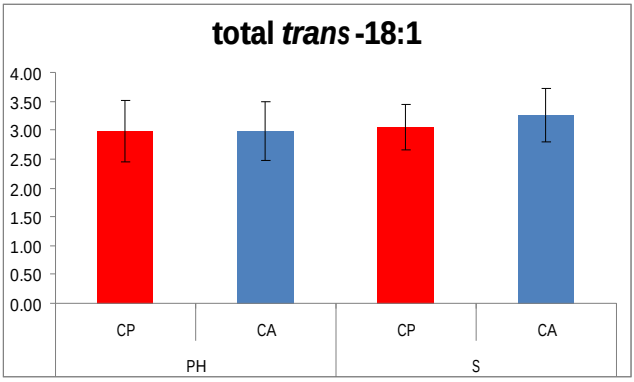

Fig.7. Concentration of **total *trans*-18:1** in milk fat from Prim'Holstein (PH) and Salers (S) cows, milked in the presence (CP) or the absence (CA) of the calf (breed × treatment interaction, $P < 0.05$)

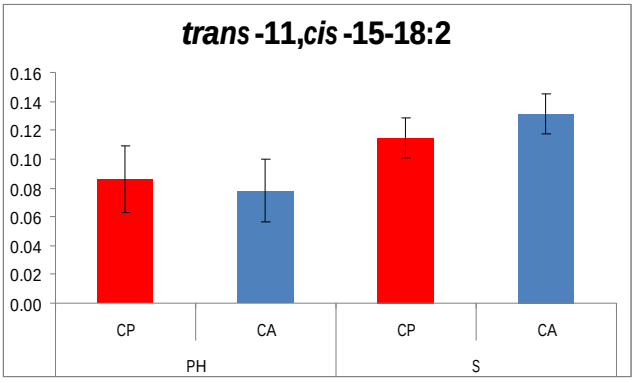

Fig.8. Concentration of ***trans*-11,*cis*-15-18:2** in milk fat from Prim'Holstein (PH) and Salers (S) cows, milked in the presence (CP) or the absence (CA) of the calf (breed × treatment interaction, $P < 0.05$)

2.4.3. Milk lipolysis

Initial lipolysis was more important ($P < 0.01$) in the milk of S cows than in the milk of PH cows. The presence of the calf tended to increase the initial lipolysis ($P < 0.10$, Table II). The presence of the calf only increased the lipolysis measured after 24 h for the PH cows ($P < 0.01$).

2.5. Discussion

2.5.1. Milk yield and composition

The average milk yield of each breed during the experimental period highlighted a large difference in production potential between the two cow breeds. Our results are in agreement with data provided by the Institut de l'Elevage [279], reporting a milk yield of 8396 kg/lactation for PH primiparous cows vs. 1954 kg/lactation for S primiparous cows. The difference in milk yield between the PH and S breeds is the result of a long selection process for milk productivity and thus could induce a difference in the size of the mammary cisterns, animals with large cisterns such as PH cows being more efficient milk producers [287].

The more significant decrease ($P < 0.05$) in total milk yield without the calf for the S than for the PH groups could be a consequence of a highly developed maternal instinct in S cows [24]. For PH group, our results are in agreement with Bar-Peled et al. [288] using Holstein cows milked 3 times daily compared to Holstein cows milked and suckled 3 times for a 6-week postpartum period. Our results agree also with Tesorero et al. [289], showing that Brahman × Holstein cows milked in the absence of their calves produced less milk (- 2 kg/day) than cows for which suckling was allowed before each milking. Likewise, Sidibé-Anago et al. [290] observed in a study with Zebu cows, that the longer the suckling period was, the higher was the total milk yield. The increasing milk yield of cows milked in the presence of the calf could be due to higher milk synthesis by the mammary gland as a result of higher udder stimulation, initiating oxytocin release and therefore milk ejection during suckling [24,291]. In contrast, De Passillé et al. [292] reported no difference with or without the calf during milking in Holstein cows and concluded that there is no obvious advantage for milk production in keeping the calf with the cow.

Milk fat content was decreased in the presence of the calf, in agreement with Mendoza et al. [293], who observed in similar experiments on Holstein cows, a decrease of approximately 22% in the fat content for cows milked in the presence of the calf. This could be explained by the fact that when a calf empties the udder after the milking, it drinks the residual milk (the alveolar milk), having a higher fat content, and therefore causes a decrease in the fat content of the milked milk (corresponding to the cisternal milk) [294,295]. In disagreement with our results, McKusick et al. [291] using East Friesian dairy ewes provided evidence that there is a reduction in the amount of

milk fat transferred from the alveoli to the cistern when ewes were separated from their lambs. Moreover, this decrease in milk fat content in the presence of the calf is observed only with S cows. One explanation could be due to the fact that the cistern of udder from S cows has a lower volume than that from PH cows. Thus the cisternal milk to the alveolar milk ratio is weaker for S cows and explains this strong decrease.

The PH groups when compared to the S groups had lower milk protein content, in agreement with data recorded in the farms [279].

Moreover, the presence of the calf decreased milk protein content for the S groups and numerically for the PH cows. This decrease might be attributed to a dilution effect, linked to higher milk productivity of S cows in the presence of the calf. Our results are in disagreement with Fröberg et al. [294] using Holstein cows, showing that suckled milk had lower protein content, whereas Mendoza et al. [293] reported no significant influence of the presence or absence of the calf on milk protein content in Holstein cows. Similarly, with dairy ewes, McKusick et al. [291] reported no inhibition on the transfer of milk protein from the alveoli to the cistern when the ewes were separated from their lambs.

Milk lactose content tended to be decreased by the presence of the calf for the PH group, whereas it increased for the S group. For the PH group, this effect may be partly due to a dilution of milk because milk yield increased in the presence of the calf. Our results are in agreement with Ontsouka et al. [295] showing lactose concentration is higher in the milk alveolar fraction, suckled by the calf, than in the cisternal fraction. In contrast, Tournadre et al. [24] reported no significant influence of S calf presence or absence during milking on milk lactose content.

Somatic cell counts in milk from PH cows decreased in the presence of the calf. Fröberg et al. [294] showed a decrease in somatic cell counts, when calf suckling was allowed, possibly due to better udder emptying or due to bacterial inhibitors present in calf saliva. One hypothesis concerning this interaction could be linked to an udder easier to be milked for PH cows than for S cows but this hypothesis should be validated on individual data.

2.5.2. Milk fatty acid profile

The cow breed had a significant effect on the concentrations of several FA in milk fat. Nevertheless, in this study we must be careful in the interpretation of the results because the effect of breed is confounded with the composition of the diet, particularly the forage to concentrate ratio. The influence of breed on milk FA composition has already been reported in previous studies [25,81,84]. A higher concentration of 4:0 and a slightly lower concentration of 14:0 in milk fat from Holstein cows compared to Montbéliarde cows have also been reported by Ferlay et al. [86]. In agreement with Ferlay et al. [86], but in disagreement with Lawless et al. [85] and Ferlay et al. [81], breed had no effect on the milk fat percentage of 16:0 in our study.

Moreover, we observed no effect of breed on 18:0 concentration, whereas Lawless et al. [85] obtained a higher 18:0 concentration in milk fat from Normande and Montbéliarde cows than in that from Holstein cows.

Breed had no effect on *cis*-9-14:1 in our study, whereas Ferlay et al. [86] reported higher concentrations of *cis*-9-14:1, *cis*-9-18:1, *cis*-9,*trans*-11-CLA and *cis*-9,*cis*-11-CLA in milk fat from Holstein cows than in milk fat from Montbéliarde cows. Nevertheless, in accordance with this latter study: *cis*-9,*trans*-11-CLA (+0.33 g/100 g) and *cis*-9,*cis*-11-CLA (+0.03 g/100 g) concentrations were higher in milk fat from S cows than from PH cows, whereas *cis*-9-16:1 concentration was higher in milk fat from PH cows than in milk fat from S cows. A small influence of cow breed on milk *cis*-9,*trans*-11-CLA content has already been reported by Lawless et al. [85] (+0.36 g/100 g of FA; Montbéliarde > Dutch Holstein/Friesian), White et al. [84] (+0.10 g/100 g; Holstein > Jersey) and Kelsey et al. [25] (+0.03 g/100 g; Holstein > Brown Swiss).

A higher milk concentration of *cis*-9,*trans*-11-CLA for the S breed than for the PH breed could be partly explained by increased activity of stearoyl coenzyme-A desaturase (SCD), as suggested by the higher desaturase ratio (*cis*-9,*trans*-11-CLA/*cis*-9-18:1) for the S breed than for the PH breed. In fact, up to 90% of the *cis*-9,*trans*-11-CLA in bovine milk is formed from the activity of this enzyme in the mammary gland [88]. Increasing milk *cis*-9,*trans*-11-CLA by increasing the activity of the SCD could be considered a possibility for improving the nutritional quality of milk.

The higher milk concentration of OBCFA for the S breed could be due to the diet rich in forage distributed to the S cows. In fact, diets based on grass forage and rich in fiber could enhance the milk concentration of these FA [18].

Treatment also had a significant influence on milk FA concentrations. For most FA, essentially FA with 18 atoms of carbon and total PUFA, the presence of the calf decreased their concentrations in milk fat, likely due to the milk richer in fat being suckled by the calf [294,295].

Concerning the influence of calf presence on milk 18:1 isomer concentrations, our results are in agreement with McKusick et al. [291], showing that 18:1 concentration tended to be higher in the alveolar milk fraction compared to the cisternal milk fraction. In disagreement with our results, this latter study showed no significant difference between alveolar and cisternal milk fractions in the concentrations of 8:0, 14:1, 16:0, 16:1, 18:0, 18:2, 18:3 and 20:0, but also for 4:0, 6:0, 10:0, 12:0, and 14:0, for which we observed no variation with respect to calf presence.

In our study, we only observed a decrease in the concentrations of the intermediates of ruminal biohydrogenation (*trans*-9-18:1, *trans*-11-18:1, *trans*-12-18:1, total *trans*-18:1, *cis*-14-18:1, *trans*-11,*cis*-15-18:2 and *cis*-9,*trans*-11-CLA) in the milk of S cows when the calf was present. This could be because the ruminal

biohydrogenation may be less complete in S than in PH cows. This fact could be explained by a lower ruminal fluid retention with a diet poor in concentrate [296].

2.5.3. Milk lipolysis

In our study, initial lipolysis was more important in the milk of S cows than in the milk of PH cows. This could be partly explained by the negative correlations between milk lipolysis and milk yield found by Chazal and Chilliard [297]. Our results are in disagreement with Ferlay et al. [81] who noted lower initial lipolysis with Tarentaise cows than with Montbéliarde cows. The Tarentaise breed produced less milk than the Montbéliarde breed. Nevertheless, Chazal and Chilliard [298] reported no difference in milk lipolysis between Friesian and Montbéliarde cows having different milk yields.

The high lipolysis in the present experiment could also be due to the cow parity. The study was performed on primiparous cows, and Chilliard and Lamberet [299] reported that lipolysis seems to be higher in primiparous that in multiparous cows.

The initial lipolysis as well as the lipolysis measured after 24 h were increased by the presence of the calf. This is contradictory to results obtained by Tournadre et al. [24], that showed for S cows that the weaker the contact between cow and calf, the greater the lipolysis after 24 h.

2.6. Conclusions

The present study reported a significant effect of cow breed on milk yield, milk protein content, FA composition, and initial lipolysis. The milk from S cows had higher protein content than that from PH cows, which could create some difficulties during cheesemaking for PH cows milk. It seems that S cows produced milk richer in FA of nutritional interest (18:3 n-3, 20:5 n-3, 22:5 n-3, and *cis*-9,*trans*-11-CLA) than did PH cows. Nevertheless, concerning lipolysis, milk from S cows had a higher level of initial FFA than milk from PH cows, suggesting that it may develop a rancid flavour.

Our study is the first to analyse the influence of calf presence during milking on milk yield, gross composition, FA profile and lipolysis. The presence of the calf during milking only increased total milk yield, and decreased the milk fat content only in S cows, and increased lipolysis at t_{24} only in PH cows, suggesting that the presence of the calf during milking could have an influence on the organoleptic characteristics of the milk. The presence of the calf also increased the milk concentration of 16:0 and decreased the milk 18:0, 18:2 n-6, 18:3 n-3 and total PUFA concentrations for the two breeds.

3. Study 2. The effects of calf presence during milking, cow parity and season on milk fatty acid composition and the lipolytic system in Salers cows

3.1. Introduction

The dairy industry is currently facing a severe economic and social crisis that could weaken the dairy production systems in mountain areas. To meet these challenges, the dairy industry must ensure the sustainability of the middle mountain production systems, including the productive efficiency of dairy cows and the quality of their products. This challenge is particularly important in mountain areas, where milk is mainly processed into cheeses having a Protected Designation of Origin (PDO) status [300]. The primary measures available to the farmer are nutritional and genetic. For this reason, the inclusion of the breed in the specifications of PDO cheeses would serve to reinforce the link to the land.

The Salers cow breed is a dual-purpose breed originating from the Auvergne region of France and oriented primarily towards beef production. Currently, Salers cows are reared primarily in suckling livestock systems, whereas only about 205 000 cows, representing no more than 2% of the French population of Salers cows, are still milked [276]. A dairy branch with a small number of individuals exists in Auvergne and allows the production of cheeses (Cantal, Salers and Saint-Nectaire) under the Auvergne PDO status. The decrease in the number of Salers cows is related to their low production level (2321 kg/lactation) [276] and to the process of milking, referred as "traditional", that requires the presence of the calf to stimulate milk ejection [24].

The available literature states that removing the calf during milking decreases the milk production of Salers cows, because milk ejection reflex is due to teat stimulation by the suckling young [301,302]. Moreover, maintaining cow-calf contact during milking considerably increases the milking time. An option to overcome this inconvenience and simplify the milking routine would be the suppression of cow-calf contact. Therefore, it is essential to evaluate the impact of this milking strategy on the milk yield, protein and fat contents and especially on the fatty acid (FA) composition because these aspects are poorly documented in the literature [301]. This information is important because milk efficiency for cheese-making is related to the ratio of the milk

fat content to the protein content and because the sensorial and nutritional qualities of cheeses are related to FA composition [28,278].

Cow-calf contact during milking could also affect the milk lipolytic system and hence modify the sensorial quality of the milk. Milk fat lipolysis is the hydrolysis of fat globule triglycerides into free FA (FFA) [60]. Spontaneous lipolysis in cold stored milk is due to the action of milk lipoprotein lipase (LPL) and endogenous lipases and varies according to physiological (including parity) or nutritional factors [20]. Nevertheless, there are few data on the effect of calf presence vs. calf removal on milk lipolysis and LPL activity.

Furthermore, previous studies indicate that milk FA profile is strongly influenced by the nature of forage (preserved vs. grazed grass) in ruminant diet [18,81]. Cow diet could also have an effect on milk lipolytic system. It has been shown that grass-based diets influence the milk lipolytic system in Tarentaise and Montbéliarde cows, but this factor received only little attention in dairy Salers cows [81,301].

In lactating cows, previous studies indicated also a minor effect of parity on milk FA composition [25]. Ultimately, little is known about the influence of parity on the milk lipolytic system.

3.2. Aims

The aim of this study was to evaluate the effects of calf presence during milking, cow parity and season on milk yield and composition, with particular emphasis on FA concentrations and the milk lipolytic system.

3.3. Materials and methods

3.3.1. Cows and diets

The study was conducted between November 2010 and June 2011 at the "Institut National de la Recherche Agronomique" (INRA) experimental farm of Marcenat in an upland area of central France (altitude 1135-1215 m; annual rainfall 1100 mm), using a protocol approved by the INRA Animal Care and Use Committee. All procedures were conducted according to French Guidelines for the use of experimental animals compliant with animal welfare and good practices [283].

Thirty Salers lactating cows (body weight (BW) - 634 kg at the beginning of the lactation period), were selected before parturition according to parity (18 primiparous and 12 multiparous), estimated calving date, their potential milk yield (defined as the best milk yield during an entire lactation of the mother plus the half of the best milk yield of the paternal grandmother as recorded by the Recording Milk Organisation), BW and body condition score (BCS) and assigned to two groups: one group of Salers

cows milked in the presence of the calf (CP, n = 9) and a second group of Salers cows milked in the absence of the calf (CA, n = 21).

The cows were housed in five pens and the calves in a separate pen located in the same building. The cows were machine-milked in a milking parlour twice daily, at 6:30 and 16:00. For cows milked in the presence of the calf, the mother-calf pair remained together 24 h after parturition, with cows being milked at the usual hours. At the end of the second milking, the young returned to the calf pen, and the dam was placed into the cow pen. For these cows, milking included a phase of calf suckling before milking (for 30 to 60 sec). The calf was then placed in a pen in front of his mother during milking. Physical contact was allowed. At the end of milking, the mother-calf pair was placed in a pen, and suckling was allowed for approximately 5 min to completely empty the udder. For the cows milked in the absence of the calf, the newborn calves were separated from their mother immediately after parturition, without any sniffing, licking or suckling.

Before calving, all cows were fed the same diet, consisting of grassland hay offered *ad libitum*. After calving, feed distribution continued to be performed collectively, and the cows were secured with head-locking stanchions. The amounts of intake were measured, on average, from 2 pens per group. The feedstuffs distributed per pen were weighted daily. Any refusals were collected and weighed daily 2 days each week (twice per day) to calculate the net intake of each group. The cows were fed 6 kg grassland hay (regrowth) per animal after morning milking and were fed concentrate according to their daily milk production during the morning and afternoon milkings, whereas grassland hay (first cut) was offered *ad libitum* in the afternoon. The control concentrate contained 26.4% barley, 20% wheat bran, 15% triticale, 10% rootlets, 5% wheat, 13.6% rapeseed meal, 4.8% sunflower meal, 2% molasses, 1.5% rapeseeds and 1.75% minerals and premix. Mineral-vitamin supplement (200 g) was distributed individually during the morning milking. During the winter season, the total dry matter (DM) intake of cows was on average 10.7 kg. The diets were formulated to cover 100% of the energy and protein requirements according to INRA [282]. The nutritive value of feedstuffs is shown in Table V.

On 26 April, the cows were turned out to pasture. They received no further concentrate until 31 October. The cows had access to successive paddocks of grassland pastures. From 21 May through 25 May, they had access to a paddock of 1.81-ha area of grassland pasture. They remained at pasture throughout the day and night. The pasture grazed consisted of 82% grasses (primarily 22% *Dactylis glomerata*, 26% ryegrass, 11% *Poa pratensis*), 1% legumes (essentially clover) and 17% dicots.

Table V. Chemical composition of feedstuffs included in the diet distributed to Salers cows

Ingredients	**Concentrate**[a]	**Grazed grass**	**Hay (first cut)**	**Hay (first cut)**	**Hay (regrowth)**
CP (% of DM)	61	-	93	104	136
OM (% of DM)	945	-	933	922	917
Energy (MJ/kg)	6.96	-	5.19	5.19	4.97
CF (% of DM)	79	-	348	358	335
ADF (% of DM)	-	-	355	345	310
Fatty acid (g/100 g of total FA)					
12:0	0.033	0.121	0.904	0.250	0.408
14:0	0.187	0.412	0.655	0.693	0.830
cis-9-14 :1	0.071	0.135	-	-	0.197
15:0	0.146	0.111	0.262	0.227	0.202
iso 16:0	0.021	4.913	6.054	6.539	7.880
16:0	15.99	14.19	23.69	23.33	24.89
cis-9-16:1	0.452	0.141	0.397	0.404	0.433
17:0	0.126	0.168	-	0.230	0.384
18:0	1.57	1.20	1.64	1.81	2.18
cis-9-18:1	24.66	2.12	2.56	2.63	2.51
cis-11-18:1	3.13	0.36	0.52	0.52	0.65
18:2 n-6	47.38	15.08	17.75	17.86	13.10
18:3 n-3	4.94	58.38	40.96	39.41	40.02
20:0	0.311	0.486	1.016	1.223	1.147
22:0	0.358	0.769	1.230	1.634	1.614
24:0	0.418	0.593	1.228	1.486	1.435
22:5 n-3	0.212	0.812	1.130	1.747	2.133

CP = crude protein; OM = organic matter; CF = crude fibre; ADF = acid-detergent fibre.
[a] Concentrate (g/kg): barley (263.5), wheat bran (200), triticale (150), rapeseed meal (136), wheat (50), sunflower meal (48), molassed sugar beet (20), rapeseed (15), salt (1) and $CaCO_3$ (9).

3.3.2. Sampling, measurement and analyses

Samples of grassland hay (first cut and regrowth) and concentrate were collected twice per week during the experimental period, then pooled to provide one sample per each 2 month-period and stored at -20°C. The DM concentration of the feed was determined after drying at 105°C for 24h. Samples of grassland hay (first cut and regrowth) and concentrate were lyophilised, sieved through a 1 mm-screen and analysed for total crude protein (CP), crude fibre (CF), acid detergent fibre (ADF), and organic matter (OM) using standard procedures [284]. The FA composition was determined from ground lyophilised samples of forages and concentrate, methylated as described by Sukhija and Palmquist [303] with modifications [51]. Tricosanoate (Sigma Saint-Quentin Fallavier, France) was used as the internal standard.

The milk suckled by the calves was determined twice per week, over four consecutive milkings, by the differences between calf body weights before and after suckling [281]. Milked milk was recorded individually at each milking with a continuous milk weighing system. Milk fat, protein, and lactose contents and somatic cell counts (SCC) were determined for individual cows from four consecutive milkings each week. One set of sub-samples (30 mL) was preserved in tubes with bronopol-B2 (Trillaud, Surgères, France) and stored at 4˚C until analysis for fat, protein, lactose contents, and SCC using infrared spectrophotometry (Galilait, Theix, France) according to standard procedures [284].

A second set of sub-samples (3 mL) for FA analyses was collected from two consecutive milkings three times during the experimental period: in the 9th week of lactation (winter season), on 16 March 2011 (winter season) and on 25 May 2011, one month after turning out to pasture (pasture season). These sub-samples were stored at -20°C before lyophilisation (ThermovacTM-20, Froilabo, Ozoir-La-Ferrière, France).

The milk FA composition was determined for the individual milk samples, obtained by pooling 40% of the evening milk and 60% of the morning milk. The FA composition was analysed in lyophilised milk samples according to Ferlay et al. [86] with some modifications. The FA methyl esters (FAME) were injected (0.6 µL) by auto-sampler into a gas chromatograph equipped with a flame ionisation detector (Agilent Technologies 7890A, Wilmington, USA). The FAME from all the samples were separated on a 100 m x 0.25mm i.d. fused-silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was maintained at 255°C and the detector temperature at 260°C. The initial oven temperature was held at 70°C for 1 min, increased to 100°C at a rate of 5°C/min (held for 2 min), and then increased by 10°C/min to 175°C (held for 42 min), and 5°C/min to a final temperature of 225°C (held for 15 min). The carrier gas was hydrogen and pressure was maintained constant (158.6 kPa) during analysis. A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain FA (C4:0 to C10:0).

Identification of FAME was accomplished by comparison to a standard mixture purchased by Nu-Chek-Prep, Inc (Elysian, MN 56028 USA). Mixtures of *cis*/*trans* (9-12) isomers of linoleic acid methyl ester and *cis* and *trans* (9-11) and (10-12) isomers of CLA methyl esters purchased by Sigma-Aldrich Corporation (38297 Saint Quentin Fallavier, France) were used to complete identification.

A third set of sub-samples was taken three times during the experimental period at morning milking on the same sampling dates used for milk FA determination to evaluate milk lipolysis by the determination of initial free FA (FFA) content and FFA content after 24 h of cold storage (4˚C). Immediately after sampling, 18-mL samples of milk were taken, heated at 60˚C for 30 min to destroy lipase activity and stored at 4˚C until analysed for initial FFA content (lipolysis at t_0). A second 18-mL sample was stored at 4˚C for 24 h, then heated and stored at 4˚C until analysed for FFA content (lipolysis at t_{24}). The milk lipolytic system was evaluated by the copper soap method [285].

A fourth set of sub-samples, obtained by pooling 40% of the evening milk and 60% of the morning milk from two consecutive milkings, three times during the experimental period, on the same sampling dates as for milk FA determination, was frozen at -20°C until analysis of milk LPL activity. Determination of milk LPL activity was measured using an artificial emulsion containing ^{3}H triolein emulsion [304].

3.3.3. Statistical analyses

Data of time-dependent changes were analysed as repeated measures using the MIXED procedure of SAS [286]. The statistical model included treatment; season; parity; treatment × season, parity × season and treatment × season × parity interactions; and residual error. The fixed effects included treatment, season, parity and their interactions. Cow was included as a random effect. A compound symmetry error structure was used because it resulted in the lowest Bayesian information criterion. Differences between treatment, parity, season and interactions were analysed with the least square means procedure [286] and were considered to be significant if $P \leq 0.05$.

3.4. Results

3.4.1. Characteristics of animals, milk yield and composition

The results for milk yield and composition and the characteristics of the experimental animals are given in Table VI.

Table VI. Influence of calf presence during milking, cow parity, season, and their interactions on changes in milk yield and composition, milk lipolytic system, BW and BCS

	Week 9[a]		**March**[a]		**Pasture**[a]			**Parity**			**Significance (*P*-value)**[d]					
	CP	**CA**	**CP**	**CA**	**CP**	**CA**	**SEM**	**P**	**M**	**SEM**	**S**	**T**	**Par**	**T × S**	**Par × S**	**T × Par**
Milked milk[b] (L/day)	9.35	11.84	7.93	8.13	7.80	6.68	0.997	7.68	9.56	0.956	***	ns	ns	***	*	ns
Suckled milk (L/day)	5.24	-	4.17	-	4.73	-	0.474	4.18	5.25	0.541	ns	-	ns	-	ns	-
Total milk yield[c] (L/day)	14.59	11.84	12.10	8.13	12.53	6.68	1.028	9.77	12.19	0.972	***	**	+	**	*	ns
Fat content (g/kg)	23.07	35.00	27.80	36.06	29.27	39.00	1.306	32.73	30.67	0.999	***	***	ns	ns	*	ns
Protein content[b] (g/kg)	32.46	33.67	35.74	36.03	37.20	36.43	0.580	35.35	35.15	0.514	***	ns	ns	*	ns	+
Lactose content[c] (g/kg)	52.13	50.95	51.42	49.52	51.37	50.22	0.588	51.45	50.42	0.505	*	+	ns	ns	*	ns
Somatic cell counts[b] (log/mL)	4.35	4.89	4.88	5.30	5.00	5.35	0.156	4.89	5.03	0.111	***	**	ns	ns	ns	ns
Lipolysis at t_0 (%)	0.48	0.34	0.37	0.43	0.32	0.31	0.042	0.35	0.41	0.033	*	ns	ns	*	ns	ns
Lipolysis at t_{24} (%)	0.63	0.46	0.65	0.71	0.59	0.44	0.076	0.52	0.64	0.059	*	ns	ns	ns	ns	+
Lipolysis $_{\Delta 24h}$ (%)	0.15	0.12	0.27	0.28	0.27	0.12	0.050	0.17	0.23	0.037	**	ns	ns	ns	ns	*
LPL activity (nmol/min/mL)	698.79	716.29	430.83	620.3	606.23	591.23	115.022	556.51	664.72	68.667	ns	ns	ns	ns	ns	ns
BW (kg)	634.4	632.9	645.5	648.1	610.3	634.4	17.32	599.9	668.6	16.89	***	ns	**	*	ns	ns
BCS	2.34	2.97	2.47	3.12	2.63	3.24	0.287	2.53	3.07	0.283	**	ns	ns	ns	ns	ns

CP = calf present during milking; CA = calf absent during milking.
P = primiparous cows; M = multiparous cows.
S = season (Week 9/March/Pasture); T = treatment (CP or CA); Par = parity (P or M); T × S = interaction between treatment and season; Par × S = interaction between parity and season; T × Par = interaction between treatment and parity.
Lipolysis$_{\Delta 24h}$ = evolution of milk lipolysis after 24 h (Lipolysis at t_0 - Lipolysis at t_{24}); LPL = Lipoprotein lipase.
BW = body weight; BCS = body condition score.
SEM = Standard Error of the Mean; ns = not significant.
[a] Number of cows: Week 9 (CP = 9; CA = 21) / March (CP = 9; CA = 17) / Pasture (CP = 9; CA = 14).
[b] $P < 0.05$, [c] $P < 0.10$ for the interaction between treatment, parity and season.
[d] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Effect of calf presence during milking. During the experimental period, the average milk yield was 13.1 and 8.9 L/day for the CP and CA cows, respectively. The total milk yield of the CP cows was greater than that of the CA cows (+32%, $P < 0.01$). The presence of the calf produced a more marked increase ($P < 0.01$) in the total milk yield of the cows during the pasture season than during the winter season. For CP cows, the average amount of milked milk was 8.4 L/day, representing 64% of the total milk yield. The average amount of suckled milk was 4.7 L/calf/day. The suckling of calves reduced milk yield at milking during the winter season, whereas it increased it during the pasture season ($P < 0.001$). The CP cows had a lower milk fat content than the CA cows ($P < 0.001$). The milk protein content was decreased by the presence of the calf during winter season, whereas it was increased during pasture season ($P < 0.05$). The calf presence decreased SCC in milk ($P < 0.01$).

Effect of parity. The multiparous cows had a higher milk fat content in the 9th week of lactation and lower milk fat content in March and in pasture season than the primiparous cows. Likewise, milk lactose content was lower during winter season, but higher during pasture season in multiparous than in primiparous cows ($P < 0.05$).

Effect of season. The season had a significant effect on milk yield and composition. The milk yield of the cows was lowest during the pasture season ($P < 0.001$). Nevertheless, the milk collected during the pasture season had higher fat ($P < 0.001$) and protein ($P < 0.001$) contents than the milk collected during the winter season. Lactose content was higher in milks collected in the 9th week of lactation than in milks collected in March and pasture season ($P < 0.05$). Milks collected during pasture season had the higher SCC than those collected during winter season ($P < 0.001$). The BW increased from the 9th week of lactation to March, then decreased from March to the pasture season ($P < 0.001$). The BCS was higher during the pasture season than during the winter season ($P < 0.01$).

3.4.2. Milk fatty acid composition

Calf presence during milking, cow parity and season had significant effects on several milk FA concentrations in Salers cows (Tables VII and VIII).

Effect of calf presence during milking. Calf presence during milking increased the milk *cis*-11-16:1 ($P < 0.01$) and 22:6 n-3 ($P < 0.05$) concentrations. In contrast, calf presence during milking decreased the milk iso 13:0 ($P < 0.001$), iso 14:0 ($P < 0.05$), 15:0 ($P < 0.01$), anteiso 15:0 ($P < 0.001$), iso 15:0 ($P < 0.01$), iso 16:0 ($P < 0.01$), *trans*-9-16:1 ($P < 0.05$), *cis*-6-18:1($P < 0.001$), *cis*-14-18:1 ($P < 0.05$), 20:0 ($P < 0.05$), total odd- and branched-chain FA (OBCFA) ($P < 0.001$), and total *trans* FA ($P < 0.01$) concentrations.

Table VII. Influence of calf presence during milking, cow parity, season, and their interactions on milk FA composition

	Week 9[a]		**March**[a]		**Pasture**[a]			**Parity**			**Significance (*P*-value)**[e]					
Fatty acid														**T**	**Par**	**T**
(g/100 g of total FA)	**CP**	**CA**	**CP**	**CA**	**CP**	**CA**	**SEM**	**P**	**M**	**SEM**	**S**	**T**	**Par**	**× S**	**× S**	**× Par**
4:0[c]	2.66	2.65	2.54	2.67	2.73	2.35	0.114	2.59	2.61	0.079	ns	ns	ns	*	ns	ns
5:0	0.03	0.02	0.03	0.02	0.05	0.05	0.008	0.04	0.03	0.005	**	ns	ns	ns	ns	ns
6:0[b]	2.12	2.11	2.01	1.97	1.83	1.45	0.083	1.84	1.99	0.065	***	ns	ns	*	ns	ns
7:0	0.02	0.02	0.02	0.02	0.03	0.03	0.003	0.02	0.03	0.002	**	ns	ns	ns	ns	ns
8:0[b]	1.32	1.35	1.27	1.22	1.07	0.82	0.050	1.11	1.24	0.042	***	ns	*	***	ns	ns
10:0[c]	3.06	3.19	3.08	2.81	2.33	1.81	0.129	2.54	2.88	0.115	***	ns	*	***	+	ns
cis-9-10:1	0.26	0.25	0.27	0.23	0.15	0.11	0.011	0.20	0.23	0.008	***	**	*	ns	ns	+
11:0	0.04	0.04	0.04	0.03	0.03	0.02	0.003	0.03	0.04	0.002	***	ns	ns	**	ns	ns
12:0	3.42	3.60	3.57	3.22	2.26	1.81	0.134	2.81	3.15	0.116	***	ns	*	**	**	ns
cis-9-12:1	0.08	0.08	0.09	0.07	0.06	0.03	0.005	0.06	0.07	0.003	***	**	*	*	*	ns
13:0	0.09	0.12	0.11	0.11	0.08	0.08	0.005	0.10	0.10	0.003	***	ns	ns	*	ns	ns
anteiso 13:0	0.01	0.02	0.02	0.02	0.02	0.01	0.001	0.02	0.02	0.001	**	ns	ns	ns	ns	ns
iso 13:0	0.04	0.05	0.04	0.05	0.04	0.05	0.002	0.05	0.04	0.001	**	***	+	ns	ns	ns
14:0	11.64	11.51	12.03	11.01	8.69	7.33	0.315	9.87	10.87	0.222	***	*	**	+	ns	ns
iso 14:0	0.18	0.22	0.20	0.23	0.14	0.17	0.010	0.20	0.18	0.008	***	*	ns	ns	ns	ns
cis-9-14:1	0.84	0.77	0.94	0.79	0.72	0.49	0.059	0.72	0.80	0.052	***	*	ns	+	ns	ns
15:0	1.20	1.37	1.29	1.38	1.13	1.21	0.037	1.27	1.25	0.027	***	**	ns	ns	ns	ns
anteiso 15:0	0.63	0.73	0.69	0.77	0.63	0.72	0.025	0.71	0.68	0.017	*	***	ns	ns	ns	ns
iso 15:0	0.36	0.43	0.41	0.45	0.31	0.37	0.017	0.41	0.37	0.013	***	**	*	ns	ns	ns
16:0	27.46	25.21	28.50	24.98	19.82	18.33	0.437	23.65	24.45	0.385	***	***	ns	**	ns	ns
iso 16:0	0.45	0.54	0.48	0.57	0.34	0.42	0.022	0.49	0.44	0.018	***	**	+	ns	*	ns
cis-9-16:1	1.48	1.18	1.49	1.20	1.20	0.96	0.070	1.27	1.23	0.062	***	**	ns	ns	ns	+
cis-11-16:1	0.05	0.04	0.05	0.04	0.04	0.03	0.003	0.04	0.05	0.002	*	**	ns	ns	ns	ns

Table VII. Influence of calf presence during milking, cow parity, season, and their interactions on milk FA composition (continued)

	Week 9[a]		**March**[a]		**Pasture**[a]			**Parity**			**Significance (*P*-value)**[e]					
Fatty acid														**T**	**Par**	**T**
(g/100 g of total FA)	**CP**	**CA**	**CP**	**CA**	**CP**	**CA**	**SEM**	**P**	**M**	**SEM**	**S**	**T**	**Par**	**× S**	**× S**	**× Par**
trans-9-16:1	0.25	0.27	0.25	0.28	0.24	0.28	0.013	0.28	0.24	0.007	ns	*	***	ns	ns	ns
trans-11-16:1	0.19	0.19	0.18	0.19	0.25	0.27	0.007	0.22	0.21	0.004	***	+	ns	ns	ns	ns
17:0	0.78	0.81	0.81	0.82	0.66	0.68	0.014	0.76	0.75	0.010	***	ns	ns	ns	ns	**
iso 17:0	0.33	0.36	0.29	0.33	0.43	0.54	0.015	0.38	0.38	0.011	***	***	ns	**	**	ns
cis-9-17:1	0.29	0.25	0.26	0.26	0.22	0.21	0.012	0.26	0.24	0.009	***	ns	+	ns	+	ns
anteiso 17:0	0.52	0.60	0.53	0.60	0.46	0.52	0.016	0.53	0.55	0.012	***	***	ns	ns	**	*
18:0	9.64	10.47	8.71	10.63	10.54	13.15	0.400	10.67	10.38	0.320	***	***	ns	*	+	ns
iso 18:0	0.08	0.08	0.07	0.07	0.06	0.06	0.003	0.08	0.07	0.002	***	ns	*	ns	ns	ns
cis-9,*trans*-13-18:2	0.14	0.13	0.13	0.13	0.40	0.36	0.011	0.20	0.22	0.007	***	ns	+	ns	ns	ns
cis-9,*trans*-12-18:2	0.08	0.08	0.07	0.08	0.18	0.16	0.006	0.10	0.11	0.004	***	ns	+	+	*	ns
trans-11,*cis*-15-18:2[d]	0.08	0.08	0.06	0.07	0.41	0.46	0.013	0.21	0.17	0.008	***	ns	***	ns	**	+
cis-9,*trans*-11-CLA[d]	0.67	0.73	0.64	0.76	2.66	2.52	0.087	1.52	1.14	0.059	***	ns	***	ns	**	ns
trans-9,*trans*-11-CLA	0.05	0.05	0.07	0.05	0.07	0.06	0.005	0.06	0.06	0.003	*	+	ns	ns	ns	ns
trans-11,*trans*-13-CLA	0.04	0.03	0.04	0.03	0.06	0.06	0.004	0.04	0.04	0.002	***	+	ns	ns	ns	ns
18:2 n-6	1.67	1.72	1.91	2.02	1.72	1.65	0.079	1.75	1.81	0.065	***	ns	ns	ns	ns	ns
18:3 n-3	0.87	0.88	0.84	0.86	0.84	0.81	0.039	0.85	0.85	0.034	ns	ns	ns	ns	ns	ns
18:3 n-6[d]	0.05	0.05	0.05	0.05	0.003	0.004	0.003	0.03	0.03	0.002	***	ns	ns	ns	ns	ns
19:0	0.13	0.12	0.14	0.12	0.26	0.25	0.007	0.17	0.18	0.005	***	ns	ns	ns	ns	*
20:0	0.20	0.23	0.21	0.24	0.18	0.20	0.009	0.22	0.20	0.007	***	*	ns	ns	ns	ns
20:2 n-6[d]	0.07	0.06	0.08	0.07	0.08	0.06	0.005	0.07	0.06	0.003	*	*	+	ns	ns	ns
20:3 n-6	0.09	0.09	0.10	0.11	0.10	0.10	0.006	0.10	0.10	0.005	**	ns	ns	ns	ns	ns
20:4 n-6	0.10	0.14	0.11	0.13	0.10	0.09	0.006	0.11	0.11	0.005	***	*	ns	***	ns	ns
20:5 n-3	0.08	0.08	0.08	0.08	0.07	0.06	0.004	0.07	0.08	0.003	***	ns	ns	*	ns	ns

Table VII. Influence of calf presence during milking, cow parity, season, and their interactions on milk FA composition (continued)

	Week 9[a]		**March**[a]		**Pasture**[a]			**Parity**			**Significance (*P*-value)**[e]					
Fatty acid														**T**	**Par**	**T**
(g/100 g of total FA)	**CP**	**CA**	**CP**	**CA**	**CP**	**CA**	**SEM**	**P**	**M**	**SEM**	**S**	**T**	**Par**	**× S**	**× S**	**× Par**
21:0	0.09	0.07	0.09	0.09	0.15	0.17	0.007	0.12	0.10	0.004	***	ns	***	+	+	ns
22:5 n-3	0.23	0.28	0.22	0.28	0.23	0.24	0.012	0.26	0.24	0.010	*	**	ns	*	+	ns
22:6 n-3	0.04	0.03	0.03	0.02	0.03	0.02	0.002	0.03	0.03	0.002	*	*	+	ns	ns	ns
24:0	0.06	0.07	0.06	0.07	0.06	0.07	0.003	0.07	0.06	0.002	*	+	*	ns	ns	ns
Total SFA	66.72	66.09	67.36	64.68	54.47	52.87	0.846	60.89	63.17	0.715	***	ns	*	ns	ns	ns
Total OBCFA	4.95	5.55	5.21	5.64	4.70	5.25	0.125	5.29	5.14	0.092	***	***	ns	ns	*	+
Total MUFA	27.27	27.78	26.46	28.85	36.53	38.47	0.725	31.81	29.97	0.609	***	+	*	ns	ns	ns
Total *trans* FA	3.97	4.30	3.71	4.29	11.15	12.09	0.244	7.18	5.99	0.156	***	**	***	ns	***	ns
Total PUFA	4.42	4.59	4.60	4.88	7.36	7.06	0.155	5.67	5.30	0.125	***	ns	*	*	*	ns
Total CLA	0.77	0.82	0.75	0.85	2.78	2.64	0.087	1.63	1.24	0.059	***	ns	***	ns	**	ns
Total n-6	1.98	2.06	2.25	2.38	2.00	1.91	0.084	2.07	2.12	0.069	***	ns	ns	ns	ns	ns
Total n-3	1.21	1.28	1.18	1.25	1.16	1.13	0.046	1.22	1.19	0.039	*	ns	ns	ns	ns	ns
Desaturase ratio																
cis-9-14:1/14:0	0.07	0.07	0.08	0.07	0.08	0.07	0.006	0.07	0.07	0.006	*	ns	ns	ns	ns	ns
cis-9-16:1/16:0	0.05	0.05	0.05	0.05	0.06	0.05	0.003	0.05	0.05	0.002	**	+	ns	ns	ns	+
cis-9-18:1/18:0	2.12	2.00	2.28	2.10	2.40	2.01	0.102	2.18	2.13	0.088	+	+	ns	ns	ns	ns
cis-9,*trans*-11-CLA/ *trans*-11-18:1	0.51	0.47	0.54	0.50	0.59	0.47	0.023	0.52	0.50	0.019	+	*	ns	*	ns	ns

CP = calf present during milking; CA = calf absent during milking.
P = primiparous cows; M = multiparous cows.
S = season (Week 9/March/Pasture); T = treatment (CP or CA); Par = parity (P or M); T × S = interaction between treatment and season; Par × S = interaction between parity and season; T × Par = interaction between treatment and parity. SFA = saturated FA; OBCFA = odd- and branched-chain FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.
SEM = Standard Error of the Mean; ns = not significant.
[a] Number of cows: Week 9 (CP = 9; CA = 21) / March (CP = 9; CA = 17) / Pasture (CP = 9; CA = 14).
[b] $P < 0.01$, [c] $P < 0.05$, [d] $P < 0.10$ for the interaction between treatment, parity and season.
[e] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table VIII. Influence calf presence during milking, cow parity, season, and their interactions on concentrations of *trans* and *cis* isomers of 18:1 in milk fat

	Week 9[a]		**March**[a]		**Pasture**[a]			**Parity**			**Significance (*P*-value)**[b]					
Fatty acid (g/100 g of total FA)	**CP**	**CA**	**CP**	**CA**	**CP**	**CA**	**SEM**	**P**	**M**	**SEM**	**S**	**T**	**Par**	**T × S**	**Par × S**	**T × Par**
trans-4-18:1	0.03	0.02	0.03	0.02	0.04	0.04	0.002	0.03	0.03	0.001	**	ns	ns	ns	ns	ns
trans-5-18:1	0.02	0.02	0.02	0.02	0.03	0.04	0.002	0.03	0.02	0.001	***	ns	ns	ns	ns	ns
trans-6-18:1	0.18	0.20	0.17	0.23	0.35	0.43	0.014	0.27	0.26	0.009	***	***	ns	+	ns	ns
trans-9-18:1	0.18	0.20	0.16	0.17	0.33	0.34	0.010	0.25	0.21	0.008	***	ns	**	ns	ns	ns
trans-10-18:1	0.20	0.22	0.17	0.21	0.37	0.36	0.016	0.26	0.25	0.011	***	ns	ns	ns	ns	ns
trans-11-18:1	1.33	1.55	1.18	1.53	4.54	5.50	0.154	2.98	2.23	0.094	***	***	***	*	***	ns
trans-13-18:1	0.31	0.33	0.30	0.31	0.73	0.76	0.022	0.43	0.49	0.016	***	ns	*	ns	ns	ns
Total *trans*-18:1	2.26	2.54	2.05	2.51	6.40	7.47	0.163	4.24	3.50	0.105	***	***	***	*	***	ns
cis-6-18:1	0.13	0.17	0.13	0.18	0.44	0.49	0.012	0.26	0.26	0.009	***	***	ns	ns	ns	ns
cis-9-18:1	20.26	20.76	19.70	21.92	24.82	26.14	0.628	22.83	21.71	0.524	***	+	ns	ns	*	ns
cis-11-18:1	0.61	0.65	0.47	0.56	0.83	0.86	0.035	0.69	0.64	0.024	***	ns	ns	ns	ns	ns
cis-12-18:1	0.11	0.13	0.13	0.15	0.27	0.24	0.011	0.17	0.18	0.008	***	ns	ns	*	ns	ns
cis-13-18:1	0.07	0.06	0.07	0.06	0.15	0.12	0.007	0.08	0.09	0.004	***	*	ns	ns	ns	+
cis-14-18:1	0.17	0.20	0.16	0.20	0.49	0.53	0.013	0.28	0.31	0.009	***	*	*	ns	*	ns
Total *cis*-18:1	21.37	21.99	20.66	23.07	27.02	28.39	0.669	24.31	23.19	0.557	***	+	ns	ns	*	ns

CP = calf present during milking; CA = calf absent during milking.
P = primiparous cows; M = multiparous cows.
S = season (Week 9/March/Pasture); T = treatment (CP or CA); Par = parity (P or M); T × S = interaction between treatment and season; Par × S = interaction between parity and season; T × Par = interaction between treatment and parity.
SEM = Standard Error of the Mean; ns = not significant.
[a] Number of cows: Week 9 (CP = 9; CA = 21) / March (CP = 9; CA = 17) / Pasture (CP = 9; CA = 14).
[b] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Effect of parity. The multiparous Salers cows had higher milk fat concentrations of 14:0 ($P < 0.01$), *trans*-13-18:1 ($P < 0.05$), *cis*-9,*trans*-13-18:2 ($P < 0.10$), and total saturated FA (SFA) ($P < 0.05$) than primiparous Salers cows. Moreover, the milk fat of multiparous Salers cows had lower concentrations of 24:0 ($P < 0.05$), iso 15:0 ($P < 0.05$), iso 18:0 ($P < 0.05$), *trans*-9-16:1 ($P < 0.001$), *trans*-9-18:1 ($P < 0.01$) and total monosaturated FA (MUFA) ($P < 0.05$) than that of primiparous Salers cows.

Effect of season. Except for anteiso 13:0, iso 13:0, *cis*-11-16:1, *trans*-9-16:1, 18:3 n-3, 20:2 n-6, 20:3 n-6, 22:6 n-3, 24:0, and desaturase ratios (*cis*-9-14:1/14:0, and *cis*-9-16:1/16:0), several FA concentrations were influenced by the season. On average, compared with milk collected in the 9th week of lactation and in March, milk collected during the pasture season had higher concentrations of 5:0 ($P < 0.01$), 7:0 ($P < 0.01$), 19:0 ($P < 0.001$), *trans*-11-16:1 ($P < 0.001$), *cis*-9,*trans*-13-18:2 ($P < 0.001$), *trans*-9,*trans*-11-CLA ($P < 0.05$), *trans*-11,*trans*-13-CLA ($P < 0.001$), and total MUFA ($P < 0.001$). Moreover, milks collected during the pasture season had the highest concentrations of *cis* (6, 11 and 13) and *trans* (4, 5, 9, 11 and 13) isomers of 18:1. In contrast, milk collected during the pasture season had lower concentrations of certain individual SFA (iso 14:0 ($P < 0.001$), 15:0 ($P < 0.001$), anteiso 15:0 ($P < 0.05$), iso 15:0 ($P < 0.001$), 17:0 ($P < 0.001$), iso 18:0 ($P < 0.001$), 20:0 ($P < 0.001$)), individual MUFA (*cis*-9-10:1 ($P < 0.001$), *cis*-9-16:1 ($P < 0.001$)), 18:2 n-6 ($P < 0.001$), total SFA ($P < 0.001$), total n-6 ($P < 0.001$), and total n-3 ($P < 0.05$) compared with milk collected during the winter season.

Effect of interactions. Several significant interactions between treatment and season are reported in Tables VII and VIII. The presence of the calf increased the concentration of 4:0 ($P < 0.05$) (Fig. 9) in the milk from cows sampled in the 9th week of lactation and during the pasture season, whereas this concentration was decreased by the presence of the calf in March.

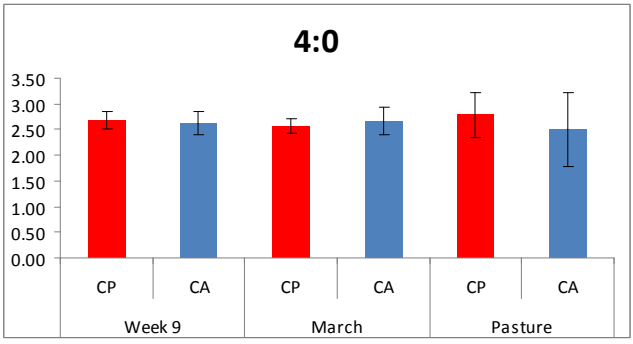

Fig.9. Concentration of **4:0** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.05$)

The presence of the calf produced a more marked increase in the milk fat concentration of 6:0 ($P < 0.05$) and the ratio between *cis*-9,*trans*-11-CLA and *trans*-11-18:1 ($P < 0.05$) during the pasture than during the winter season. Moreover, calf presence increased the 16:0 ($P < 0.01$) (Fig. 10) concentration more markedly in milk collected in March than in that collected during the 9th week of lactation and the pasture season. In contrast, the presence of the calf more markedly decreased the concentrations of iso 17:0 ($P < 0.01$) (Fig. 11), 18:0 ($P < 0.05$) (Fig. 12), and *trans*-11-18:1 ($P < 0.05$) (Fig. 13) in milk fat during the pasture than during the winter season, whereas calf presence decreased the milk 22:5 n-3 ($P < 0.05$) concentration more markedly during the winter season than during the pasture season.

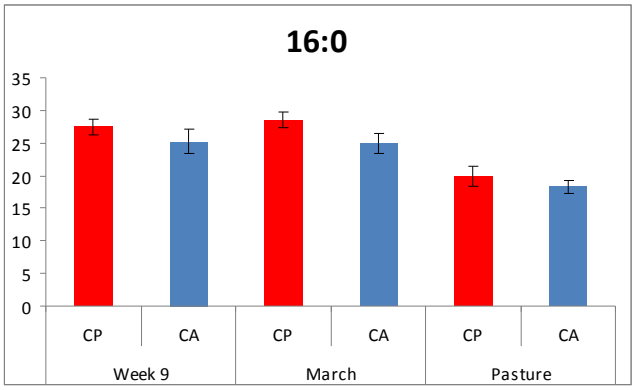

Fig.10. Concentration of **16:0** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.01$)

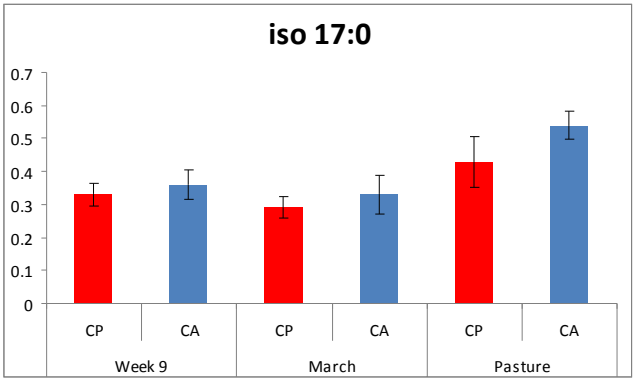

Fig.11. Concentration of **iso 17:0** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.01$)

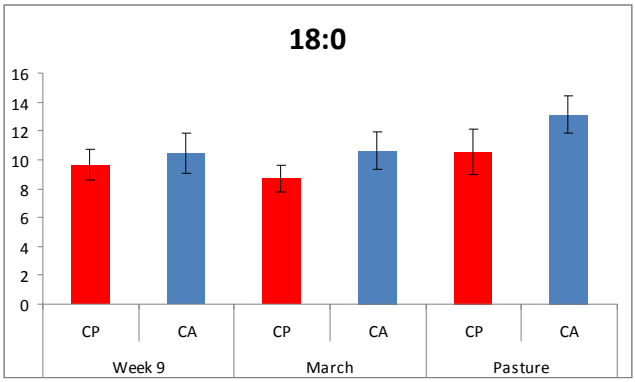

Fig.12. Concentration of **18:0** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.05$)

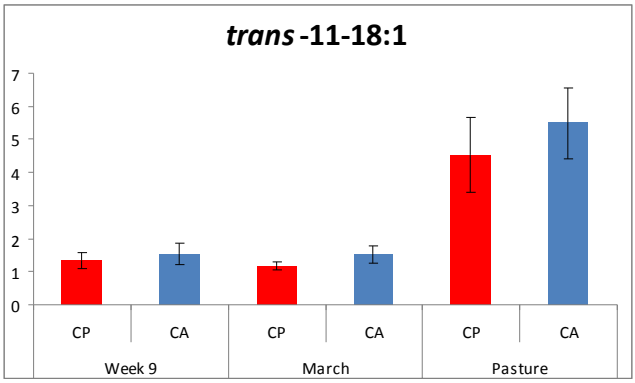

Fig.13. Concentration of ***trans*-11-18:1** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.05$)

The presence of the calf decreased the 8:0 ($P < 0.001$), and 10:0 ($P < 0.001$) concentrations in milk from cows milked during the 9th week of lactation, whereas it increased these FA concentrations in milk fat from cows milked during March and during the pasture season. The presence of the calf decreased milk *cis*-12-18:1 ($P < 0.05$), 20:4 n-6 ($P < 0.001$) (Fig. 14), and total PUFA ($P < 0.001$) concentrations in the 9th week of lactation and in March and increased the abovementioned FA concentrations during the pasture season.

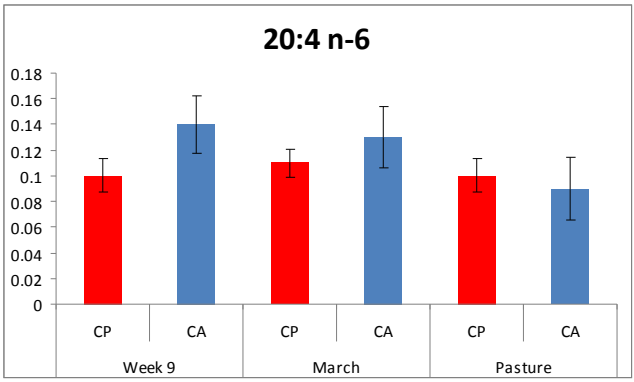

Fig.14. Concentration of **20:4 n-6** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.001$)

The presence of the calf increased the 20:5 n-3 ($P < 0.05$) (Fig. 15) concentration during the pasture season but had no effect during the winter season.

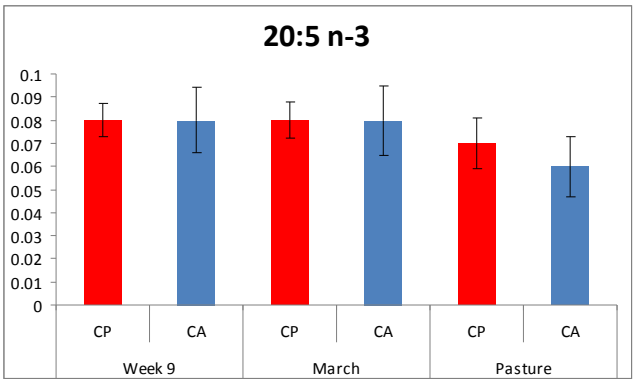

Fig.15. Concentration of **20:5 n-3** in milk fat from Salers cows, milked in the presence (CP) or the absence of the calf (CA), according to season (Week 9/March/Pasture) (treatment × season interaction, $P < 0.05$)

Parity had a significant effect on several milk FA concentrations according to the season (10:0, 12:0, 18:0, 21:0, *cis*-9-12:1, iso 16:0, anteiso 17:0, *cis*-9-17:1, *cis*-9,*trans*-12-18:2, and 22:5 n-3). Indeed, the milk concentrations of total *trans*-18:1, total CLA, total *trans* FA (Fig. 16), *trans*-11,*cis*-15-18:2 (Fig. 17), and *cis*-9,*trans*-11-CLA (Fig. 18) from the primiparous cows were higher than those from the multiparous cows, with a more marked difference during the pasture season. The milk concentrations of total OBCFA (Fig. 19) and iso 17:0 from the primiparous cows were higher during the winter and lower during the pasture period than those from the multiparous cows.

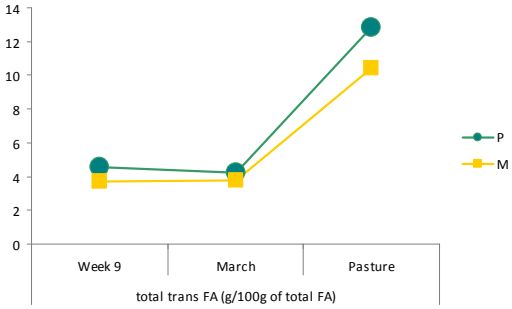

Fig.16. Concentration of **total *trans* FA** in milks from primiparous (P) and multiparous (M) cows, collected on the 9th week of lactation, on March and on pasture season (parity × season interaction, $P < 0.001$)

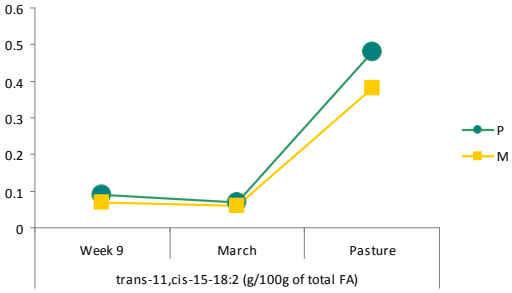

Fig.17. Concentration of ***trans*-11,*cis*-15-18:2** in milks from primiparous (P) and multiparous (M) cows, collected on the 9th week of lactation, on March and on pasture season(parity × season interaction, $P < 0.01$)

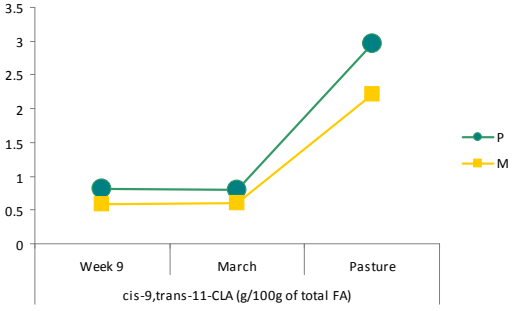

Fig.18. Concentration of ***cis*-9,*trans*-11-CLA** in milks from primiparous (P) and multiparous (M) cows, collected on the 9th week of lactation, on March and on pasture season(parity × season interaction, $P < 0.01$)

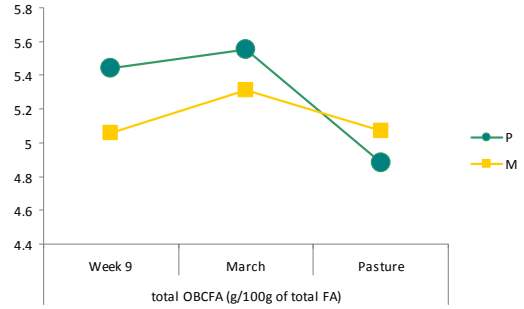

Fig.19. Concentration of **total OBCFA** in milks from primiparous (P) and multiparous (M) cows, collected on the 9th week of lactation, on March and on pasture season(parity × season interaction, $P < 0.05$)

Calf presence decreased milk 17:0 ($P < 0.01$) concentration in fat from the primiparous cows, whereas it increased this FA concentration in milk fat from the multiparous cows. The calf presence decreased more markedly the concentration of anteiso 17:0 ($P < 0.05$) in milk fat from the primiparous than from the multiparous cows. The presence of the calf increased the milk 19:0 concentration ($P < 0.05$) for multiparous cows, whereas it did not change with primiparous cows.

3.4.3. Milk lipolytic system

Effect of calf presence during milking. The presence of the calf had no influence on milk lipolysis and LPL activity. However, a treatment by season interaction ($P < 0.05$) changed the initial lipolysis. The presence of the calf increased the initial FFA concentrations in milks collected in the 9th week of lactation, decreased initial FFA concentrations in milks collected in March and did not change the initial FFA concentrations in milks collected during the pasture season.

Effect of parity. Milk lipolysis and LPL activity were not affected by cow parity. Nevertheless, a significant interaction ($P < 0.05$) between the treatment and parity was observed for the lipolysis$_{\Delta 24h}$. The presence of the calf decreased the lipolysis$_{\Delta 24h}$ in the primiparous cows whereas it increased it in the multiparous cows.

Effect of season. Initial lipolysis and lipolysis measured after 24 h were greater during the winter season than during the pasture season ($P < 0.05$). In contrast, LPL activity was not affected by season.

3.5. Discussion

3.5.1. Milk yield and composition

Effect of calf presence during milking. The present study showed a significant increase in total milk yield from cows milked in the presence of the calf, in agreement with Cozma et al. [301], who reported a higher milk yield (+5.6 L/day) for primiparous Salers cows milked in the presence of the calf. Our results agree also with Tournadre et al. [24], who compared Salers cows suckled before milking with Salers cows for which

only the visual presence of the calf (without physical contact with the cow) was allowed before and during milking. Moreover, Álvarez-Rodríguez et al. [305] have observed that *ad libitum* nursing in Parda de Montaña cows allowed a higher milk yield (+1.7 kg/day) than nursing only once daily. The higher milk yield of Salers cows milked in the presence of the calf could be due to an oxytocin-mediated milk ejection reflex initiated by the presence of the suckling young through nervous stimulation of the udder [24,302]. In contrast to our results, Mendoza et al. [293] observed a decrease in milk yield from Holstein cows with the calf presence, whereas De Passillé et al. [292] reported no effect of calf presence during milking on milk production in Holstein cows.

The principal effect of calf presence during milking was a decrease in milk fat content, in agreement with Cozma et al. [301] with Salers cows and Mendoza et al. [293] with Holstein cows. Moreover, nursing *ad libitum* induced a lower milk fat content in Parda de Montaña cows than nursing once-daily [305]. In contrast, Tournadre et al. [24] have shown that the fat content in milked milk was lower if cow-calf contact was reduced in Salers cows. A lower fat content in milked milk in CP cows may be related to the removal of residual milk (alveolar milk) by the calves. This fraction of the milk is richer in fat than the cisternal milk (available milk), which corresponds to the milk obtained after milking [294].

The presence of the calf decreased milk protein content during the winter season but increased it during the pasture season. This result was most likely due to a dilution effect because the milk yield of the cows in the presence of the calf was higher during the winter season. Cozma et al. [301] also reported a lower protein content for Salers cows milked in the presence of the calf during 210 days of lactation during winter season. In contrast, Mendoza et al. [293] reported no difference in milk protein content with or without the calf during milking in Holstein cows.

Effect of parity. Milk yield was higher in the multiparous cows than in the primiparous cows at the beginning of lactation, but decreased afterwards, resulting in similar production for both parities toward the end of lactation. The lower milk yield in primiparous cows at the beginning of lactation could be linked to a lower density of secretory cells and to a less secretory activity of cells in mammary gland from primiparous cows, as reported by Miller et al. [306] in Holstein cows.

Concerning the milk fat content, it was higher for the multiparous than for the primiparous cows in the 9th week of lactation, whereas it was lower in March and during pasture season (parity × season interaction). Nevertheless, Miller et al. [306] reported no influence of parity on milk fat content in Holstein cows. We observed no differences in milk protein content between primiparous and multiparous Salers cows in accordance with this latter study.

Effect of season. The milk yield of the Salers cows was significantly lower during the pasture season than during the winter season ($P < 0.001$). Our results are in

agreement with previous studies using Holstein cows, which have shown a lower milk production for grazing cows than for cows fed a total mixed ration [84,307,308]. The primary reason for this difference could be the advanced stage of lactation during this period of the experiment [308].

In our study, milk fat content increased when cows were turned out to pasture from the winter diet, in agreement with Khanal et al. [308], but in disagreement with White et al. [84] and Lerch et al. [307] showing that the grazing cows had significantly lower milk fat content than the indoor cows. Moreover, cows had higher milk protein content on pasture season compared to winter season in the present study, in disagreement with Ferlay et al. [81] reporting a lower milk protein content for the grazing cows compared to the cows fed diet rich in mountain natural grassland hay. The higher milk fat and protein contents during the pasture season compared to the winter season reported in our study might be attributed to a concentration effect, because milk yield decreased on the pasture season compared to the winter season.

3.5.2. Milk fatty acid composition

Effect of calf presence during milking. The experimental treatment had a significant effect on the milk concentrations of several FA. The range of the values of milk FA concentrations in our study, except for anteiso 17:0, agrees with that observed by Cozma et al. [301] with bulk milks from primiparous Salers cows fed diets rich in preserved grass.

For most FA, mainly OBCFA, FA with 18 atoms of carbon (18:0, *cis*-6-18:1, *cis*-14-18:1, *trans*-11-18:1), 22:5 n-3, and total *trans* FA, the presence of the calf decreased their concentrations in milk fat. This effect can be explained by a higher fat content in milk suckled by the calf [293]. Similar results have been reported by Cozma et al. [301], who studied the influence of calf presence during milking on the FA profile in bulk milk from Prim'Holstein and Salers cows. Moreover, McKusick et al. [291] showed in ewes that 18:1 FA concentration tended to be higher in the alveolar milk, suckled by the offspring, compared with the cisternal milk. Indeed, calf presence had no effect on milk *cis*-9,*trans*-11-CLA in our study, whereas Cozma et al. [301] reported a significant decrease in this FA concentration in the presence of the Salers calf.

Effect of parity. The influence of parity on the milk FA composition was generally minor in the present study relative to the effects of season and treatment. We observed lower concentrations of *trans*-11-18:1 (+0.75 g/100 g) and *cis*-9,*trans*-11-CLA (+0.38 g/100 g) in milk from the multiparous than from the primiparous cows, in contrast to Kelsey et al. [25], who had reported no effect of parity on these FA concentrations in milk from Holstein and Brown Swiss cows under the same feeding practices. The lack of parity effect on the desaturase ratios suggests that the activity of the Δ9-desaturase is not modified by the lactation rank and cannot explain the lower *cis*-9,*trans*-11-CLA concentration in milk for the multiparous cows. Moreover, Kay et al. [309] showed no

correlation between temporal changes in the Δ9-desaturase system during 16 weeks of lactation and *cis*-9,*trans*-11-CLA content in milk fat from Holstein cows and suggested that an increase in milk fat *cis*-9,*trans*-11-CLA content is due to an increased production of *trans*-11-18:1 in the rumen rather than to an altered Δ9-desaturase activity and/or expression.

In the present study, milk fat from the multiparous cows had higher concentrations of 8:0 (+0.13 g·100 g^{-1}), 10:0 (+0.34 g·100 g^{-1}), 12:0 (+0.34 g·100 g^{-1}), and 14:0 (+1 g·100 g^{-1}) than that from the primiparous cows, in accordance with Kelsey et al. [25]. Nevertheless, in disagreement with this latter study, our results showed any significant effect of parity on milk 4:0, 6:0, *cis*-9-14:1, 15:0, *cis*-9-16:1, 17:0, 18:0, *cis*-9-18:1, and *trans*-4-18:1 concentrations.

Overall, the milk fat from the multiparous cows was richer in short- and medium-chain FA (SMCFA) with 8 to 14 carbons and poorer in long-chain FA (LCFA) with up to 18 carbons than that from the primiparous cows. This difference could be due, in part, to the higher level of fatty acid synthetase in the mammary tissue of multiparous than of primiparous cows reported by Miller et al. [306], which suggests that the mammary gland of multiparous cows is more active for the *de novo* synthesis of SMCFA. Moreover, the differences in the milk FA profile between cows with different parities could be linked to a higher proportion of grass in the diet ingested by the primiparous than by the multiparous cows because dietary PUFA are powerful inhibitors of *de novo* lipogenesis in the mammary gland [20].

Effect of season. The season appears to be the main factor affecting milk FA composition. In fact, the stable concentrations for the majority of the FA in milk collected between the 9th week of lactation and March, when the diet was constant during the winter season, indicated that the effect of lactation stage was negligible in this study. Therefore, the differences observed in FA composition between milks collected during the winter or pasture season were due primarily to seasonal changes in the basal diet (conserved grass vs. grazed grass).

The milk from the grazed grass contained less SMCFA (from 6:0 to 16:0) than the milk from the winter diets based on concentrate and hay. This result is consistent with Ferlay et al. [81], who have shown that the SMCFA concentration in milk fat from cows grazed young grass was significantly less than that from cows fed a high-forage diet. Similarly, Craninx et al. [310] and Lerch et al. [311] have reported lower concentrations of all individual SMCFA in milk from grazing cows than in milk from cows fed indoor diets. Most likely, this decrease is due, in part, to the inhibitory effect of dietary PUFA provided in higher amounts by the pasture diet on acetyl-CoA carboxylase activity in the mammary gland [20]. In contrast, our study reported no seasonal variation in 4:0 concentration between the winter and pasture diets. This FA

is synthesised, in part, by metabolic pathways not involving acetyl-CoA carboxylase and is thus less susceptible to regulation by dietary factors [18].

For the most of OBCFA (13:0, iso 14:0, 15:0, anteiso 15:0, iso 15:0, iso 16:0, 17:0, anteiso 17:0, *cis*-9-17:1, iso 18:0), the present study reported a lower concentration in milk collected during the pasture than during the winter season. This difference could be linked to the inhibitory effect of the PUFA provided in important amounts by pasture on the activity or number of rumen bacteria that synthesized OBCFA [11].

The increase in the milk concentrations of 18:0 (+1.98 g/100 g), *cis*-9-18:1 (+4.82 g/100 g), and *trans*-11-18:1 (+3.62 g/100 g) during the pasture season is typically observed in cows grazing pasture vs. cows fed winter diets [81,311] and can be attributed to the production of these FA as intermediates of the ruminal biohydrogenation of PUFA provided in higher amounts by the pasture diet [18]. Milk 18:0 and *cis*-9-18:1 could also originate from body fat mobilization, as suggested by the decrease in BW during the pasture season. This outcome could be due to the increased energy expenditure by cows resulting from walking during the pasture season [60]. Moreover, the increase in milk fat concentration of *cis*-9,*trans*-11-CLA (+1.89 g/100 g) with pasture feeding could be mainly attributed to an increased escape of *trans*-11-18:1 from the rumen, and its Δ9-desaturation in the mammary gland [20]. The effect of stage of lactation could also partly influence the concentration of this FA, because Lawless et al. [85] reported a slight increase (+0.28 g/100 g) in milk fat *cis*-9,*trans*-11-CLA from 70 and 115 days in milk from grazing cows of different breeds.

A decrease in milk 18:2 n-6 concentration (-0.14 g/100 g) was observed when cows turned out to pasture, in agreement with Khanal et al. [308] and White et al. [84]. This could be explained by the higher 18:2 n-6 amount intake with diets containing concentrate based on cereals rich in 18:2 n-6 than with pasture. Surprisingly, the nature of the diet had no effect on the milk 18:3 n-3 concentration, whereas most published studies have reported higher values for pasture diets than for winter diets [4]. One explanation of this difference could be that the diets were rich in grass during the experimental period.

Whatever the season, the milk fat from the primiparous cows had higher concentrations of several intermediates of biohydrogenation of PUFA (*trans*-18:1, total CLA, total *trans* FA, *trans*-11,*cis*-15-18:2, and *cis*-9,*trans*-11-CLA) than that from the multiparous cows, suggesting a less complete ruminal biohydrogenation of PUFA in the primiparous cows. Moreover, the higher milk concentration of total OBCFA in the primiparous than in the multiparous cows could be due to a higher content of crude fibre and a lower level of concentrate in the diet ingested by the primiparous cows [11].

3.5.3. Milk lipolytic system

Effect of calf presence during milking. In the present study, calf presence increased initial lipolysis for the cows milked during the 9th week of lactation and decreased it for the cows milked in March, whereas it had no influence on initial lipolysis for the cows milked during the pasture season. Nevertheless, the presence of the calf had no influence on lipolysis measured after 24 h or on LPL activity. Our results agree only in part with Cozma et al. [301], who noted in Salers cows (DIM = 40-70) that initial lipolysis and lipolysis after 24 h were increased by the presence of the calf. In contrast, Tournadre et al. [24], using Salers cows, reported no significant effect of cow-calf contact on initial lipolysis, whereas lipolysis after 24 h was higher for cows for which no physical contact with the calf was allowed than for cows suckled before milking. In our study, the high initial lipolysis in the presence of the calf for milk collected on the 9th week of lactation could be due, in part, to suckling by the offspring. Suckling can cause milk agitation in the udder and incorporation of air into milk, both known as factors inducing lipolysis [312].

Effect of parity. Cow parity had no influence on milk lipolysis and LPL activity in the present study, in disagreement with Chilliard and Lamberet [299], who have reported that lipolysis was higher for primiparous than for multiparous cows. The lack of a parity effect could be linked to the absence of a difference in milk yield between the primiparous and multiparous cows in our study.

Effect of season. In the present study, initial lipolysis and lipolysis measured after 24 h were more important in milk collected during the winter season than in milk collected during the pasture season. Our results agree with Chilliard and Lamberet [299] and Lerch et al. [311]. Nevertheless, Chilliard and Lamberet [299] have noted a negative correlation between milk lipolysis and milk yield. Moreover, in contrast with our results, they have reported a higher lipolysis in milk from cows in late lactation. The results of the present study are also contradictory to the results of Ferlay et al. [81] with Tarentaise and Montbéliarde cows, which showed no difference in milk lipolysis between grazing cows and cows fed mountain natural grassland hay.

3.6. Conclusions

The present study reported a significant effect of calf presence during milking on milk yield, composition, FA profile and lipolysis. The presence of the calf increased the total milk yield and decreased the milk fat content whatever the season. It also decreased the milk protein content during the winter season, whereas it increased it during the pasture season. Throughout the experimental period, the presence of the calf increased the milk concentration of 16:0 and decreased the milk total OBCFA concentration. Moreover, calf presence only increased initial lipolysis in milk collected

during the 9th week of lactation. This result suggested that calf presence could influence the organoleptic characteristics of the milk.

Some differences, albeit minor, were observed in milk FA composition relative to cow parity. The primiparous cows produced milk poorer in SMCFA (8:0, 10:0, 12:0 and 14:0) and richer in *trans*-11-18:1 and *cis*-9,*trans*-11-CLA than did the multiparous cows. Cow parity had no influence on the milk lipolytic system.

The season was the principal factor affecting milk FA composition and lipolysis. Compared with the winter diet, the pasture diet increased milk 18:0, *cis*-9-18:1, *trans*-11-18:1 and *cis*-9,*trans*-11-CLA concentrations, whereas it decreased the milk medium-chain FA and OBCFA concentrations, indicating that pasture could increase the milk concentrations of FA with putative nutritional effects on human health, as previously demonstrated in Holstein cows.

4. Study 3. Fatty acid, vitamin A, and cholesterol concentrations and oxidative stability in milk fat from Carpathian goats fed alfalfa hay-based diets supplemented with hemp seed oil

4.1. Introduction

Romania is one of the first countries for goat breeding in the European Union (EU), with a goat population of 1.350.000 heads in December 2012, equaled to France concerning the number of goats (1.313.000 heads) [313]. Goat livestock in Romania increased by 56% over the period from 2005 to 2012, achieving a milk production of 229.000 t, the fourth largest production among the European countries in 2011 [313]. The increase in Romanian goat milk production in recent years is due to increasing demand of consumers concerning to the dietary value and putative effects on human health of goat milk [136]. Goat milk is obtained mainly from Carpathian goats, the main goat breed reared in Romania representing about 75% of the total goat population [314].

Milk fat is one of the most important components of the nutritional quality of goat milk, containing fatty acids (FA) and fat-soluble vitamins that exhibit numerous health benefits. Goat milk has high concentrations of caproic (6:0), caprylic (8:0), and capric (10:0) acids which are known to exhibit antiobesity and antidiabetic properties [12]. Butyric acid (4:0) and the major isomer of conjugated linoleic acid (CLA), *cis*-9,*trans*-11, have shown anticarcinogenic effects both *in vitro* and *in vivo* [10,28]. Vitamin A has been reported to exert a major role in cell growth and differentiation as well as in vision and reproduction [17,117]. In contrast, some saturated FA (SFA) (12:0, 14:0, 16:0), *trans*-18:1 FA, and cholesterol if they are consumed in excess have been associated with an increase in cardiovascular disease risk [3,28].

All these aspects underline the interest of modulating milk fat composition by ruminant diet. It has been known that ruminant diet strongly influences the milk FA profile [18], as well as the milk vitamin A [315], and cholesterol contents [23]. For both ruminant species (cows and goats), supplementation of diet with plant oils rich in polyunsaturated FA (PUFA) is an efficient mean to modify milk FA profile by decreasing SFA and increasing oleic (*cis*-9-18:1), linoleic (18:2 n-6) or α-linolenic (18:3

n-3) acids (according to the nature of plant oil), along with several *trans* isomers of 18:1, conjugated and non-conjugated 18:2 [18,20]. There is also evidence that adding plant lipids to cow diets increased milk vitamin A and decreased cholesterol contents, respectively [22].

Over the last decades, different lipid sources have been used to supplement the ruminant diet to increase energy intake and/or modify milk FA composition [18]. Previous experiments have studied the effects on milk FA profile of goats feeding vegetable oils rich in either 18:2 n-6 (e.g., sunflower or soybean oils) or 18:3 n-3 (e.g., linseed or rapeseed oils) [104,105,316]. However, no previous study has used hemp seed oil (HSO) as lipid supplement in ruminant diets.

HSO is obtained from industrial hemp (*Cannabis sativa* L.), an annual herbaceous plant that has been used for centuries for its fiber in textile industry and oilseed production. Until the year 1989, Romania was the first in hemp cultivation among the European countries and the fourth worldwide, with 45.000 ha cultivated. Subsequently, Romanian hemp industry began to decline due to strict European regulations, limiting production of industrial hemp because it can be easily confounded with high-delta^9-tetrahydrocannabinol (THC) hemp types, mainly *Cannabis indica* (marijuana). Thus, Romania currently cultivates industrial hemp over an area of only 200 ha [317], but great efforts are made to restore Romanian hemp industry to its once important place in world agriculture. HSO is rich in 18:2 n-6 and 18:3 n-3 (59.8% and 18.2% of total FA, respectively) as well as in γ-tocopherol (0.5 g/L) [318].

Feeding a lipid supplement rich in PUFA is expected to increase the levels of beneficial PUFA in milk [18]. Nevertheless, this change in milk FA profile could also have negative aspects, because milk PUFA are susceptible to be oxidized and may thus decrease the oxidative stability of milk associated with a putative increase in milk negative flavors [21,60]. In this context, it is important to measure the concentration of malondialdehyde (MDA) in milk, MDA being a secondary lipid peroxidation product and a common marker of milk oxidation status [277].

4.2. Aims

The aim of the present study was to evaluate the effect of a diet supplemented with HSO on the FA, vitamin A, and cholesterol concentrations and oxidative stability in goat milk.

4.3. Materials and methods

4.3.1. Goats and diets

The experiment was conducted with 10 Carpathian goats in midlactation (at 13 ± 1 wk of lactation and 37.4 kg BW on average at the beginning of the experiment)

between 5 July and 4 August 2011. Goats were selected and divided up into two groups of 5 goats based on the homogeneity of their body weight, milk yield and milk fat content to receive one of the two diets during the experimental period (31 days). The feed distribution was collectively per group. The animals were gradually adapted to the experimental diets (4 days adjustment period). The control group (C) received daily a basal diet consisting of 60% alfalfa hay and 40% concentrate. The experimental group (HSO) received the same diet in which a part of the concentrate was substituted by 93 g/day of HSO (4.7% of dry matter (DM); Canah International, Salonta, Romania). An extra amount of soybean meal was incorporated into the concentrate of the experimental diet to balance nitrogen intake between the two diets (Table IX). The diets were formulated in an attempt to cover 100% of the energy and protein requirements of the animals [282].

Table IX. Effect of hemp seed oil in the diet on dry matter intake and fatty acid intake in dairy goats

	C	HSO
DMI (kg/d)		
Total	2.03	1.98
Alfalfa hay	1.15	1.35
Concentrate	0.88	0.5
Soybean meal	-	0.13
FA intake (g/d)		
14:0	0.21	0.42
16:0	10.72	45.60
18:0	2.01	13.17
cis-9-18:1	8.20	44.19
18:2 n-6	18.21	206.11
18:3 n-3	4.74	72.64

DMI = dry matter intake.
C = control diet; HSO = diet supplemented with hemp seed oil.

Goats were milked manually, twice daily, at 8:00 and 16:00. The two groups were housed in the same stall, separated by a metallic grill, had free access to water, and were fed two equal meals, after milking. The feedstuffs distributed and refusals were recorded on d 1, 8, 15, 22, and 29 of experimental period. Body weight of goats was recorded at the beginning and at the end of the experimental period. Goats were weighed after the milking and before the morning meal. All experimental procedures were approved by the Committee of Bioethics in Scientific Research of the University of Medicine and Pharmacy Cluj-Napoca (Romania) in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

4.3.2. Sampling, measurement and analyses

Samples of alfalfa hay (100 g), concentrate (100 g), and feed refusals (10 g) were collected on d 1, 8, 15, 22, and 29 of experimental period and stored at -20˚C. Feed DM

was determined after drying at 105˚C for 24 h. Fatty acid composition was determined on ground lyophilized samples of alfalfa hay and concentrate, methylated as described by Sukhija and Palmquist [303] with modifications [51]. Tricosanoate (Sigma Saint-Quentin Fallavier, France) was used as internal standard.

Milk yield of individual goats was recorded once weekly (from two consecutive milkings). Milk samples were collected from each goat over two consecutive milkings on d 1, 8, 15, 22, and 29 of experimental period. Each sample of individual milk was obtained by pooling 60% of the morning milk with 40% of the evening milk. After sampling, two sets of milk sub-samples were generated.

The first set of sub-samples (40 mL) was stored at 4˚C until determination of milk fat, protein, and lactose contents using an automated milk analyser (Ekomilk Ultrasonic Milk Analyzers, EON Trading LLC, USA) that analyses milk composition by ultrasonic technology. The analysis was performed in accordance with the recommendations of Venturoso et al. [319].

A second set of milk sub-samples (40 mL) was lyophilised (Ilshin Laboratory Floor Model Freeze Dryers, USA) and stored at -80˚C until analyses for determination of milk FA, vitamin A, cholesterol and MDA concentrations.

Milk FA composition was determined as described by Ferlay et al. [86] with some modifications. Briefly FA methyl esters (FAME) were prepared by direct methylation: 2 mL 0.5 M sodium methanolate and 1 mL hexane were mixed with the lyophilised milk at 50°C for 15 min, followed by the addition of 1 mL 12 N HCl 5% in methanol (v/v) at 50°C for 15 min. The FAME were washed with a saturated K_2CO_3 solution and recovered with 1.5 mL hexane. The FAME were injected (0.6 µL) by auto-sampler into a gas chromatograph equipped with a flame ionisation detector (Agilent Technologies 7890A, Wilmington, USA). The FAME from all the samples were separated on a 100 m × 0.25mm i.d. fused-silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was maintained at 255°C and the detector temperature at 260°C. The initial oven temperature was held at 70°C for 1 min, increased by 5°C/min to 100°C (held for 2 min), and then increased by 10°C/min to 175°C (held for 42 min), and 5°C/min to a final temperature of 225°C (held for 15 min). The carrier gas was hydrogen, and pressure was maintained constant (158.6 kPa) during analysis. A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain FA (C4:0 to C10:0). Identification of FAME was accomplished by comparison to a standard mixture (Nu-Chek-Prep, Inc Elysian, MN 56028 USA). Mixtures of *cis*/*trans* (9-12) isomers of linoleic acid methyl ester and *cis* and *trans* (9-11) and (10-12) isomers of CLA methyl esters purchased from Sigma-Aldrich Corporation (38297 Saint Quentin Fallavier, France) were used to complete the identification.

Milk vitamin A concentration was determined by HPLC using the method described by Andrei et al. [320]. Briefly, 200 mg of lyophilised milk were mixed with 40 mL ethyl ether (containing butyl-hydroxytoluene 0.0025%) and 40 mL petroleum ether. The upper etheric phase was collected and evaporated to dryness using an evaporator (ConcentratorPlus, Eppendorf, Germany). After adding 30 mL 5% KOH in ethanol 96% to the residue, the mixture was agitated in the dark for 3 h using a magnetic stirrer. Saponification was stopped by addition of 30 mL water. Vitamin A was then extracted twice with 30 mL hexane. The hexanoic phases were washed twice with water, collected, and evaporated to dryness (ConcentratorPlus, Eppendorf, Germany). The residues were dissolved in 1 ml hexane and injected into an HPLC system equipped with pumps (Shimadzu LC-20 AT), a photodiode-array detector (SPD-M20A Prominence) and a reversed-phase chromatographic column (Supelco Discovery RP18, nucleosil 5µm, 250 × 4.6 mm, Merck) with a mobile phase of acetonitrile:methanol (85:15, v/v) in isocratic system. Quantification was obtained from calibration curves using a standard solution of *trans*-total retinol (Sigma-Aldrich Corporation, Saint Quentin Fallavier, France) having the following concentrations: 5, 10, 14.28, 30, 50, 75, 100 µg/ml.

The cholesterol concentration in lyophilised milk was determined according to the method of Folch et al. [321]. Milk total lipid fraction containing cholesterol was extracted with 20 mL methanol and 40 mL chloroform. The mixture was agitated for 15 min and then washed in a separating funnel. The lower chloroformic phase was dried over Na_2SO_4 anhydrous, evaporated to dryness and recovered in 1 mL chloroform. Cholesterol was derivatised with 480 µL mixture of BSTFA (bis-trimethylsilyl-trifluoroacetamide) - TMCS (trimethylchlorosilane) (2:1) for 2 h at 60°C and then quantified by gas chromatography. 2 µL of derivatised standards and samples were injected into a gas chromatograph equipped with a flame ionisation detector (Shimadzu GC – 2010, Kyoto, Japan). A fused silica capillary column Varian CP-Sil 5CB (25 m × 0.25 mm) with 0.25 µm film thickness was used in this study. Injector temperature was set at 260°C, and detector temperature at 290°C. The initial oven temperature was held at 130°C for 3 min, increased by 101°C/min to 200°C (held for 3 min), and then increased by 20°C/min to a final temperature of 290°C (held for 5 min). The carrier gas was helium, with a flow rate of 1.9 mL/sec. The internal standard (Sigma-Aldrich Corporation, Saint Quentin Fallavier, France) and cholesterol peaks were determined by area measurement.

MDA concentration was determined using a spectrophotometric method as described by Pintea et al. [322]. One 200-mg sample of lyophilised milk was properly mixed with 750 µL acetic acid 20% with a vortex mixer. Afterward 750 µL thiobarbituric acid (TBA) 0.8% was added. Mixture was vigorously agitated in a vortex and placed in a water bath at 95˚C for 60 min. After cooling, 1-mL aliquots were measured followed by the addition of a 2 mL butanol-pyridine (15:1) mixture. The

mixture was then homogenized and centrifuged at 2500 rpm for 5 min (Hettich Centrifuge Rotofix 32A, Tuttlingen Germany). The upper phase containing the pink MDA-TBA complex was separated and measured at 532 nm using an UV-Vis spectrophotometer (Jenway 6315, Essex, England). TMP (1,1,3,3-tetramethoxypropane) was used as the MDA standard, with acidic hydrolysis prior to the TBA reaction. A stock solution was prepared by mixing 82 µL TMP with 40 mL distilled water and 40 drops HCl 37%. The solution was brought at 50 mL with distilled water and kept at room temperature for 2 h to hydrolyse TMP into MDA. From the stock solution a working solution (100 nmol/L) was prepared. Standard solutions of 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10 and 12.5 nmol/L of MDA were then obtained by successive dilutions.

4.3.3. Statistical analyses

Data recorded at day before the addition of oil were used as covariates. Data for milk yield, milk composition, and FA, vitamin A, cholesterol, and MDA concentrations in milk were analyzed as repeated measures using the MIXED procedure of SAS [286]. The statistical model included: day, diet, goat, diet × day interaction, covariate and residual error. Fixed effects included covariate, day, diet and interaction. Goat was the random effect.

4.4. Results

4.4.1. Animal performance

Results for milk yield and composition are reported in Table X.

HSO supplementation did not alter milk yield and milk lactose content. Throughout the entire experiment, HSO diet resulted in a significant increase in milk fat (+0.78%; $P < 0.001$) and protein (+0.13%; $P < 0.01$) contents compared to C diet. Changes in milk fat content were affected by time on diet for both C and HSO diets ($P < 0.05$). For C diet, milk fat content decreased between d 1 and 15 from 2.81% to 2.56%, then increased to 3.06% on d 22 and finally decreased to 2.56% on d 29. For HSO diet, milk fat content increased between d 1 and 8 from 3.19% to 3.84%, then decreased to 3.09% on d 15, and increased to 3.79% on d 22 and finally decreased to 3.65% on d 29. HSO supplementation increased milk protein content on d 1, 8, and 15, but had no effect on milk protein content on d 22 and 29 (diet × time interaction, $P < 0.05$).

Table X. Effect of dietary supplementation of hemp seed oil on milk yield and composition in dairy goats

	Days of supplemented diet														**Significance (*P*-value)[a]**		
	Day 1		***Day 8***		***Day 15***		***Day 22***		***Day 29***								
	C	**HSO**	**SEM**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**SEM**	***Diet***	***Time***	***Diet × Time***
Milk yield (L/day)	1.25	1.30	368.574	1.14	1.18	1.32	1.31	1.19	1.31	1.30	1.34	1.30	1.35	504.962	ns	***	ns
Fat content (%)	2.73	3.51	0.106	2.81	3.19	2.65	3.84	2.56	3.09	3.06	3.79	2.56	3.65	0.207	***	*	ns
Protein content (%)	3.16	3.29	0.030	3.16	3.30	3.09	3.45	3.12	3.26	3.25	3.25	3.18	3.17	0.054	**	ns	*
Lactose content (%)	4.25	4.22	0.061	4.22	4.30	4.17	4.31	4.25	4.27	4.27	4.11	4.34	4.11	0.077	ns	ns	+

C = control diet; HSO = diet supplemented with hemp seed oil.
SEM = Standard Error of the Mean; ns = not significant.
[a] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

4.4.2. Milk fatty acid concentrations

The effects of dietary treatments on milk FA composition are presented in Tables XI and XII. Supplementation with HSO to the diet resulted in marked changes in milk FA composition compared to C diet. HSO supplementation decreased milk concentrations of 18:3 n-6 ($P < 0.05$), 20:4 n-6 ($P < 0.01$), and 22:4 n-6 ($P < 0.05$), whereas it increased milk *cis*-9,*trans*-12-18:2 ($P < 0.01$) concentration.

Nevertheless, for most FA, responses to dietary HSO supplementation varied according to time on diet. Interestingly, milk concentrations of 10:0 ($P < 0.10$), 12:0 ($P < 0.01$) (Fig. 20), 14:0 ($P < 0.001$) (Fig. 21), *cis*-9-14:1 ($P < 0.001$), 16:0 ($P < 0.001$) (Fig. 22), iso 16:0 ($P < 0.05$), anteiso 17:0 ($P < 0.001$), total saturated FA (SFA) ($P < 0.001$), and desaturase ratios (*cis*-9-14:1/14:0 ($P < 0.01$) and *cis*-9-18:1/18:0 ($P < 0.001$)) declined with HSO diet, except for d 22 of lipid supplementation in which the above mentioned FA increased. Similarly, milk concentrations of iso 17:0 ($P < 0.001$), 18:0 ($P < 0.01$) (Fig. 23), *trans*-11-18:1 ($P < 0.001$) (Fig. 24), *trans*-16-18:1 ($P < 0.01$), *cis*-9,*trans*-13-18:2 ($P < 0.01$), *trans*-11,*cis*-15-18:2 ($P < 0.001$), total monosaturated FA (MUFA) ($P < 0.001$), and total PUFA ($P < 0.001$) increased with HSO supplementation, except for d 22 of oil addition in which these FA concentrations decreased.

Moreover, the decreases in *cis*-9-10:1 ($P < 0.001$), 11:0 ($P < 0.05$), *cis*-9-12:1 ($P < 0.001$), 13:0 ($P < 0.001$), anteiso 13:0 ($P < 0.001$), and iso 14:0 ($P < 0.05$) concentrations, in response to HSO supplementation were transient, such that concentrations of these FA were comparable with those in milk from C diet towards the end of the experiment.

Milk fat concentrations of 15:0 ($P < 0.001$), anteiso 15:0 ($P < 0.01$), *cis*-9,*trans*-11-CLA/*trans*-11-18:1 ($P < 0.001$), and total odd- and branched-chain FA (OBCFA) ($P < 0.001$) were decreased by HSO on d 1, 8, and 15 of supplementation and increased on d 22 and 29 of supplementation. Likewise, HSO increased *cis*-12-18:1 ($P < 0.001$) milk fat concentration on d 1, 8, and 15 of supplementation and decreased this FA concentration on d 22 and 29 of supplementation.

Between d 1 and 22 of the experiment, HSO decreasedin the milk *trans*-11-16:1 ($P < 0.01$), 17:0 ($P < 0.001$), *cis*-9-17:1 ($P < 0.01$), and 20:5 n-3 ($P < 0.10$) concentrations, whereas on d 29 the concentrations of these FA increased with lipid supplementation. In a similar manner, HSO increased milk 4:0 (Fig. 25), *trans*-6-18:1 ($P < 0.001$), *trans*-9-18:1 ($P < 0.001$), *trans*-10-18:1 ($P < 0.05$), and 18:2 n-6 ($P < 0.001$) concentrations between d 1 and 22 of supplementation, whilst it decreased these FA concentrations on d 29 of supplementation.

Table XI. Effect of dietary supplementation of hemp seed oil on fatty acid profile in milk fat of dairy goats

				Days of supplemented diet											**Significance (*P*-value)**[a]		
				Day 1		***Day 8***		***Day 15***		***Day 22***		***Day 29***					
	C	**HSO**	**SEM**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**SEM**	***Diet***	***Time***	***Diet × Time***
FA (g/100 g of total FA)																	
4:0	2.35	2.45	0.057	2.17	2.37	2.25	2.28	1.97	2.37	2.69	2.72	2.69	2.49	0.085	ns	***	**
5:0	0.06	0.04	0.006	0.05	0.05	0.10	0.04	0.05	0.04	0.04	0.05	0.04	0.04	0.013	ns	ns	+
6:0	2.67	2.69	0.062	2.66	2.66	2.66	2.52	2.34	2.61	2.90	2.87	2.80	2.76	0.139	ns	*	ns
7:0	0.06	0.05	0.004	0.06	0.05	0.08	0.05	0.06	0.05	0.04	0.03	0.04	0.05	0.007	+	**	ns
8:0	2.98	2.88	0.092	3.10	2.94	3.08	2.72	2.69	2.82	3.11	2.99	2.91	2.94	0.194	ns	ns	ns
9:0	0.10	0.07	0.007	0.12	0.09	0.14	0.08	0.11	0.08	0.07	0.06	0.06	0.07	0.013	*	**	ns
10:0	9.94	8.71	0.334	11.09	9.84	11.55	7.83	9.77	8.54	8.46	8.58	8.84	8.78	0.702	*	+	+
cis-9-10:1	0.20	0.14	0.012	0.25	0.18	0.27	0.12	0.22	0.12	0.13	0.13	0.14	0.14	0.018	***	***	***
11:0	0.13	0.09	0.011	0.15	0.12	0.20	0.09	0.15	0.09	0.07	0.07	0.07	0.07	0.019	*	***	*
12:0	4.50	3.39	0.240	5.09	4.40	5.84	2.92	5.10	3.39	2.99	3.12	3.48	3.11	0.411	**	**	**
cis-9-12:1	0.11	0.06	0.011	0.13	0.09	0.17	0.05	0.14	0.06	0.04	0.05	0.05	0.05	0.016	**	***	***
13:0	0.11	0.08	0.006	0.13	0.10	0.15	0.08	0.13	0.09	0.08	0.07	0.08	0.08	0.010	**	***	***
anteiso 13:0	0.05	0.03	0.003	0.06	0.04	0.08	0.03	0.06	0.03	0.02	0.03	0.02	0.03	0.006	***	***	***
iso 13:0	0.04	0.04	0.002	0.04	0.03	0.04	0.04	0.03	0.04	0.03	0.03	0.03	0.05	0.004	ns	ns	+
14:0	9.5	7.9	0.341	10.1	9.2	11.1	6.2	10.4	7. 6	6.9	8.1	8.8	8.5	0.575	**	**	***
iso 14:0	0.12	0.10	0.006	0.14	0.09	0.10	0.07	0.13	0.09	0.10	0.12	0.12	0.13	0.012	*	**	*
cis-9-14:1	0.14	0.08	0.014	0.17	0.11	0.21	0.07	0.20	0.08	0.06	0.08	0.09	0.07	0.020	**	***	***
trans-9-14:1	0.01	0.01	0.002	0.005	0.01	0.01	0.01	0.01	0.01	0.007	0.01	0.01	0.01	0.003	ns	*	ns
15:0	1.14	0.91	0.030	1.24	0.90	1.35	0.75	1.30	0.88	0.83	0.99	0.97	1.03	0.056	***	*	***
anteiso 15:0	0.40	0.34	0.015	0.43	0.29	0.38	0.24	0.42	0.33	0.37	0.41	0.41	0.42	0.028	**	**	**
iso 15:0	0.22	0.21	0.014	0.23	0.18	0.21	0.18	0.23	0.22	0.23	0.22	0.21	0.23	0.023	ns	ns	ns
16:0	24.5	21.3	0.651	25.6	23.8	26.1	17.4	25.4	18.3	19.8	23.6	25.7	23.5	11.007	**	**	***
iso 16:0	0.26	0.20	0.018	0.26	0.17	0.27	0.17	0.29	0.20	0.22	0.24	0.25	0.24	0.025	+	ns	*
cis-11-16:1	0.07	0.06	0.007	0.07	0.07	0.06	0.05	0.08	0.04	0.07	0.06	0.06	0.08	0.011	ns	ns	*
trans-6-16:1	0.09	0.10	0.007	0.08	0.09	0.10	0.12	0.11	0.12	0.07	0.07	0.09	0.09	0.010	ns	***	ns
trans-11-16:1	0.34	0.29	0.014	0.31	0.27	0.29	0.24	0.38	0.19	0.39	0.37	0.30	0.38	0.031	*	**	**
17:0	0.98	0.79	0.032	1.06	0.91	1.10	0.72	1.10	0.60	0.81	0.80	0.81	0.93	0.057	***	*	***
iso 17:0	0.49	0.68	0.036	0.45	0.46	0.45	0.97	0.57	0.98	0.53	0.51	0.43	0.49	0.064	**	***	***
cis-9-17:1	0.31	0.23	0.015	0.31	0.30	0.31	0.19	0.39	0.16	0.29	0.24	0.23	0.25	0.034	***	ns	**
anteiso 17:0	0.99	0.71	0.040	1.05	0.73	1.05	0.62	1.17	0.60	0.83	0.84	0.85	0.78	0.067	***	ns	***

Table XI. Effect of dietary supplementation of hemp seed oil on fatty acid profile in milk fat of dairy goats (continued)

	Days of supplemented diet														**Significance (*P*-value)[a]**		
	Day 1			***Day 8***		***Day 15***		***Day 22***		***Day 29***							
	C	**HSO**	**SEM**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**SEM**	***Diet***	***Time***	***Diet × Time***
FA (g/100 g of total FA)																	
18:0	9.2	11.2	0.620	7.0	9.9	5.4	9.9	6.5	9.8	15.1	13.1	12.0	13.4	0.999	*	***	**
iso 18:0	0.08	0.08	0.005	0.13	0.14	0.07	0.07	0.11	0.06	0.06	0.05	0.05	0.06	0.012	ns	***	ns
cis-9,*trans*-13 -18:2	0.26	0.35	0.016	0.24	0.30	0.26	0.44	0.27	0.43	0.30	0.27	0.23	0.32	0.028	***	**	**
cis-9,*trans*-12 -18:2	0.08	0.11	0.006	0.08	0.10	0.08	0.13	0.07	0.11	0.09	0.10	0.09	0.11	0.014	**	ns	ns
trans-9,*trans* -12-18:2	0.02	0.03	0.003	0.03	0.02	0.01	0.04	0.03	0.03	0.02	0.03	0.02	0.03	0.006	ns	ns	+
trans-11, *cis*-15-18:2	0.11	0.26	0.022	0.06	0.10	0.10	0.48	0.06	0.33	0.20	0.19	0.15	0.21	0.038	***	***	***
cis-9,*trans*-11 -CLA	0.48	1.87	0.127	0.38	0.83	0.53	3.61	0.48	3.71	0.59	0.63	0.41	0.54	0.201	***	***	***
18:2 n-6	2.36	2.69	0.152	2.28	2.30	2.76	4.41	2.95	2.98	1.87	1.92	1.94	1.86	0.240	ns	***	***
18:3 n-3	0.89	1.16	0.144	0.76	0.88	0.75	1.36	0.83	0.91	1.12	1.16	0.96	1.50	0.221	ns	ns	ns
18:3 n-6	0.06	0.05	0.005	0.06	0.04	0.07	0.06	0.09	0.05	0.05	0.04	0.03	0.02	0.009	*	***	ns
20:0	0.24	0.33	0.018	0.21	0.26	0.21	0.33	0.21	0.38	0.30	0.31	0.28	0.38	0.027	**	*	***
20:2 n-6	0.04	0.05	0.006	0.03	0.04	0.06	0.05	0.03	0.07	0.03	0.04	0.04	0.05	0.012	ns	ns	ns
20:3 n-3	0.04	0.03	0.003	0.04	0.03	0.04	0.02	0.04	0.03	0.03	0.03	0.03	0.04	0.007	ns	ns	ns
20:3 n-6	0.06	0.04	0.004	0.04	0.06	0.09	0.04	0.06	0.04	0.05	0.03	0.04	0.03	0.007	*	**	**
20:4 n-6	0.16	0.14	0.006	0.16	0.15	0.17	0.14	0.19	0.13	0.17	0.14	0.13	0.13	0.012	**	+	ns
20:5 n-3	0.12	0.09	0.012	0.17	0.13	0.14	0.09	0.13	0.07	0.08	0.07	0.06	0.09	0.018	ns	**	+
21:0	0.09	0.10	0.011	0.08	0.08	0.09	0.08	0.08	0.10	0.09	0.11	0.09	0.14	0.015	ns	+	ns
22:0	0.11	0.12	0.012	0.09	0.11	0.13	0.11	0.11	0.12	0.13	0.12	0.11	0.16	0.016	ns	+	+
22:4 n-6	0.03	0.02	0.003	0.03	0.02	0.03	0.01	0.03	0.02	0.03	0.03	0.03	0.03	0.006	*	ns	ns
22:5 n-3	0.21	0.18	0.015	0.25	0.22	0.24	0.18	0.25	0.16	0.19	0.15	0.14	0.20	0.027	ns	+	+
22:6 n-3	0.08	0.07	0.006	0.10	0.08	0.08	0.07	0.09	0.07	0.07	0.05	0.05	0.07	0.010	ns	**	ns
23:0	0.05	0.05	0.008	0.04	0.02	0.05	0.03	0.04	0.04	0.05	0.06	0.05	0.08	0.010	ns	**	*
24:0	0.06	0.06	0.008	0.05	0.05	0.05	0.04	0.05	0.05	0.07	0.07	0.06	0.10	0.011	ns	*	+
Total SFA	71.45	65.56	0.711	72.94	69.84	74.32	56.44	70.62	60.40	67.04	70.16	72.34	70.92	15.218	***	***	***
Total OBCFA	5.43	4.57	0.125	5.81	4.42	5.98	4.29	6.11	4.53	4.57	4.70	4.66	4.91	0.216	***	*	***
Total MUFA	22.40	26.01	0.651	21.14	23.72	19.10	31.06	22.56	29.08	26.94	23.80	22.26	22.40	14.246	***	*	***
Total *trans* FA	6.40	13.41	0.678	5.33	7.77	6.64	22.65	6.46	22.13	7.33	7.50	6.22	6.99	11.395	***	***	***
Total PUFA	5.20	7.27	0.277	4.92	5.46	5.64	11.24	5.84	9.30	5.10	4.98	4.52	5.36	0.467	***	***	***

Table XI. Effect of dietary supplementation of hemp seed oil on fatty acid profile in milk fat of dairy goats (continued)

	Days of supplemented diet														**Significance (*P*-value)[a]**		
	Day 1			***Day 8***		***Day 15***		***Day 22***		***Day 29***							
	C	**HSO**	**SEM**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**SEM**	***Diet***	***Time***	***Diet × Time***
FA (g/100 g of total FA)																	
Total n-6	2.55	2.84	0.156	2.45	2.45	3.01	4.57	3.18	3.14	2.03	2.05	2.10	1.99	0.248	ns	***	**
Total n-3	1.32	1.54	0.167	1.31	1.35	1.25	1.73	1.33	1.26	1.49	1.47	1.22	1.91	0.258	ns	ns	ns
Desaturase ratio																	
cis-9-14:1/14:0	0.02	0.01	0.001	0.02	0.01	0.02	0.01	0.02	0.01	0.009	0.01	0.01	0.009	0.002	*	***	**
cis-9-18:1/18:0	2.21	1.46	0.177	2.56	1.78	2.77	1.43	2.87	1.30	1.36	1.45	1.49	1.33	0.232	**	***	***
cis-9,*trans*-11-CLA/ *trans*-11-18:1	0.52	0.44	0.040	0.57	0.50	0.66	0.45	0.74	0.42	0.29	0.41	0.36	0.40	0.059	ns	***	***

C = control diet; HSO = diet supplemented with hemp seed oil.
SFA = saturated FA; OBCFA = odd- and branched-chain FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.
SEM = Standard Error of the Mean; ns = not significant.
[a] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table XII. Effect of dietary supplementation of hemp seed oil on concentrations of *trans* and *cis* isomers of 18:1 in goat milk fat

	Days of supplemented diet																	
	Day 1			*Day 8*		*Day 15*		*Day 22*		*Day 29*				**Significance (*P*-value)[a]**		
	C	**HSO**	**SEM**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**SEM**	***Diet***	***Time***	***Diet × Time***
FA (g/100 g of total FA)																	
trans-4-18:1	0.03	0.04	0.003	0.02	0.02	0.02	0.04	0.02	0.04	0.03	0.04	0.03	0.03	0.005	*	ns	*
trans-5-18:1	0.02	0.03	0.003	0.02	0.03	0.02	0.03	0.01	0.04	0.03	0.03	0.04	0.03	0.005	+	ns	**
trans-6-18:1	0.20	0.33	0.020	0.17	0.27	0.21	0.47	0.17	0.49	0.21	0.23	0.23	0.20	0.030	***	***	***
trans-9-18:1	0.21	0.39	0.016	0.18	0.29	0.22	0.56	0.21	0.62	0.21	0.26	0.22	0.21	0.027	***	***	***
trans-10-18:1	0.29	0.47	0.047	0.24	0.40	0.40	0.85	0.35	0.63	0.23	0.25	0.24	0.22	0.077	*	***	*
trans-11-18:1	1.07	4.94	0.381	0.68	1.81	0.90	9.34	0.72	10.25	1.92	1.82	1.16	1.50	0.664	***	***	***
trans-12-18:1	0.28	0.54	0.022	0.24	0.39	0.27	0.77	0.25	0.84	0.33	0.35	0.30	0.34	0.036	***	***	***
trans-13-18:1	0.39	0.78	0.066	0.24	0.39	0.32	1.05	0.28	1.13	0.58	0.68	0.52	0.65	0.119	***	**	**
trans-16-18:1	0.33	0.47	0.035	0.26	0.37	0.26	0.46	0.24	0.53	0.49	0.47	0.41	0.52	0.051	**	*	**
Total *trans*-18:1	2.86	7.96	0.514	2.08	3.94	2.66	13.53	2.29	14.53	4.07	4.11	3.19	3.67	0.884	***	***	***
cis-9-18:1	17.22	15.84	0.897	16.58	17.58	13.87	14.89	17.66	12.32	20.83	17.72	17.14	16.71	18.461	ns	+	ns
cis-11-18:1	0.58	0.52	0.031	0.61	0.48	0.58	0.58	0.59	0.48	0.59	0.56	0.54	0.49	0.058	ns	ns	ns
cis-12-18:1	0.27	0.45	0.033	0.27	0.28	0.33	0.89	0.30	0.70	0.23	0.17	0.24	0.20	0.061	**	***	***
cis-13-18:1	0.16	0.17	0.012	0.20	0.17	0.19	0.21	0.18	0.20	0.11	0.13	0.14	0.14	0.020	ns	***	ns
cis-15-18:1	0.09	0.07	0.011	0.12	0.08	0.10	0.07	0.05	0.04	0.09	0.08	0.08	0.10	0.016	ns	**	ns
Total *cis*-18:1	18.29	17.07	0.907	17.74	18.61	15.03	16.66	18.76	13.77	21.83	18.67	18.11	17.66	18.611	ns	ns	ns

C = control diet; HSO = diet supplemented with hemp seed oil.
SEM = Standard Error of the Mean; ns = not significant.
[a] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Fig.20. Concentration of **12:0** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.01$)

Fig.21. Concentration of **14:0** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.001$)

Fig.22. Concentration of **16:0** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.001$)

Fig.23. Concentration of **18:0** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.01$)

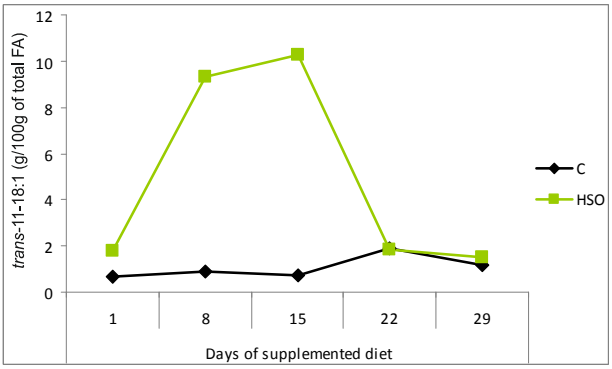

Fig.24. Concentration of ***trans*-11-18:1** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.001$)

Fig.25. Concentration of **4:0** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.01$)

Supplementation with HSO increased milk concentrations of *trans*-12-18:1 ($P < 0.001$), *trans*-13-18:1 ($P < 0.01$), *cis*-9,*trans*-11-CLA ($P < 0.001$) (Fig. 26), total *trans*-18:1 ($P < 0.001$), and total *trans* FA ($P < 0.001$) with a more marked increase on d 8 and 15 of supplementation. Likewise, HSO supplementation increased 20:0 ($P < 0.001$) milk fat concentrations, with a more marked increase on d 8, 15, and 29 of supplementation.

Fig.26. Concentration of ***cis*-9,*trans*-11-CLA** in milk fat of goats fed none (C) or 93 g/d of hemp seed oil (HSO) (diet × time interaction, $P < 0.001$)

Initially, 20:3 n-6 ($P < 0.01$) milk fat concentration increased in response to HSO supplementation, but started to decline after d 8 of supplementation.

Nevertheless, HSO supplementation did not influence the concentrations of 6:0, 8:0, iso 13:0, *trans*-9-14:1, iso 15:0, *trans*-6-16:1, iso 18:0, *cis*-9-18:1, *cis*-11-18:1, *cis*-13-18:1, *cis*-15-18:1, 18:3 n-3, 20:2 n-6, 20:3 n-3, 21:0, 22:6 n-3 and total n-3 in milk fat.

4.4.3. Milk vitamin A and cholesterol concentrations

Milk vitamin A and cholesterol concentrations were not affected by HSO supplementation, but they were influenced by time on diet (Table XIII). For both C and HSO diets, milk vitamin A concentration increased from d 22 onwards ($P < 0.05$), whereas milk cholesterol concentration tended to be decreased from d 15 onwards ($P < 0.10$).

Table XIII. Effect of dietary supplementation of hemp seed oil on vitamin A, cholesterol, and MDA concentrations in milk of dairy goats

	Days of supplemented diet														**Significance (*P*-value)[a]**		
	Day 1			***Day 8***		***Day 15***		***Day 22***		***Day 29***							
	C	**HSO**	**SEM**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**C**	**HSO**	**SEM**	***Diet***	***Time***	***Diet × Time***
Vitamin A (μg/ml)	0.166	0.146	0.019	0.161	0.126	0.134	0.126	0.141	0.125	0.198	0.162	0.193	0.190	0.025	ns	*	ns
Cholesterol (mg/100 g)	14.96	12.50	11.435	16.48	14.95	15.78	17.01	13.12	9.90	15.54	10.59	13.87	10.04	20.822	ns	+	ns
MDA (nmol/g)	1.36	1.61	0.378	0.14	0.41	0.56	1.74	0.51	1.40	0.63	1.30	4.96	3.19	0.845	ns	***	ns

C = control diet; HSO = diet supplemented with hemp seed oil.
MDA = malondialdehyde.
SEM = Standard Error of the Mean; ns = not significant.
[a] Significance (*P*-value): ns: $P > 0.10$; +: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

4.4.4. Milk MDA concentration

HSO supplementation had no influence on milk MDA concentration (Table XIII). Nevertheless, numerically, we observed a higher value for HSO than for C diets. Moreover, for both C and HSO diets, an important increase of MDA on d 29 of supplementation was observed ($P < 0.001$).

4.5. Discussion

4.5.1. Animal performance

Diet supplementation with vegetable oils generally has no influence on milk yield, increases milk fat content, and determines variable effects on milk protein and lactose contents in mid-lactation goats (review of Chilliard et al. [60]). In our study, milk yield was not affected by HSO supplementation. Our results agree with Bernard et al. [104] who reported no significant influence of grass hay diets supplemented with 5.8% sunflower or linseed oil on milk yield in Alpine goats. Likewise, Bouattour et al. [105] observed in a study with Murciano-Granadina goats fed diets based on dehydrated fescue that soybean oil supplementation (2.5%) did not modify milk yield. The lack of increase in milk yield with HSO supplementation reported in our study might be partly related to a lack of increase in milk lactose content with HSO diet compared to C diet, due to the role of lactose in the regulation of milk osmolarity [323]. In disagreement with our results, Ollier et al. [324] using Alpine goats provided evidence that there is a decrease in milk yield (-0.41 kg/d) when goats were fed high-forage diets supplemented with 4.1% whole intact rapeseeds.

The increase in milk fat content as a response to HSO supplementation is in agreement with Bouattour et al. [105], who reported an increase of 0.67% for Murciano-Granadina dairy goats fed diets based on dehydrated fescue supplemented with 2.5% soybean oil. Likewise, Li et al. [325] using Saanen goats found that supplementing an alfalfa hay diet with 5% safflower oil or linseed oil enhanced the fat content of milk with 0.45% and 0.62%, respectively. Similarly, Ollier et al. [324] observed in a study with Alpine goats, that supplementing a high-forage diet with 4.1% whole intact rapeseeds increased milk fat content with 0.6%. In fact, recent *in vitro* studies indicated that mammary lipogenesis in goats is not affected and may even be stimulated by PUFA RBH intermediates [326].

The HSO supplementation to the diet enhanced milk protein content on d 1, 8, and 15 in our study on dairy goats, in agreement with Bernard et al. [104], who found that supplementation with 5.8% linseed oil of a diet based on grass hay increased milk protein content (+0.08%) with Alpine goats. In contrast, Li et al. [325] reported no significant effect of 5% safflower or linseed oil supplementation of an alfalfa hay diet on milk protein content in Saanen goats. This increase in protein content may improve milk cheese making properties.

In the present study, HSO supplementation had no effect on milk lactose content. Our results are in agreement with Li et al. [325] using Saanen goats fed 5% safflower or linseed oil in a diet based on alfalfa hay, compared to Saanen goats fed a diet based on alfalfa hay without oil. In disagreement with our results, Bernard et al. [104] showed that Alpine goats fed diets based on grass hay supplemented with 5.8% sunflower oil or linseed oil had higher milk lactose content than goats fed the same basal diets without oil. Likewise, with Alpine dairy goats, Ollier et al. [324] showed an increase in milk lactose content, when whole intact rapeseeds (4.1%) were added to a high forage diet (+0.07%). Differences in results for milk lactose content between these latter studies and our study could be due to differences in the level of lipid supplementation.

4.5.2. Milk fatty acid concentrations

In the present study, HSO supplementation increased milk 4:0, without effect on 6:0 and 8:0 concentrations. An increase in the milk content of 4:0 (+0.31 - 0.37 g/100 g of total FA) has already been reported by Bernard et al. [104] when supplementing 5.8% sunflower oil or linseed oil to diets based on grass hay. Nevertheless, only in partial agreement with our results, Bouattour et al. [105] observed no significant changes in the concentrations of FA from 4:0 to 8:0 in goat milk fat after diet supplementation with 2.5% soybean oil. Likewise, Chilliard and Ferlay [20] reported no changes in 4:0 to 8:0 contents after supplementing alfalfa hay-based diets with vegetable oils rich in PUFA (5% - 6% of total DM intake). The increase in the milk fat content of 4:0 could be due to the fact that this short-chain FA (SCFA) is partly synthesized by metabolic pathways not involving acetyl-CoA carboxylase [20].

HSO in the diet decreased the milk concentrations of medium-chain FA (MCFA) (10:0, 12:0, 14:0, and 16:0) derived from *de novo* synthesis, in agreement with previous studies in dairy goats [104,105]. Decreases in the concentrations of FA synthesised *de novo* were accompanied by reductions in milk concentrations of most OBCFA (13:0, anteiso 13:0, iso 14:0, 15:0, anteiso 15:0, iso 16:0, 17:0, and anteiso 17:0), in agreement with Bernard et al. [104], who reported an inverse association between 5.8% sunflower oil and linseed oil supplementation to the diet and OBCFA content in goat milk fat. OBCFA are largely synthesized by rumen bacteria, so that reductions in milk concentrations of these FA could be due to an inhibitory effects of dietary PUFA on rumen microbial activity [11]. Furthermore, the decline in the milk fat contents of 10:0 to 16:0 observed in our experiment could be due also to a negative effect of intermediates of RBH of dietary PUFA on activity of ACC or FAS [20]. Moreover, the significant decrease in milk content of MCFA without changes in most SCFA concentrations could be due to the inhibitory effect of long-chain FA supplied to the mammary gland on the ratio of acetyl-CoA carboxylase to FA synthetase activities [316]. According to Martínez Marín et al. [316], decreasing the ratio of acetyl-CoA carboxylase to FA synthetase activities in the mammary cell has been shown to orient the pattern

of FA synthesized toward the shorter chain types *in vitro*. In this respect, Bernard et al. [323] reported a decrease in the ratio of acetyl-CoA carboxylase to FA synthetase activities without reduction of the secretion and content of 4:0 to 8:0 in milk fat of goats fed diets supplemented with sunflower or linseed oil.

In agreement with Bouattour et al. [105] and Bernard et al. [104], lipid supplementation increased the milk fat percentage of 18:0 in our study. The 18:0 in milk fat may have multiple origins. It may come from 18:0 ingested with diet, it may be obtained from a complete RBH of dietary PUFA or, when animals are in negative energy balance, it may be derived from lipid store mobilization [20]. The latter origin was not likely in the present study because the goats gained weight. Moreover, the higher amounts of 18:0, 18:2 n-6, and 18:3 n-3 ingested with HSO than with C diets (Table IX) suggest that most 18:0 in milk from goats fed HSO would come from dietary 18:0 and a complete RBH of PUFA provided by diet, respectively.

The lack of *cis*-9-18:1 response to lipid supplementation reported in our study is in agreement with dairy goats data reviewed by Chilliard et al. [60] and Chilliard and Ferlay [20], although some studies showed positive responses of *cis*-9-18:1 to vegetable oils supplementation [105,325], but not that of Bernard et al. [104] who reported no increases in *cis*-9-18:1 concentration in milk fat when supplementing grass hay-based diets with 5.8% linseed oil. Furthermore, about 80% of *cis*-9-18:1 secreted in milk originates from Δ-9 desaturation of 18:0 in the mammary gland [18]. In agreement with Chilliard and Ferlay [20], who indicated an inhibition of Δ-9 desaturase activity in the mammary gland with diets which increase the availability of PUFA, our study reported a decrease in *cis*-9-14:1/14:0, the best indicator of Δ-9 desaturase activity [88], as well as a decrease in *cis*-9-18:1/18:0 and *cis*-9-*trans*-11-CLA/*trans*-11-18:1, in response to HSO supplementation. Our results suggest that the desaturation capacity of the mammary gland is limited with the increased supply of 18:0 to the mammary gland, which could partly explain the lack of variation in *cis*-9-18:1 milk concentration with HSO supplementation.

In the present study, HSO diet significantly increased the concentration of milk fat *trans*-18:1 isomers compared to C diet. Increases in *trans*-18:1 FA in milk fat are frequently reported when goats are supplemented with vegetable oils and represent most likely the result of an incomplete RBH of dietary PUFA, producing a large number of MUFA and PUFA intermediates along the RBH pathways [20,60]. However, the most important increase among *trans*-18:1 isomers reported in our study was for *trans*-11-18:1 (+3.87 g/100g of total FA). This increase could be attributed to the high intakes of 18:2 n-6 and 18:3 n-3 with HSO diet, because both FA have *trans*-11-18:1 as main intermediate in their RBH pathways [18].

HSO supplementation has been shown to be an effective mean of increasing *cis*-9,*trans*-11-CLA (+1.39 g/100 g of total FA) in milk fat. Our results are in agreement

with Bouattour et al. [105], showing that the level of *cis*-9,*trans*-11-CLA in the milk of goats fed 2.5% soybean oil was much higher (+1.35 g/100 g of total FA) than that in the control. Our results agree also with Li et al. [325], showing an important increase in *cis*-9,*trans*-11-CLA (+0.95 - 1.61 g/100 g of total FA) milk fat concentration after feeding dairy goats with an alfalfa hay diet supplemented with 5% safflower or linseed oil. *Cis*-9,*trans*-11-CLA is mainly formed in the mammary gland via the action of Δ-9 desaturase on *trans*-11-18:1, but it can also be obtained during 18:2 n-6 RBH [20]. Nevertheless, the close association between increases in milk *cis*-9,*trans*-11-CLA and *trans*-11-18:1 concentrations in response to HSO supplementation confirms that Δ-9 desaturation of *trans*-11-18:1 is the main process involved in milk *cis*-9,*trans*-11-CLA synthesis [104]. Furthermore, in goats, the increase in milk fat *cis*-9,*trans*-11-CLA concentration in response to vegetable oils has been shown to reach its maximum values two weeks after the beginning of supplementation and to persist for at least ten weeks of lipid supplementation [18]. In our study, however, *cis*-9,*trans*-11-CLA responses to HSO supplementation were transient, with the maximum values obtained during the second and third weeks after the beginning of supplementation and decreasing from fourth week. Hypothesis concerning this result could be linked to an adaptation of the rumen microbial ecosystem to lipid supplementation or to slight changes in forage/concentrate ration during the experiment.

In the present study, HSO diet resulted in increases in milk 18:2 n-6 (+0.33 g/100 g of total FA) concentration. These increases were similar to the increases (+0.41 g/100 g of total FA) reported by Bouattour et al. [105] after supplementing goat diet with soybean oil (2.5% of total DM intake). Our results were to be expected, given that HSO is a rich source of 18:2 n-6. Nevertheless, the relatively low increase in 18:2 n-6 content in our study is in agreement with previous studies in goats, indicating that when supplementing diets with seeds or oils rich in 18:2 n-6 such as sunflower or soybean, the 18:2 n-6 proportion in milk fat rarely exceeds control values by more than 1.5% [18]. In contrast, milk 18:3 n-3 concentration was not significantly altered when HSO was supplemented. The lack of increase in 18:3 n-3 with HSO supplementation although dietary 18:3 n-3 concentration was greater in the HSO diet compared to C diet could be partially attributed to the fact that most of 18:3 n-3 in the supplemented diet was supplied in the free form (oil) and thus was highly susceptible to be transformed by RBH [107]. In agreement with our results, addition of 5.8% linseed oil to grass hay-based diets did not modifiy 18:3 n-3 concentration in goat milk fat [104]. Our results agree also with Li et al. [325], showing that supplementation of an alfalfa hay diet with 5% safflower oil had no effect on 18:3 n-3 milk fat concentration in Saanen goats.

4.5.3. Milk vitamin A and cholesterol concentrations

The range of the values of milk vitamin A concentration in our study, are comparable with that observed by Kondyli et al. [327] with raw goat milk of the indigenous Greek breed during lactation (0.11-0.16 μg/ml). Vitamin A in milk may be

derived from forages, concentrates (cereals, oilseeds) and mineral-vitamin supplements in ruminant diet ot it may be formed from dietary precursors, such as β-carotene [21]. The present study showed no effect of HSO supplementation on milk concentration of vitamin A, suggesting that there were only small differences in vitamin A and β-carotene intakes between C and HSO diet. Our results are in disagreement with Puppel et al. [112] using Polish Holstein Friesian cows, showing that supplementation of diet for 21 days with different lipid sources (300g/d of fish oil, 500g/d of Opal linseed, 500g/d of Szafir linseed, 150 g/d of fish oil or 250 g/d of Opal linseed, 150 g/d of fish oil and 250 g/d Szafir linseed) increased milk α-retinol concentration in all dietary treatments by 23 to 183%. Similarly, with Holstein Friesian cows, Nałęcz-Tarwacka et al. [22] reported an increase in milk vitamin A concentration (+0.147 mg/L) when diet was supplemented with linseed (200 g/d) for 28 days.

Nevertheless, our study reported an increase in milk vitamin A concentration with time for both C and HSO diets, suggesting an effect of lactation stage on vitamin A in milk. Our results agree with Jensen et al. [92], who observed a variation in milk retinol concentration during the first 24 weeks of lactation of Holstein-Friesian cows.

In the present study, HSO supplementation had no influence on milk cholesterol concentration. Similarly, with Holstein cows, Rafalowski and Park [328] reported no effect of graded increases of whole sunflower seed (10 to 30% of the concentrate) in the diet on cholesterol in milk. This could be explained by the fact that milk cholesterol is mainly synthesised through processes independent of the ruminant diet [102]. In disagreement with our results, Precht et al. [23] showed that addition of 275 g or 550 g rapeseed oil or corresponding quantities of whole meal from rapeseed decreased milk cholesterol concentration by 8 and 13%. Likewise, Reklewska et al. [113] reported a lower cholesterol concentration (-32%) in milk of cows fed *ad libitum* a total mixed ration supplemented with a low dose of linseed (21 g/d) for seven weeks than in milk of control cows.

For both C and HSO diets, milk cholesterol concentration was lower on d 15, 22, and 29 than on d 1 and 8. This variation might be attributed to an effect of lactation stage, linked to a reduction in mammary gland capacity for serum cholesterol uptake with the progress of lactation. Nevertheless, this hypothesis is not supported by the results of Strzałkowska et al. [102], showing an increase in milk cholesterol content with the progress of lactation in Polish Holstein-Friesian cows fed TMR throughout the year.

4.5.4. Milk MDA concentration

Previous studies have shown that increasing concentrations of unsaturated FA in milk, particularly of 18:2 n-6 and 18:3 n-3, will increase the susceptibility of milk to oxidation [329]. Nevertheless, even though milk PUFA concentrations were higher with HSO diet than with C diet in the present study, HSO supplementation had no influence on milk MDA concentration. Our results are in agreement with Reklewska et al. [113],

who reported no difference in milk MDA content between cows fed *ad libitum* a total mixed ration supplemented with a low dose of linseed (21 g/d) for seven weeks and control cows. The lack of modifications in milk oxidative stability with lipid supplementation in our study could be due to the important amount of vitamin E provided by HSO, because vitamin E in association with other antioxidant sources has been reported to protect plasma lipids against lipoperoxidation in dairy cows receiving linseed rich in 18:3 n-3 [330]. Similarly, single intramuscular administration of a high dosage of vitamin E to lactating goats improved the peroxidation resistant mechanisms of the overall body and those specifically within mammary gland [331]. In disagreement with our results, Puppel et al. [112] reported an increase in milk MDA concentration when 300g/d of fish oil, 500g/d of Opal linseed, 500g/d of Szafir linseed, 150 g/d of fish oil and 250 g/d of Opal linseed or 150 g/d of fish oil and 250 g/d Szafir linseed where supplemented to dairy cows diet for 21 d. Our contradictory result could be explained by the fact that fish oil is rich in n-3 FA with long chain. This supplementation could produce milks more susceptible to be oxidized.

Surprisingly, for both C and HSO diets, milk MDA concentration increased markedly at the end of the experiment.

4.6. Conclusions

HSO in the diet altered strongly milk FA composition, whereas it had no effect on milk vitamin A, cholesterol, and MDA concentrations. Changes in milk FA composition were characterised by a decrease in FA synthesised *de novo* (10:0-16:0) and an increase in 4:0, 18:0, *cis*-9,*trans*-11-CLA and PUFA concentrations, indicating that vegetable oils can be used to increase the milk content of FA having a putative positive effect on human health. Nevertheless, milk fat *cis*-9,*trans*-11-CLA responses to HSO supplementation were rapid, but the levels of enrichment declined after d 15. Likewise, large increases in detrimental *trans*-18:1 concentrations were observed. Furthermore, improvements in the fat composition of milk can be reached without inducing detrimental effects on animal performance.

5. General conclusions

The studies on the influence of cow breed, cow-calf contact, cow parity, and season on fatty acid (FA) profile and lipolytic system of milk allowed us to conclude the following:

- ***cow breed*** had a significant effect on milk FA composition and initial lipolysis. With the same feeding, the concentrations of short- and medium-chain FA were higher in milk fat from Prim'Holstein than from Salers cows. In contrast, Salers cows produced milk richer in FA with nutritional interest (18:3 n-3, 20:5 n-3, 22:5 n-3, and *cis*-9,*trans*-11-CLA) than did Prim'Holstein cows. Nevertheless, milk from Salers cows had a higher level of initial free FA than milk from Prim'Holstein cows, suggesting that it may develop a rancid flavour;

- ***calf presence during milking*** increased the concentration of 16:0 and decreased the 18:0, 18:2 n-6, 18:3 n-3, and total polyunsaturated FA concentrations in bulk milks from Salers and Prim'Holstein cow breeds. Likewise, the presence of the calf increased the concentration of 16:0 and decreased the total odd- and branched-chain FA concentration in individual milks from Salers cows. Because calf presence had a variable effect on milk FA concentrations, it is difficult to establish if this treatment positively or negatively influenced the content of milk in FA with nutritional importance. Moreover, the presence of the calf increased lipolysis at t_{24} in bulk milks from Prim'Holstein cows and also increased initial lipolysis in individual milks collected during the 9^{th} week of lactation from Salers cows, suggesting that calf presence could influence milk organoleptic characteristics;

- ***cow parity*** had a minor influence on milk FA composition. The primiparous cows produced milk poorer in short- and medium-chain FA and richer in *trans*-11-18:1 and *cis*-9,*trans*-11-CLA than did the multiparous cows. Cow parity had no influence on the milk lipolytic system;

- the ***season*** had a significant strong effect on milk FA composition and lipolysis. Compared with the winter diet, the pasture diet increased milk 18:0, *cis*-9-18:1, *trans*-11-18:1, and *cis*-9,*trans*-11-CLA concentrations, whereas it decreased the milk medium-chain FA and odd- and branched-chain FA concentrations, indicating that pasture could increase the milk concentrations of FA with putative nutritional effects on human health. Our results are in agreement with the literature.

The study on the effect of ***diet supplementation with hemp seed oil (HSO)*** on the FA, vitamin A, and cholesterol concentrations and oxidative stability in goat milk conducted us to the following conclusions:

- feeding HSO to dairy goats changed strongly milk FA composition with a decrease in FA synthesised *de novo* (10:0-16:0) and an increase in 4:0, 18:0, *cis*-9,*trans*-11-CLA and polyunsaturated FA concentrations, suggesting that vegetable oils can be used to increase the milk content of FA having a putative positive effect on human health. However, despite changes induced by HSO supplementation in the milk FA profile that pointed to an enhanced nutritional value of the milk fat, large increases in *trans* FA, mainly *trans*-18:1, were also observed;

- HSO in the diet had no effect on milk vitamin A and cholesterol concentrations, indicating that lipid supplementation is not useful in modulating the content of these liposoluble components in milk;

- diet supplementation with HSO had no influence on milk malondialdehyde concentration, suggesting that HSO oil did not affect the oxidative stability of milk.

6. Originality and innovative contributions of the thesis

The original contribution of the thesis consists in the simultaneous approach of the factors affecting milk fat composition, both nutritional and linked to animal. This topic is of particular importance due to the numerous effects that milk liposoluble components exert on human health.

The present work presents the first research regarding the influence of calf presence during milking on fatty acid composition in milk from primiparous and multiparous Salers and Prim'Holstein cows. This research provides also new information about the effect of both cow breed and parity on the content of fatty acids in milk. Moreover, it is the first work to describe the milk fatty acid profile of Salers cow breed.

Through the complexity of the research conducted, the thesis provides new data regarding the effect of ruminant diet on milk fat composition. First, it complements other studies in cows on the influence on the nature of forage (preserved vs. grazed grass) on milk fatty acid profile. Then, it presents the results of a complex study regarding the influence of diet supplementation with hemp seed oil on fatty acid, vitamin A, and cholesterol concentrations in goat milk. It is the first study to use hemp seed oil as a lipid supplement in ruminant diet, as well as the first research to evaluate the effect of adding a vegetable oil to goat diet on milk vitamin A and cholesterol contents. Likewise, it is the first study to characterise the milk liposoluble fraction of Carpathian goat breed.

The present work also reports new information regarding the effect of breed, calf presence during milking, parity, and diet on milk lipolytic system in cows. This part of the research was conducted in the context of the important influence of lipolytic system on milk organoleptic properties, which are essential for Salers cows milk further used for the production of traditional cheeses having a PDO status.

Finally, this thesis has as primary benefit the provision of valuable information on the influence of cow breed, cow-calf contact, cow parity, and ruminant diet on milk fat composition, particularly milk fatty acid composition. With all these data available, ruminant breeders and professionals from dairy industry can intervene in the modulation of milk fat composition, in order to provide to the consumer milk and dairy products with numerous health benefits.

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