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## Influence of calf presence during milking on yield, composition, fatty acid profile and lipolytic system of milk in Prim'Holstein and Salers cow breeds

Anamaria Cozma · Bruno Martin · Marlène Guiadeur ·  
Philippe Pradel · Emilie Tixier · Anne Ferlay

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**Abstract** Milk fat content and its fatty acid (FA) profile are important components of both the milk nutritional and technological qualities. The present study was conducted to evaluate the influence of calf presence during milking and of cow breed on milk yield, gross composition, FA profile and milk lipolytic system. A total of 30 Prim'Holstein (PH) and Salers (S) primiparous lactating cows were milked in the presence or absence of their calves during 210 days of lactation and bulk milks were collected during 6 weeks in a restricted part of the whole experimental period for analyses. Calf presence increased milk yield and decreased the milk fat content in S cows, whereas no difference was observed for the PH cows. The PH milk had higher levels of short- and medium-chain FA and C18:2 *n*-6, whereas S milk had higher levels of C18:2 *cis*-9,*trans*-11 (conjugated linoleic acid, CLA), C18:3 *n*-3 and C20:5 *n*-3. The S milk also had higher initial lipolysis. Calf presence during milking increased the C16:0 percentage and decreased C18:0, C18:2 *n*-6, C18:3 *n*-3, total polyunsaturated FA percentages in milk fat. Calf presence decreased the milk *trans*-C18:1 level only in S cows. *Cis*-9,*trans*-11-CLA level increased in the calf presence in PH cows, whereas it decreased in S cows. Calf presence during milking increased milk lipolysis after 24 h only in the PH cows. Calf presence during milking had significant effects on the milk yield, gross and fine composition, and lipolysis but these effects differed according to the breed.

A. Cozma · B. Martin · M. Guiadeur · E. Tixier · A. Ferlay (✉)  
INRA, UMR1213 Herbivores, 63122 Saint-Genès-Champanelle, France  
e-mail: anne.ferlay@clermont.inra.fr

A. Cozma · B. Martin · M. Guiadeur · E. Tixier · A. Ferlay  
VetAgro Sup, UMR Herbivores, BP 10448, 63000 Clermont-Ferrand, France

A. Cozma  
Faculty of Pharmacy, Department of Environmental Chemistry and Hygiene, Iuliu Hațieganu  
University of Medicine and Pharmacy, 6 Pasteur Street, Cluj-Napoca, Romania

P. Pradel  
INRA, UE1296, Monts d'Auvergne, 63210 Orcival, France

## Prim 荷斯坦奶牛和Salers 奶牛泌乳期间牛犊的存在对奶的产量、组成、脂肪酸以及脂解作用的影响

**摘要**：乳脂肪含量以及它的脂肪酸组成是衡量奶的营养特性和加工品质很重要的成分。目前的研究主要是评价不同品种奶牛在泌乳期间小牛犊的存在对产奶量、组成、脂肪酸组成以及脂解作用的影响。30 Prim 荷斯坦(PH) Salers初产奶牛(S) 210天的泌乳期间,牛犊在存在或不存在的条件下,对6个星期在限制的时间内采集的奶进行分析。对于S奶牛而言,牛犊的存在增加了产奶量,但是降低了乳脂肪的含量,然而对于PH奶牛而言,牛犊的存在对产奶量和脂肪含量影响不显著。PH奶牛乳中短链、中链脂肪酸和C18:2 n-6含量较高,然而S奶牛乳中共轭亚油酸(CLA),亚麻酸(C18:3 n-3)和二十碳五稀酸(C20:5 n-3)含量较高。S奶牛乳中初始脂解度较高。泌乳期间牛犊的存在增加了乳脂肪中棕榈酸(C16:0)的含量,降低了硬脂酸(C18:0),亚油酸(C18:2 n-6),亚麻酸(C18:3 n-3)和总多不饱和脂肪酸的百分含量。牛犊的存在只降低了S奶牛乳中反式油酸(*trans*-C18:1)的含量。对于PH奶牛,牛犊的存在增加了*Cis*-9, *trans*-11-CLA的含量,然而,对于S奶牛,牛犊的存在却降低了*Cis*-9, *trans*-11-CLA的含量。对于PH奶牛,泌乳期间牛犊的存在增加了奶在24h的脂解作用。两个奶牛品种至少有一种奶牛在泌乳期间,牛犊的存在对奶的产量,组成和脂解作用有影响。

**Keywords** Calf presence · Cow breed · Milk fatty acids · Lipolytic system

**关键词** 牛犊存在 · 牛品种 · 乳脂肪酸 · 脂解作用

## 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 (Chilliard et al. 2007; Martin et al. 2005). The potential human health benefits of some milk FA have already been identified. Butyric acid (C4:0) is known to exhibit anti-carcinogenic effects (Mills et al. 2011; Shingfield et al. 2008). Medium-chain FA (C8:0-C12:0) may reduce the risk of metabolic syndrome and have also been shown to possess antimicrobial properties (Mills et al. 2011). The isomer *cis*-9,*trans*-11 of conjugated linoleic acid (CLA) has demonstrated the ability to prevent various types of cancer, hypertension and atherosclerosis, as well as to improve immune function and bone health in both animal models and in human cells (Bhattacharya et al. 2006; Mills et al. 2011; Shingfield et al. 2008). There is a lack of consistent data in human studies because mixtures of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers of CLA instead of purified *cis*-9,*trans*-11 isomer were used.

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 (Chilliard et al. 2007). Few experiments have been conducted comparing Holstein and Jersey breeds (Kelsey et al. 2003; White et al. 2001). In the present experiment, two cow breeds were selected: Prim'Holstein (PH), the main cow breed used for milk production in France, 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 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 (Institut de l'Élevage 2009), and the S cows have been replaced by more specialised PH or Montbéliarde cows. The decrease in the number of Salers cows is related to their low production level (2,400 vs. 9,000 kg/lactation for Prim'Holstein) (Institut de l'Élevage 2009) and to the very time-consuming process of milking, referred to as “traditional”, that requires the presence of the calf to stimulate milk ejection (Tournadre et al. 2008). A range of studies has focused on breed effect using Holstein, Jersey, Montbéliarde and Normande breeds (Ferlay et al. 2006; Kelsey et al. 2003; White et al. 2001), 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 (Combellas et al. 2003; Tournadre et al. 2008). 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 cheesemaking, 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 (Ferlay et al. 2006). The development of a rancid flavour in cold stored milk is due to FFA, especially to free butyric acid (Chilliard et al. 2003). 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 (Chilliard et al. 2003).

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 Materials and methods

### 2.1 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 (1,135–1,215 m above sea level) region of Auvergne (Cantal, France). The duration of the study was 6 weeks from December 14, 2009. A total of 36 PH (38 ± 11.6 days in milk (DIM)—on December 15, 2009, 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 15 SCA cows, 6 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 0600 and 1530 hours. 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 (1973).

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% *D. 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 two pens per group. The feedstuffs distributed per pen were weighed 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 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 (Institut National de la Recherche Agronomique INRA 2007).

Before December 15th, calves were fed only mother's milk. After this date, calves were fed progressively in addition to mother's 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 (Veissier 1999).

## 2.2 Milk sampling 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 15, 17 and 21, 2009, and January 6, 11, 13 and 20, 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 (Association of Official Analytical Chemists (AOAC) 1997). The somatic cell count data were 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. (2010) with some modifications. Briefly, 2 mL of 0.5 mol.L<sup>-1</sup> 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<sub>2</sub>CO<sub>3</sub> 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×0.25 mm 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 (C4:0 to C10:0). 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 (Jellema et al. 1991).

### 2.3 Statistical analyses

Data from individual milks were analysed by variance analysis using the GLM procedure (SAS Institute 2003), with breed (B), treatment (T, presence or absence of the calf during milking) and interaction between breed and treatment ( $B \times T$ ) as main effects.

Data for bulk milk composition, FA profile and lipolytic system were analysed by the mixed models procedure of SAS software (SAS Institute 2003) with B, T and  $B \times T$  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  $0.05 < P \leq 0.10$ .

## 3 Results

### 3.1 Milk yield and composition

Milk yield, milk fat, protein and lactose contents and somatic cell counts are shown in Table 1. During the experimental period, the average milk yield was 26.9 and 11.5 L.day<sup>-1</sup> 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  $\times$  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,  $P < 0.01$ ).

### 3.2 Milk fatty acid profile

The milk FA composition is presented in Tables 2 and 3. The milk fat of PH cows had higher percentages of individual saturated FA (SFA) (C4:0 ( $P < 0.01$ ), C6:0 ( $P < 0.001$ ), C7:0 ( $P < 0.01$ ), C8:0 ( $P < 0.001$ ), C9:0 ( $P < 0.001$ ), C10:0 ( $P < 0.01$ ), C11:0 ( $P < 0.001$ ), C12:0 ( $P < 0.05$ ), anteiso-C17:0 ( $P < 0.05$ )), total monounsaturated FA (MUFA) ( $P < 0.10$ ), and individual MUFA (*trans*-11-C16:1 ( $P < 0.01$ ), *cis*-9-C16:1

**Table 1** Influence of cow breed, calf presence during milking, and their interaction on milk yield and composition and milk lipolytic system

	PH		S		SEM <sup>a</sup>	Significance ( <i>P</i> value) <sup>b</sup>		
	CP	CA	CP	CA		B	T	B×T
Milked milk <sup>c</sup> (L.day <sup>-1</sup> )	22.9 <sup>a</sup>	26.6 <sup>a</sup>	9.1 <sup>b</sup>	8.7 <sup>b</sup>	2.89	***	ns	+
Suckled milk <sup>c</sup> (L.day <sup>-1</sup> )	4.2	–	5.2	–	0.78	*		
Total milk yield <sup>c</sup> (L.day <sup>-1</sup> )	27.1 <sup>a</sup>	26.6 <sup>a</sup>	14.3 <sup>b</sup>	8.7 <sup>c</sup>	2.87	***	**	*
Fat content <sup>d</sup> (g.kg <sup>-1</sup> )	31.81 <sup>a</sup>	33.31 <sup>a</sup>	26.83 <sup>b</sup>	35.30 <sup>a</sup>	1.67	ns	***	*
Protein content <sup>d</sup> (g.kg <sup>-1</sup> )	30.08	30.84	31.58	35.04	0.95	**	*	ns
Lactose content <sup>d</sup> (g.kg <sup>-1</sup> )	52.03 <sup>b</sup>	53.27 <sup>a</sup>	53.10 <sup>a</sup>	52.50 <sup>b</sup>	0.17	ns	+	***
Somatic cell counts <sup>d</sup> (log.mL <sup>-1</sup> )	4.49 <sup>b</sup>	5.04 <sup>a</sup>	4.93 <sup>a</sup>	4.90 <sup>a</sup>	0.11	ns	*	**
Lipolysis at <i>t</i> <sub>0</sub> <sup>d</sup> (%)	0.79	0.66	0.84	0.83	0.04	**	+	ns
Lipolysis at <i>t</i> <sub>24</sub> <sup>d</sup> (%)	0.98 <sup>a</sup>	0.75 <sup>b</sup>	0.97 <sup>a</sup>	0.93 <sup>a</sup>	0.04	*	**	*
Lipolysis <sub>Δ24h</sub> <sup>d</sup> (%)	0.19 <sup>a</sup>	0.09 <sup>b</sup>	0.13 <sup>c</sup>	0.10 <sup>b,c</sup>	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, Lipolysis<sub>Δ24h</sub> evolution of milk lipolysis after 24 h (Lipolysis at *t*<sub>0</sub>–Lipolysis at *t*<sub>24</sub>), SEM standard error of the mean, ns not significant

Within-row means with different letters differ at *P*<0.05

<sup>a</sup> For individual milks, root SEM is presented

<sup>b</sup> Significance (*P* value): ns, *P*>0.10; +*P*<0.10; \**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001

<sup>c</sup> Individual milks

<sup>d</sup> Bulk milks. The number of samples was 7, 7, 7, and 7 for PHCP, PHCA, SCP and SCA groups, respectively

(*P*<0.05), *cis*-11-C20:1 (*P*<0.001), *trans*-5-C18:1 (*P*<0.05), *trans*-6, 8-C18:1 (*P*<0.01), *trans*-10-C18:1 (*P*<0.001), *trans*-12-C18:1 (*P*<0.001), *trans*-13-C18:1 (*P*<0.001), *cis*-9-C18:1 (*P*<0.10), *cis*-11-C18:1 (*P*<0.001), *cis*-12-C18:1 (*P*<0.001), *cis*-13-C18:1 (*P*<0.001), *cis*-14-C18:1 (*P*<0.001), total *cis*-C18:1 (*P*<0.05) and C18:2 *n*-6 (*P*<0.001) than that of S cows. In contrast, PH cows had lower milk fat concentrations of C14:0 (*P*<0.10), C15:0 (*P*<0.001), anteiso-C15:0 (*P*<0.001), iso-C16:0 (*P*<0.001), C17:0 (*P*<0.001), iso-C17:0 (*P*<0.001), *cis*-9,*cis*-11-CLA (*P*<0.001), *trans*-9,*trans*-11-CLA (*P*<0.05), C20:0 (*P*<0.001), C20:2 *n*-6 (*P*<0.10), C22:0 (*P*<0.001), C20:5 *n*-3 (*P*<0.001), and C22:5 *n*-3 (*P*<0.05), if compared to S cows.

Calf presence during milking increased *cis*-9-C14:1 (*P*<0.001), C16:0 (*P*<0.01), *cis*-9-C16:1 (*P*<0.001), C17:0 (*P*<0.10), and anteiso-C17:0 (*P*<0.001) concentrations, whereas it decreased the concentrations of C8:0 (*P*<0.10), anteiso-C15:0 (*P*<0.01), C18:0 (*P*<0.05), several MUFA (*trans*-10-C18:1 (*P*<0.10), *trans*-13-C18:1 (*P*<0.01), *cis*-12-C18:1 (*P*<0.05), C18:2 *n*-6 (*P*<0.001), C20 (*P*<0.05), C20:4 *n*-6 (*P*<0.001), C22:5 *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 2 and 3). The presence of the calf decreased milk concentrations of *cis*-14-C18:1 (*P*<0.10) and C18:3 *n*-3 (*P*<0.10) more markedly for S than for PH cows. In contrast, the presence of the calf decreased the milk concentration of C20:3 *n*-6 (*P*<0.001) more markedly for PH than for S cows. The milk fat C15:0 (*P*<0.001), *cis*-9,

**Table 2** Influence of cow breed, calf presence during milking, and their interaction on milk FA composition

	PH		S		SEM	Significance ( <i>P</i> value) <sup>a</sup>		
	CP	CA	CP	CA		B	T	B×T
Fatty acid (g.100 g <sup>-1</sup> of FA) <sup>b</sup>								
C4:0	2.89	2.86	2.77	2.74	0.07	**	ns	ns
C5:0	0.029 <sup>a</sup>	0.023 <sup>b</sup>	0.019 <sup>c</sup>	0.020 <sup>b</sup>	0.001	***	ns	*
C6:0	2.30	2.30	2.14	2.13	0.02	***	ns	ns
C7:0	0.02	0.02	0.02	0.02	0.001	**	ns	ns
C8:0	1.41	1.45	1.28	1.30	0.02	***	+	ns
C9:0	0.03	0.03	0.02	0.02	0.002	***	ns	ns
C10:0	3.07	3.21	2.86	2.93	0.10	**	ns	ns
C10:1	0.26	0.25	0.25	0.24	0.01	+	ns	ns
C11:0	0.05	0.05	0.04	0.04	0.003	***	ns	ns
C12:0	3.34	3.50	3.21	3.28	0.14	*	ns	ns
C12:1	0.06	0.08	0.07	0.07	0.008	ns	+	+
C13:0	0.06	0.08	0.07	0.07	0.008	ns	+	+
iso-C13:0	0.03 <sup>c</sup>	0.03 <sup>c</sup>	0.04 <sup>b</sup>	0.05 <sup>a</sup>	0.001	***	**	*
anteiso-C13:0	0.01	0.01	0.02	0.02	0.003	ns	ns	ns
C14:0	11.12	11.12	11.44	11.32	0.35	+	ns	ns
iso-C14:0	0.11 <sup>c</sup>	0.12 <sup>c</sup>	0.20 <sup>b</sup>	0.23 <sup>a</sup>	0.004	***	***	**
<i>cis</i> -9-C14:1	0.83	0.74	0.80	0.72	0.05	ns	***	ns
iso-C15:0	0.25 <sup>c</sup>	0.25 <sup>c</sup>	0.37 <sup>b</sup>	0.41 <sup>a</sup>	0.01	***	***	***
anteiso-C15:0	0.49	0.51	0.70	0.73	0.02	***	**	ns
C15:0	1.07 <sup>c</sup>	1.04 <sup>c</sup>	1.34 <sup>b</sup>	1.41 <sup>a</sup>	0.03	***	ns	***
C16:0	27.27	26.48	27.52	26.38	0.58	ns	**	ns
iso-C16:0	0.27	0.27	0.45	0.49	0.01	***	+	ns
<i>trans</i> -11-C16:1	0.20	0.20	0.18	0.19	0.006	**	ns	ns
<i>cis</i> -9-C16:1	1.30	1.17	1.23	1.15	0.02	*	***	ns
<i>cis</i> -11-C16:1	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.03 <sup>b</sup>	0.002	***	**	*
C17:0	0.71	0.70	0.80	0.78	0.01	***	+	ns
iso-C17:0	0.36	0.37	0.41	0.42	0.01	***	ns	ns
anteiso-C17:0	1.30	1.17	1.23	1.15	0.02	*	***	ns
C18:0	10.34	10.91	10.44	10.58	0.43	ns	*	ns
iso-C18:0	0.06 <sup>b</sup>	0.07 <sup>b</sup>	0.08 <sup>a</sup>	0.07 <sup>b</sup>	0.003	*	ns	*
<i>cis</i> -9, <i>trans</i> -13-C18:2	0.14	0.15	0.13	0.14	0.008	ns	ns	ns
<i>trans</i> -11, <i>cis</i> -15-C18:2	0.08 <sup>c</sup>	0.07 <sup>c</sup>	0.11 <sup>b</sup>	0.13 <sup>a</sup>	0.007	***	ns	*
C20:0	0.17	0.17	0.20	0.21	0.006	***	*	ns
<i>cis</i> -11-C20:1	0.07	0.07	0.05	0.06	0.004	***	ns	ns
<i>cis</i> -9, <i>trans</i> -11-CLA	0.57 <sup>c</sup>	0.53 <sup>d</sup>	0.86 <sup>b</sup>	0.90 <sup>a</sup>	0.03	***	ns	***
<i>cis</i> -9, <i>cis</i> -11-CLA	0.05	0.04	0.07	0.07	0.006	***	ns	ns
<i>trans</i> -11, <i>trans</i> -13-CLA	0.03	0.03	0.03	0.02	0.003	ns	ns	ns
<i>trans</i> -9, <i>trans</i> -11-CLA	0.03	0.02	0.03	0.03	0.003	*	ns	ns

**Table 2** (continued)

	PH		S		SEM	Significance ( <i>P</i> value) <sup>a</sup>		
	CP	CA	CP	CA		B	T	B×T
C18:2 <i>n</i> -6	2.00	2.13	1.43	1.54	0.09	***	***	ns
C18:3 <i>n</i> -3	0.82	0.85	0.91	1.00	0.02	***	***	+
C20:2 <i>n</i> -6	0.03	0.04	0.04	0.04	0.003	+	ns	ns
C22:0	0.07	0.06	0.10	0.10	0.004	***	ns	ns
C20:3 <i>n</i> -6	0.07	0.09	0.07	0.08	0.003	ns	***	+
C20:4 <i>n</i> -6	0.11	0.12	0.12	0.12	0.003	ns	***	ns
C20:5 <i>n</i> -3	0.07	0.07	0.08	0.08	0.003	***	ns	ns
C22:5 <i>n</i> -3	0.13	0.16	0.16	0.17	0.01	*	**	ns
C22:6 <i>n</i> -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 <sup>c</sup>	4.77 <sup>d</sup>	5.82 <sup>b</sup>	5.94 <sup>a</sup>	0.10	***	ns	**
Total MUFA	27.60	27.45	26.46	27.03	0.72	+	ns	ns
Total <i>trans</i> FA	4.36 <sup>c</sup>	4.31 <sup>c</sup>	4.77 <sup>b</sup>	5.04 <sup>a</sup>	0.22	***	+	*
Total PUFA	4.38	4.55	4.29	4.60	0.13	ns	***	ns
Total CLA	0.68 <sup>c</sup>	0.62 <sup>d</sup>	0.99 <sup>b</sup>	1.03 <sup>a</sup>	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								
<i>cis</i> -9-C14:1/C14:0	0.07	0.06	0.07	0.06	0.003	*	***	ns
<i>cis</i> -9-C16:1/C16:0	0.02	0.02	0.02	0.02	0.001	ns	ns	+
<i>cis</i> -9-C18:1/C18:0	2.02	1.91	1.91	1.93	0.04	+	*	ns
<i>cis</i> -9, <i>trans</i> -11-CLA/ <i>cis</i> -9-C18:1	0.027 <sup>a</sup>	0.025 <sup>b</sup>	0.043 <sup>c</sup>	0.044 <sup>c</sup>	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, SEM standard error of the mean, ns not significant, CLA conjugated linoleic acid, SFA saturated FA, OBCFA odd- and branched-chain FA, MUFA monounsaturated FA, PUFA polyunsaturated FA, short-chain FA from C4:0 to C12:0, medium-chain FA from C13:0 to *cis*-9-C17:1, long-chain FA from C18:0 to C22:6 *n*-3

Within-row means with different letters differ at  $P < 0.05$ .

<sup>a</sup> Significance (*P* value): ns,  $P > 0.10$ ; + $P < 0.10$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

<sup>b</sup> The number of samples were 7, 7, 7, and 7 for PHCP, PHCA, SCP and SCA groups, respectively

*trans*-11-CLA ( $P < 0.001$ ), total CLA ( $P < 0.001$ ), 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-C18:0 concentration did not change in the presence of the calf with PH cows and increased with S cows. In the case of iso-C13:0 ( $P < 0.05$ ), iso-C15:0 ( $P < 0.001$ ), iso-C14:0 ( $P < 0.01$ ) (Table 2), *trans*-9-C18:1 ( $P < 0.01$ ) (Table 3), *trans*-11-C18:1 ( $P < 0.05$ ), *trans*-12-C18:1 ( $P < 0.10$ ), total *trans*-C18:1 ( $P < 0.05$ ), *trans*-11,*cis*-15-C18:2 ( $P < 0.05$ ), and total *trans* FA ( $P < 0.05$ ) (Table 2), 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 C5:0 ( $P < 0.05$ ) and

**Table 3** 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 ( <i>P</i> value) <sup>a</sup>		
	CP	CA	CP	CA		B	T	B×T
Fatty acid (g.100 g <sup>-1</sup> of FA) <sup>b</sup>								
<i>trans</i> -4-C18:1	0.02	0.02	0.02	0.01	0.002	ns	ns	ns
<i>trans</i> -5-C18:1	0.02	0.02	0.01	0.01	0.002	*	ns	ns
<i>trans</i> -6,8-C18:1	0.25	0.25	0.23	0.24	0.02	**	ns	ns
<i>trans</i> -9-C18:1	0.21 <sup>a</sup>	0.21 <sup>a</sup>	0.19 <sup>b</sup>	0.21 <sup>a</sup>	0.009	*	+	**
<i>trans</i> -10-C18:1	0.30	0.31	0.18	0.20	0.02	***	+	ns
<i>trans</i> -11-C18:1	1.40 <sup>b</sup>	1.42 <sup>b</sup>	1.89 <sup>a</sup>	1.96 <sup>a</sup>	0.11	***	ns	*
<i>trans</i> -12-C18:1	0.25	0.25	0.18	0.21	0.02	***	*	+
<i>trans</i> -13-C18:1	0.46	0.49	0.33	0.39	0.02	***	**	ns
Sum of <i>trans</i> -C18:1	2.97 <sup>b</sup>	2.98 <sup>b</sup>	3.05 <sup>b</sup>	3.26 <sup>a</sup>	0.18	***	*	*
<i>cis</i> -9-C18:1	20.86	20.82	19.90	20.30	0.61	+	ns	ns
<i>cis</i> -11-C18:1	0.74	0.75	0.58	0.60	0.02	***	ns	ns
<i>cis</i> -12-C18:1	0.16	0.17	0.09	0.12	0.02	***	*	ns
<i>cis</i> -13-C18:1	0.09	0.08	0.05	0.06	0.006	***	ns	ns
<i>cis</i> -14-C18:1	0.25	0.26	0.21	0.24	0.01	***	***	+
Sum of <i>cis</i> -C18:1	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

Within-row means with different letters differ at  $P < 0.05$

<sup>a</sup> Significance (*P* value): ns,  $P > 0.10$ ; + $P < 0.10$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

<sup>b</sup> The number of samples were 7, 7, 7, and 7 for PHCP, PHCA, SCP and SCA groups, respectively

decreased C12:1 ( $P < 0.10$ ) and C13:0 ( $P < 0.10$ ) concentrations with PH cows, but had no influence on these FA concentrations with S cows.

### 3.3 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 1). The presence of the calf only increased the lipolysis measured after 24 h for the PH cows ( $P < 0.01$ )

## 4 Discussion

### 4.1 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'Élevage (2009), reporting a milk yield of 8,396 kg/lactation for PH primiparous cows vs. 1,954 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 (Ayadi et al. 2003).

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 (Tournadre et al. 2008). For PH group, our results are in agreement with Bar-Peled et al. (1998) using Holstein cows milked three times daily compared to Holstein cows milked and suckled three times for a 6-week postpartum period. Our results agree also with Tesorero et al. (2001), showing that Brahman  $\times$  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. (2008) 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 (McKusick et al. 2002; Tournadre et al. 2008). In contrast, De Passillé et al. (2008) 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. (2010), 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) (Fröberg et al. 2008; Ontsouka et al. 2003). In disagreement with our results, McKusick et al. (2002) 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 (Institut de l'Élevage 2009).

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. (2008) using Holstein cows, showing that suckled milk had lower protein content, whereas Mendoza et al. (2010) 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. (2002) 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. (2003) showing lactose concentration is higher in the milk alveolar fraction, suckled by the calf, than in the cisternal fraction. In contrast, Tournadre et al. (2008) 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. (2008) 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.

#### 4.2 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 (Ferlay et al. 2006; Kelsey et al. 2003; White et al. 2001). A higher concentration of C4:0 and a slightly lower concentration of C14:0 in milk fat from Holstein cows compared to Montbéliarde cows have also been reported by Ferlay et al. (2010). In agreement with Ferlay et al. (2010), but in disagreement with Lawless et al. (1999) and Ferlay et al. (2006), breed had no effect on the milk fat percentage of C16:0 in our study. Moreover, we observed no effect of breed on C18:0 concentration, whereas Lawless et al. (1999) obtained a higher C18:0 concentration in milk fat from Normande and Montbéliarde cows than in that from Holstein cows.

Breed had no effect on *cis*-9-C14:1 in our study, whereas Ferlay et al. (2010) reported higher concentrations of *cis*-9-C14:1, *cis*-9-C18:1, *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<sup>-1</sup>) and *cis*-9,*cis*-11-CLA (+0.03 g.100 g<sup>-1</sup>) concentrations were higher in milk fat from S cows than from PH cows, whereas *cis*-9-C16:1 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. (1999) (+0.36 g.100 g<sup>-1</sup> of FA; Montbéliarde>Dutch Holstein/Friesian), White et al. (2001) (+0.10 g.100 g<sup>-1</sup>; Holstein>Jersey) and Kelsey et al. (2003) (+0.03 g.100 g<sup>-1</sup>; 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-C18:1) 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 (Arnould and Soyeurt 2009). 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 (Chilliard et al. 2007).

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 (Fröberg et al. 2008; Ontsouka et al. 2003).

Concerning the influence of calf presence on milk C18:1 isomer concentrations, our results are in agreement with McKusick et al. (2002), showing that C18:1 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 C8:0, C14:1, C16:0, C16:1, C18:0, C18:2, C18:3 and C20:0, but also for C4:0, C6:0, C10:0, C12:0 and C14:0, 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-C18:1, *trans*-11-C18:1, *trans*-12-C18:1, total *trans*-C18:1, *cis*-14-C18:1, *trans*-11,*cis*-15-C18:2 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 (Chilliard et al. 1995).

### 4.3 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 (1986). Our results are in disagreement with Ferlay et al. (2006) 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 (1987) 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 (1984) reported that lipolysis seems to be higher in primiparous than in multiparous cows.

The initial lipolysis, as well as the lipolysis measured after 24 h, was increased by the presence of the calf. This is contradictory to results obtained by Tournadre et al. (2008), that showed for S cows that the weaker the contact between cow and calf, the greater the lipolysis after 24 h.

## 5 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 cheese-making for PH cows' milk. It seems that S cows produced milk richer in FA of nutritional interest (C18:3 *n*-3, C20:5 *n*-3, C22:5 *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 C16:0 and decreased the milk C18:0, C18:2  $n-6$ , C18:3  $n-3$  and total PUFA concentrations for the two breeds. Further investigations using individual milk samples will be needed in order to confirm these results.

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