Comparative dynamics of milk fatty acids for primiparous and multiparous Holstein cows in early lactation

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Abstract

The milk fatty acid (FA) profile is a valuable indicator of a cow's nutritional and metabolic status, potentially aiding in assessing metabolic status at the individual cow level. However, limited knowledge exists regarding milk fat composition changes with parity. Understanding these changes during early lactation could enhance our understanding of dairy cow physiology. This study aimed to investigate whether milk FA composition differed between Holstein cows of different parities in early lactation. We characterized the milk FA profiles from day 7 to day 60 postpartum in primiparous (PP) and multiparous (MP) cows. A total of 26 Holstein Friesian dairy cows, including 12 PP and 14 MP, were included in the study and divided into two groups based on parity. Milk samples were collected on days 7, 14, 21, 30, and 60 post-calving and analysed for milk FA profiles, including saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total *de novo* FA, mixed FA, and preformed FA, using MilkoScan FT^{+} 300 equipped with Fourier-transform infrared spectra. Blood samples were collected on days 7, 14, and 21 postpartum for the analysis of non-esterified fatty acids, beta-hydroxybutyrate (BHBA), glucose, and triglycerides, as well as for evaluating body condition scores (BCS). Partial Least Squares Discriminant Analysis (PLS-DA) was used to analyse the changes in milk composition over time in PP and MP. The results of PLS-DA showed changes in milk FA over lactation in both groups. PP had higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP ($P < 0.05$). MP had higher levels of SFA, *de novo* FA, and SCFA compared to PP ($P < 0.05$). PP had higher BHBA levels in milk, suggesting a more severe negative energy balance post-calving compared to MP. However, the average postpartum BCS and preformed FA were higher and BCS loss and *de novo* FA were lower in PP than in MP. In the early lactation stage of dairy cows, the cow mobilizes stored body fat to meet high energy demands, leading to an increase in metabolic products such as ketone in the blood. PP cows may release higher levels of preformed FA during early lactation because their higher BCS. PP cows might also be more inclined to allocate energy to continued growth and milk production in the early stages of lactation. This could lead to a higher release of preformed fatty acids from stored body fat to meet the demands of milk production. These findings could inform nutritional management strategies to better meet the requirements of cows in early lactation.

Keywords: body condition score, energy balance, parity.
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Introduction

The milk fatty acid (FA) profile serves as a crucial indicator of a dairy cow's nutritional and metabolic status, reflecting factors such as dietary intake, ruminal biohydrogenation, and mammary lipogenesis (McFadden and Corl, 2009). Factors like breed, parity, lactation stage, and feeding practices also influence the FA composition of milk (Poulsen *et al*., 2012; O'Callaghan *et al*., 2020). The FA profile of bovine milk is complex, comprising approximately 400 different FAs derived from mammary gland synthesis and circulating plasma (Giannuzzi *et al*., 2022). During early lactation, the mobilization of adipose reserves and diet processing result in changes in milk FA composition (Chilliard *et al*., 2000). These changes can be indicative of the cow's energy status and metabolic health (Giannuzzi *et al*., 2022). Specific FAs, such as c9-18:1, are mobilized from body reserves during negative energy balance (NEB) in early lactation, reflecting the severity of NEB and serving as indicators of energy status (Bastin *et al*., 2011; Gross *et al*., 2011). Conversely, short- and medium-chain FAs (e.g., C14:0) are synthesized *de novo* in the mammary gland and decrease in proportion during NEB (Churakov *et al*., 2021).

Non-esterified fatty acids (NEFA) in circulation reflect body reserve mobilization and dry matter intake (DMI), while beta-hydroxybutyrate (BHBA) reflects fat oxidation completeness in the liver (Adewuyi *et al*., 2005). NEFA released from lipid stores are either taken up by the udder to provide milk triglycerides or are oxidized in the liver as an alternative energy source (Duffield, 2000). The plasma NEFA concentration is therefore an index of lipid mobilization, with a rise in NEFA pre-partum suggestive of an energy deficit at this time. Elevated NEFA and BHBA concentrations indicate an increased risk of fatty liver and ketosis (Leblanc, 2010). These dysfunctions mainly concern the failure of individual animals to cope with complex nutritional and metabolic processes and to adapt to large variations in them during early lactation (Mulligan and Doherty, 2008). Although poor adaptation may start before calving, often it happens without clinical signs (Trevisi and Minuti, 2018; Mezzetti *et al*., 2020). Other markers of EB include changes in body condition score (Thorup *et al*., 2012; Chebel *et al*., 2018).

Parity significantly influences milk FA profile and yield, potentially due to differences in energy requirements and FA synthesis between primiparous (PP) and multiparous (MP) cows (Wilms *et al*., 2022; Bilal *et al*., 2014; Contarini *et al*., 2014; O'Callaghan *et al*., 2020). Limited information exists regarding milk fat composition changes with parity, particularly in early lactation, which could offer insights into dairy cow physiology (Contarini *et al*., 2014). Therefore, this study aims to investigate potential differences in milk FA composition between PP and MP Holstein cows in early lactation, shedding light on the physiological variations associated with parity in dairy cows.

Material and methods

Cows management and experimental design

There were 26 Holstein Friesian dairy cows, including 12 PP and 14 MP, were included in the study and divided into two groups based on parity. The cows entered the study during the first week after calving and stayed until 60 days in milk. All cattle were fed a total mixed ration (TMR) twice a day (at 0500h and 1400h) and had free access to clean water. The TMR formulation followed the NRC (2001) guidelines and included bermudagrass hay, alfalfa hay, corn silage, soybean hulls, brewers' grains, and a concentrate consisting of corn and soybean meal. Cattle were milked twice daily (0500 and 1600). All cows were housed together in a free stall facility equipped with rubber beds and solid concrete floors, which were scraped clean by a tractor six times a day. During the study period, the cows did not have access to pasture.

Blood collection and biochemical profile examination

Blood samples were collected at d 7, 14, and 21 post-calving. Aseptic jugular venipuncture was performed using a 20-G needle and sterile vacutainers (EDTA, heparinized, or clot activators). EDTA vacutainers were employed to collect blood samples for BHBA, glucose, and triglyceride analysis. Clot activator vacutainers were used to obtain serum samples for NEFA analysis, while heparinized vacutainers were utilized for plasma biochemical profile analysis. After collection, the blood samples were centrifuged at 1,500 g for 15 minutes to separate serum and plasma, which were then stored at -20 °C until further analysis.

Blood samples were also analyzed immediately after sampling for BHBA and glucose using test kits (Optium Beta Ketone Test Strips and Optium Blood Glucose Test Strips; FreeStyle, Abbott, USA). Serum samples were utilized for the analysis of NEFA using a Hitachi 704 Analyzer (Hitachi, Japan). Plasma biochemical samples were used to analyze triglyceride using a 7170 Chemistry Analyzer (Hitachi, Japan).

Milk sampling and FA profile analysis

Milk samples were collected from individual cows at 7, 14, 21, 30 and 60days in milk by the AFIMEN management system (Afimilk Ltd., Israel). The DHI (dairy herd improvement) laboratory analyzed and recorded each milk FA profiles, including saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total *de novo* FA, mixed FA, and preformed FA, using MilkoScan FT+ 300 equipped with Fourier-transform infrared spectra. (FOSS, Denmark).

BCS records

Trained personnel evaluated the body condition score (BCS) of the cows using a 5-point scale (1 = thin, 5 = fat) (Edmonson *et al*., 1998) at 7, 14, 21 days postpartum. The BCS assessment was consistently performed by the same observer. BCS loss refers to the difference in BCS between day 21 and day 7 postpartum.

Statistical analysis

Statistical analysis included the use of ANOVA and Tukey's post hoc test to assess differences in biochemical profile, FA profiles and BCS among the different parities. To analyze the variations in FA profiles among PP and MP dairy cows during experiment period, we used Partial Least Squares Discriminant Analysis (PLS-DA). We used the FA profiles data as the independent variable matrix and the different parities as the dependent variable.

Results

PP had higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP ($P < 0.05$, Table 1). MP had higher levels of SFA, *de novo* FA, and SCFA compared to PP ($P < 0.05$). The BHBA value of PP was higher than 1.2 mmol/L, which is the threshold for subclinical ketosis. The average postpartum BCS and preformed FA were higher and BCS loss and de novo FA were lower in PP than in MP.

PLS-DA was used to analyse the changes in milk composition over time in PP and MP. PLS-DA demonstrated the evolution of the milk fatty acid profile from days 7 to 30 post

parturition in PP and MP cows (Figure 1). Using PLS-DA, we analyzed the changes in milk composition from day 7 to day 60 postpartum in primiparous and multiparous cows. The results of the PLS-DA revealed that as lactation days increased, there were changes in the distribution of samples, reflecting the changes in milk fatty acids over the course of lactation in primiparous and multiparous cows. For example, multiparous cows tended to cluster towards the negative direction of Component 2 on days 14 and 21, and towards the positive direction of Component 1 on days 30 and 60. The samples from primiparous cows were more dispersed, tending to cluster towards the negative direction of Component 1 and Component 2 on days 30 and 60.

Discussion

The composition of milk fat is strongly influenced by the stage of lactation (Palmquist *et al*., 1993). Initially, the proportion of SCFA produced via de novo synthesis is low,

Table 1. BCS, biochemical profile, milk fatty acid (FA) composition in primiparous and multiparous cows during experiment period.

Item	Primiparous cows	Multiparous cows	P-Value
BCS loss	0.02	0.19	< 0.05
BCS	3.06	2.88	< 0.05
Blood parameters			
NEFA (mmol/l)	0.48	0.47	0.88
BHBA (mmol/l)	1.25	0.94	0.06
Glucose (mg/dl)	56.88	56.52	0.86
Triglyceride (mg/dl)	18.16	17.54	0.79
Milk parameters			
Fat (%)	4.03	3.90	0.51
Protein (%)	3.21	3.11	0.24
Fatty acid composition (g/100 g of fatty acids)			
Total Saturated FA	63.45	65.97	< 0.05
Total Unsaturated FA	31.81	28.80	< 0.05
Mono Unsaturated FA	29.05	26.04	< 0.05
Poly Unsaturated FA	2.75	2.75	0.94
De novo FA	19.42	21.94	< 0.05
Mixed FA	28.93	30.43	0.06
Preformed FA	43.19	38.88	< 0.05
Trans FA	3.07	3.57	< 0.05
SCFA	7.84	9.03	< 0.05
MCFA	42.75	43.96	0.36
LCFA	42.55	39.55	< 0.05
C14:0	8.62	9.16	0.07
C16:0	27.37	28.02	0.39
C18:0	13.27	12.61	< 0.05
C18:1	27.43	25.12	< 0.05

BCS: body condition score; BCS loss refers to the difference in BCS between day 21 and day 7 postpartum. NEFA: Non-esterified fatty acids; BHBA: beta-hydroxybutyrate; FA: fatty acid; *de novo* FA: C4 to C14; Mixed FA: C16, C16:1, and C:17; Preformed FA: Greater than or equal to C18; SCFA: C4 to C10; MCFA: C12 to C16; LCFA: C18.

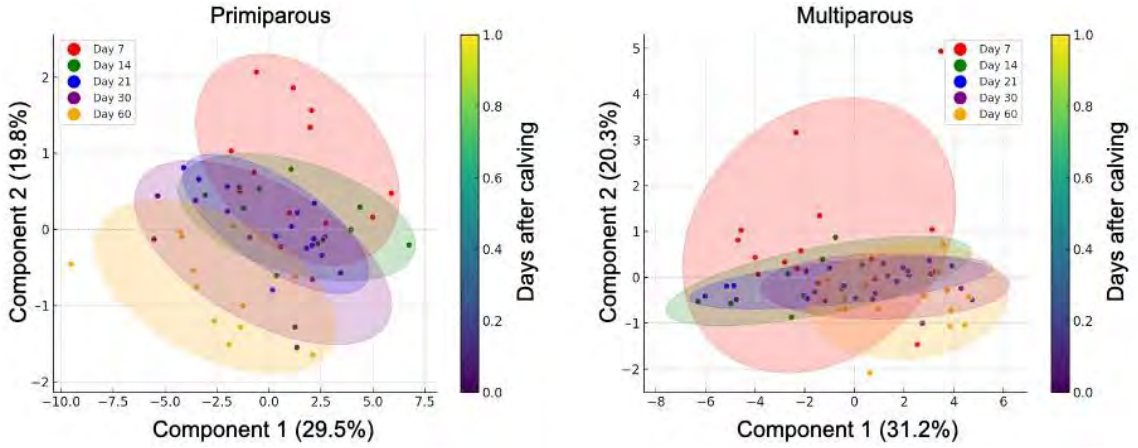

Figure 1. Partial least square discriminant analysis (PLS-DA) depicting the changes in milk fatty acid profile occurring as milk transition from days 7 to 60 post parturition [days 7, 14, 21, 30, 60 post parturition (red, green, blue, violet, yellow, respectively)].

but it increases steadily until at least 8 to 10 weeks into lactation. We used the milk composition data as the independent variable matrix and the different parities as the dependent variable in PLS-DA. By calculating the main components obtained, we were able to identify patterns in milk fatty acid composition among different parities. The results showed that as the days in lactation increased, the distribution of milk FAs from different parities changed, reflecting the trends in milk FA composition over the lactation period.

As lactation progresses, the concentrations of preformed FA decrease, while those of *de novo* FA and mixed-origin FA (e.g., 16:0) increase (Kay *et al*., 2005). The milk FA profiles reflect changes in the cow's energy balance (Churakov *et al*., 2021). PP cows exhibited higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP cows in this study ($P < 0.05$, see Table 1). In contrast, MP cows had higher levels of SFA, *de novo* FA, and SCFA compared to PP cows ($P < 0.05$). These results indicate differences in metabolism between PP and MP cows during the early lactation stage.

During the early lactation stage of dairy cows, the cow mobilizes stored body fat to meet high energy demands, resulting in an increase in metabolic products such as ketones in the blood (Leblanc, 2010). In our study, PP cows had higher blood ketone levels than MP cows, indicating that PP cows experienced higher energy demands. Blood glucose levels are tightly regulated by homeostasis and may not serve as a reliable indicator for monitoring or investigating health status (Herdt, 2000). Therefore, Van *et al*. (2020) and our study did not find differences in blood glucose levels based on parity.

The common NEB is compensated for by the mobilization of fat from body reserves during the first weeks after parturition in dairy cows, leading to the release of preformed FA; C18:1c9 is the predominant UFA in adipocytes and is primarily released through lipolysis during NEB (Rukkwamsuk *et al*., 2000). Subsequently, preformed long-chain non-esterified fatty acids (≥C18) derived from plasma are incorporated into milk fat and inhibit the de novo synthesis of SCFA (C4-C14) by the mammary gland (Bauman and Davis, 1974). Therefore, blood NEFA concentrations are related to milk LCFA concentrations. Additionally, according to Churakov *et al*. (2021), during this experiment, PP cows with C18:1 > 26 (Table 1) have already reached a state of NEB (-30 MJ NEL/d), and blood BHBA > 1.2 mmol/L has reached the threshold for subclinical ketosis, indicating a more severe NEB after calving than MP cows.

BCS is widely recognized as a reliable indicator of a dairy cow's nutritional status, body fat content, and early lactation dry matter intake (Roche *et al*., 2009). Furthermore, the health of dairy cows may be compromised if they lose more than 0.25 BCS points during the first month of lactation (Roche *et al*., 2009). However, in PP cows, the average postpartum BCS was higher and BCS loss was lower than in MP cows, suggesting less mobilization of body fat stores for energy production in milk fat. This indicates significant differences in energy metabolism compared to MP cows in our study.

In our study, PP cows, with their higher BCS, may release higher levels of preformed FA during early lactation. At the start of their first lactation, the competing demands of the mammary gland are superimposed on the requirements for growth (Etherton, 1982). Both insulin and insulin-like growth factor I (IGF-I) have positive growth-promoting effects (Oksbjerg *et al*., 2004), with IGF-I being the primary regulator of postnatal muscle hypertrophy, stimulating protein synthesis, and inhibiting degradation (Etherton, 1982). Studies comparing the metabolic data from PP and MP cows showed consistently higher concentrations of IGF-I throughout the period from –1 to +7 weeks after calving in PP cows (Wathes *et al*., 2007). These results suggest that the differing endocrine background in less mature animals may limit the partitioning of nutrients into milk (Wathes *et al*., 2007; Bilal *et al*., 2014; Contarini *et al*., 2014; O'Callaghan *et al*., 2020). This may be related to differences in energy requirements and partitioning, as well as differences in FA synthesis between PP and MP cows (Miller *et al*., 2006). Our findings could inform nutritional management strategies to better meet the requirements of cows in early lactation.

Conclusion

In our study, PP cows demonstrated higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP cows. Conversely, MP cows exhibited higher levels of SFA, *de novo* FA, and SCFA compared to PP cows. PP cows with BHBA reached the threshold for subclinical ketosis, indicating a NEB after calving compared to MP cows. However, the average BCS postpartum and preformed FA were higher, and BCS loss and *de novo* FA were lower in PP than in MP cows. PP cows also appeared to prioritize energy allocation towards continued growth during the early lactation stages. These observations may be attributed to differences in energy requirements and partitioning, as well as variations in FA synthesis between PP and MP cows. This understanding could inform nutritional management practices during early lactation to better address the distinct needs of cattle in different parity groups.

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