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Importance of Correlations between Colostrum-Blood Fatty Acid Components in Dairy Cattle for Animal Health

Research Article

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ABSTRACT

In order to evaluate the metabolic status of Holstein cows, correlations were determined between colostrum-blood and milk-blood fatty acids. 27 fatty acids were detected in colostrum, 30 in milk and 25 in blood. The highest fatty acid ratio in colostrum and milk was C16:0 (36.13%; 32.54%), while in blood it was C18:1n9c (22.28%; 20.4%). The largest difference between the fatty acid levels in colostrum and blood was determined in linoleic acid (3.68%; 20.37%). As a result of the analysis, it was determined that colostrum saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) contents were higher than blood (SFA: 61.52%; 42.10%; MUFA: 27.93%; 26.00%, respectively), while polyunsaturated fatty acids (PUFA) content was lower than blood (PUFA: 4.90%; 25.99%, respectively). In the correlation analysis between colostrum and blood fatty acids; A statistically significant positive correlation ($P<0.01$) was found between colostrum and blood fatty acids ($r^2=0.768$) and colostrum-blood SFA ($r^2=0.894$), colostrum-blood MUFA ($r^2=0.932$) and colostrum-blood PUFA ($r^2=0.980$) values. Regression graphs showed that changes in colostrum SFA, MUFA and PUFA were largely dependent on changes in blood SFA, MUFA and PUFA ($R^2=0.800$, $R^2=0.868$, $R^2=0.961$, respectively). Therefore, it was determined that changes in colostrum and milk fatty acids made a significant contribution to the estimation of blood fatty acids.

KEY WORDS blood, colostrum, correlation, fatty acids, gas chromatography.

INTRODUCTION

Dairy cows are a very important component in the world livestock industry because these animals have an economically important place. Colostrum is rich in protein, fat, trace elements, and vitamins essential for calves' physiology (Guo *et al*. 2024). Calf feeding management during the neonatal and pre-weaning periods has a significant impact on rearing success. It also significantly impacts the health and performance of calves in later life (Machado and Ballou, 2022). Providing colostrum, especially in the days following birth, is crucial for calf rearing success, as severe diarrhea is a leading cause of neonatal calf mortality. Adequate and prompt colostrum provision (within 2–3 hours of birth) is crucial for the development of passive immunity in calves (Pyo *et al*. 2020; Lopez and Heinrichs, 2022). The content of colostrum given to newborn calves is directly related to the prevention of diseases and calf losses. Bovine colostrum contains various bioactive and nutritional components, including fatty acids (FAs), amino acids or minerals. The role of fatty acids in a living organism is extremely diverse. This is closely related to the structure of biologically active molecules with different carbon chain lengths and degrees of saturation. Saturated fatty acids (SFA), in particular, are a source of energy and play an important role in preventing lipid oxidation of cell membranes. There are approximately 400 identified fatty acids in milk fat (Amores and Virto, 2019). Colostrum fatty acid compo-

nents consist of approximately 65–75% saturated, 24–28% monounsaturated, and 4–5% polyunsaturated fatty acids. Furthermore, palmitic and oleic acids being the predominant fatty acids, accounting for 40% and 21% of total fatty acids, respectively (O'Callaghan *et al.* 2020). Colostrum is rich in lipid molecules essential for newborns, including high concentrations of n-3 PUFA, palmitic acid (C16:0), phospholipids and cholesterol (O'Callaghan *et al.* 2020). Colostrum fatty acids are crucial for meeting the energy needs of newborn animals and for other important structural and metabolic functions (Wilms *et al.* 2022; Silva *et al.* 2024). Linoleic acid (C18:2 n6) and α-linolenic acid (C18:3 n3), essential fatty acids, are the precursors of all n-6 and n-3 long-chain polyunsaturated fatty acids (LCPUFAs). LCPUFAs have important functions in the brain and retina (Cambiaggi *et al.* 2023).

Fatty acids in milk originate from lipids or de novo synthesis in the mammary gland. The source of fatty acids in milk varies depending on the chain length of the fatty acids: (>Cl4) lipoprotein-triglycerides in the blood provide the formation of long-chain fatty acids in milk, and fatty acids are delivered to the mammary gland by hydrolysis in the capillary endothelium via lipoprotein Iipase (Dudi *et al.* 2021). It has been determined that there is a relationship between the lipase activity of lipoprotein and the uptake of triglyceride fatty acids by the mammary gland (Kimura *et al.* 2023). The contribution of phospholipid fatty acids to long-chain fatty acids in milk is based on the low concentration in chylomicrons and very low density lipoproteins (VLDLs) and the low hydrolysis rate of phospholipids by lipoprotein lipase. Therefore, triglycerides in chylomicrons and VLDLs constitute the main source of long-chain fatty acids in milk, even if there is no additional uptake of phospholipids and free fatty acids by the mammary gland (Gugliucci, 2023). When free fatty acids are released from adipose tissue, they are likely incorporated into VLDLs in the liver before being transported to the mammary gland for milk fat synthesis (Yuan *et al.* 2019). Medium-chain fatty acids (<C16) are produced by de novo synthesis occurring in the mammary gland (Kupczyński *et al.* 2024) (Figure 1). The concentration and composition of FAs change significantly under various physiological and pathological conditions (Qin *et al.* 2018). Determination of their content in various biological substrates such as colostrum and milk, including blood plasma, can be an important diagnostic tool (Caron *et al.* 2018). In particular, determining the changes and relationships between colostrum and blood fatty acids associated with calf feeding allows early detection of systemic disorders and diseases associated with fatty acid imbalance and thus improves the healthy growth quality of animals.

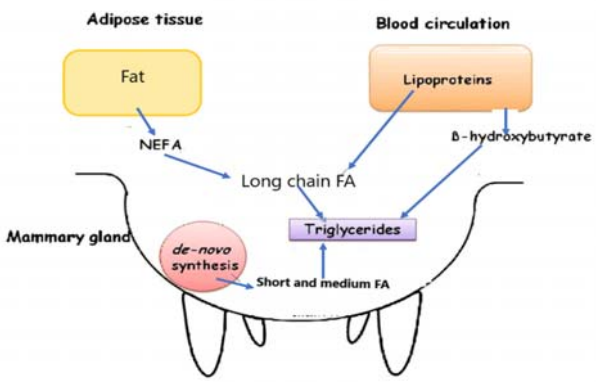

Figure 1 Fatty acids that are synthesized or transferred from the blood and muscle tissue of cows to the mammary glands (Dudi *et al.* 2021)

It has been determined that administering certain herbal supplements (banana or lemongrass) to the feed results in positive changes in milk and blood fatty acids (improving MUFA and PUFA levels, particularly n-3 fatty acids) (Rahman *et al.* 2024). Therefore, determining the correlation levels between blood and colostrum fatty acids is important. Based on the correlation level, it can be determined that low fatty acid levels detected in colostrum are similar to those in blood fatty acids. This can be corrected with feed supplements, resulting in better health for both cows and calves. Many researchers emphasize the importance of examining certain types of FA as biomarkers of metabolic homeostasis disorders and early detection of pathological conditions in the body (Caron *et al.* 2018; Bogie *et al.* 2020).

Improving knowledge of the fatty acid composition of postpartum colostrum and blood of dairy cows can improve the understanding of dairy cow physiology, the importance of the relationship between colostrum and blood and the needs of newborn calves. At the same time, animal welfare is becoming a phenomenon of increasing importance and therefore the avoidance of chronic stress in the animal is one of the prerequisites for animal welfare. Especially in scientific research, it is necessary to improve the environment and treat the animals in a way that will cause the least stress so that they do not experience stress while taking samples. Applying some manipulations to the animal during sampling, especially during blood sampling, causes stress for both the practitioner and the animal in free and mobile animals. Finding various health indicators in the easily sampled secretions of animals is therefore of great importance. In recent years, the term bioindicator has become a priority in studies in this sense.

The hypothesis of this experiment is to determine whether colostrum fatty acid levels are related to blood fatty acid levels by looking at the relationship between

them and to be able to comment on this relationship by looking at the colostrum of the animal, which is a less stressful process instead of the stress caused by blood sampling. The aim of the study was to characterize the colostrum-blood and milk-blood fatty acids profile of Holstein cows by Gas Chromatography, and also to determine the relationship between colostrum and blood and milk and blood fatty acids and the importance of this relationship in cattle breeding.

MATERIALS AND METHODS

Animal material

Holstein cows raised in Çukurova University Faculty of Agriculture, Revolving Fund Dairy Cattle Research and Application Unit were used in this study. Healthy animals with similar characteristics (weight 600-670 kg, body condition score between 2.5-3, 3-5 years of age, at least one birth, no reproductive problems, 45-60 days postpartum) were used in the study. It was determined by the veterinarian of the enterprise that the animals used in the study did not have any disease as a result of anamnesis information and clinical examinations.

The farm has 150 dairy cows and 40 of them are healthy and have similar characteristics. In order to determine the number of animals to be used in the study, it was taken into consideration that the smallest sample size to represent this population was 5% at 5% confidence interval. Therefore, since the number of dairy cows to be used in the study was determined as 10% of the total number of cows (=40/0.10), although it was sufficient to take samples from 4 cows, it was found appropriate to take samples from 6 animals to increase the accuracy of the data.

Total mixed ration (TMR) feeding system was applied to the 6 cows used in the study and the ratio of mixed feed: roughage in TMR composition was 60:40. The cows were fed with a total feed mixture containing corn silage, alfalfa, wheat straw and concentrate (18% crude protein and 2650 kcal/metabolic energy (ME)/kg). Total mix rations are prepared daily and given to the animals in two meals at 07:00 in the morning and 16:00 in the afternoon.

Data collection and method

Colostrum and milk samples are collected after the udders are washed with plenty of water and dried before milking. Colostrum and milk samples were collected as described by Bendall. Colostrum is milked in the morning and evening on the first day after the cows give birth. Milk samples are also taken during the morning and evening milkings. Samples are taken after the foremilk accumulated in the teat is milked. Milk and colostrum samples are filled into 250 mL sample containers and transported for analysis, maintaining a cold chain.

Blood samples were collected as described in Windberger *et al*. (2023).

Blood samples were collected by early morning venipuncture. Samples were prepared by mixing a 10 mL aliquot of blood with 10% sodium citrate in a 30 mL injection bottle sealed with a Teflon-coated septum. Colostrum and blood samples were rapidly frozen and stored at -80 °C until they were prepared for analysis in a gas chromatography device.

Bligh and Dyer (1959) method was used for lipid extraction from animal samples. Methyl esters were prepared by transmethylation using 2 M KOH in methanol and n-hexane according to the method of Ichihara *et al*. (1996); 10 mg of extracted oil was dissolved in 2 ml of hexane, followed by the addition of 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2 minutes at room temperature to mix the liquid well. After centrifugation at 4000 rpm for 10 min, it was taken to the hexane layer for GC analysis.

Samples were analyzed for fatty acid compositions by GC Clarus 500 with autosampler (Perkin Elmer, USA) equipped with a flame ionization detector and silica capillary SGE column (30 m×0.32 mm, ID×0.25 um, BP20 0.25 UM, USA). After the injector and detector temperature were set to 220 °C and 280 °C, respectively, the oven temperature was maintained at 140 °C for 5 min, raised to 200 °C at a rate of 4 °C/min and then to 220 °C at a rate of 1 °C/min. The size of the samples was set to 1 µL and the carrier gas was operated at 16 psi. A 1:100 split ratio was used as separation. Two replicate GC analyses were performed to determine fatty acids and the results obtained were expressed as the mean value in % GC area and ± standard deviation.

Correlation analysis and multiple regression analysis were used to determine whether there was any relationship between dependent variables and independent variables, and the degree, direction and significance levels of the relationship. Scatter graphs were used to visually express the results of the analysis.

Statistical analysis

Statistical analyses of fatty acids in colostrum, milk and blood samples taken from the animals were performed using SPSS 16.0 package program. Analysis of variance (ANOVA) was applied to the data obtained and the comparison analysis was performed according to $P < 0.05$ level of significance.

The regression model is under the influence of one dependent variable and multiple independent variables. In statistical theory, this multiple regression relationship is generally expressed as follows (Göktaş and İşçi, 2010):

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_K X_K + \varepsilon$$

In the current study, fatty acids found in colostrum, milk and blood and their SFA, MUFA and PUFA variables were used and correlation analysis was performed to determine the relationship between colostrum-blood fatty acids, colostrum-blood SFA, colostrum-blood MUFA, colostrum-blood PUFA and milk-blood fatty acids, milk-blood SFA, milk-blood MUFA, milk-blood PUFA ratios and each variable was treated separately as a dependent variable and the relationships were indicated with regression analysis scatter plots.

RESULTS AND DISCUSSION

The results of colostrum and blood fatty acids and saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contents of cows were shown in detail in Table 1.

As a result of the analysis of the study, 27 fatty acids were detected in colostrum and 25 in blood. When Table 1 was examined, it was seen that there were differences in the ratios of fatty acids in colostrum and blood. Eicosanoic acid and nervonic acid were determined in colostrum fatty acids but not in blood fatty acids. Although the fatty acid with the highest rate in colostrum is palmitic acid (36.13), the fatty acid with the highest rate in blood is oleic acid (22.28). The sum of the two fatty acids with the highest rates in colostrum, palmitic acid and oleic acid, constitutes 59.1% of all fatty acids (36.13, 22.97). The sum of the highest levels in blood, oleic acid, linoleic acid and palmitic acid (22.28, 20.37, 19.98), constitutes 63.63% of all fatty acids. The greatest difference between the fatty acids in colostrum and blood of animals was seen in linoleic acid (3.68% and 20.37% in colostrum and blood, respectively). The proportion of linoleic acid in blood was approximately 5.5 times that in colostrum (Table 1).

In colostrum, SFAs except stearic acid, behenic acid and lignoceric acid from the SFA group were detected at higher content than blood. The reason why the SFA content of colostrum was higher than blood was that palmitic acid was determined at 36.13% in colostrum while it was determined at 19.98% in blood. In Table 1, it was seen that the difference between other fatty acids except margaric acid and arachidic acid in the SFA analysis of colostrum and blood was statistically significant ($P<0.05$). When MUFA ratios were examined, other fatty acids except heptadecenoic acid were detected at higher rates in colostrum. The reason for the high MUFA content in colostrum may also be due to the fact that eicosanoic acid and nervonic acid were not detected in blood. When colostrum and blood MUFA were compared, it was seen that only heptadecenoic acid had statistically significant differences ($P<0.05$) (Table 1).

When colostrum and blood SFA, MUFA and PUFA were examined, the biggest difference was in PUFA content (Figure 2). PUFA content were 4.90% in colostrum, 25.99% in blood and were detected at higher rates in blood as seen in Table 1. All PUFA content in blood are higher than in colostrum. The biggest difference was that linoleic acid was 20.37% in blood, but 3.68% in colostrum. The level of linoleic acid in blood was approximately 5.5 times higher than that of colostrum. The difference between colostrum and blood PUFA rates for PUFAs other than eicosadienoic acid was statistically significant ($P<0.05$) (Table 1). In the study, colostrum and blood ∑n6 contents were determined as 3.92%, 20.97%, respectively; ∑n3 contents were found as 0.63%, 5.02%, respectively. As a result of the analysis, it was determined that ∑n6 and ∑n3 contents were high in the blood (Table 1).

Milk and blood fatty acids and SFA, MUFA and PUFA contents of cows were shown in detail in Table 2.

As a result of the analysis of the study, 30 fatty acids were detected in milk and 25 in blood. In Table 2, eicosadienoic acid (C20:2n6) was not detected in milk despite being found in blood. In addition, arachidonic acid (C20:4n6), eicosapentaenoic acid (EPA) (C20:5n3), eicosatrienoic acid (C20:3n3), eicosanoic acid (C20:1), undecanoic acid (C11:0) and butyric acid (C4:0) were detected in milk but not in blood. Although the fatty acid with the highest level in milk was palmitic acid (C16:0) (32.54%), the fatty acid with the highest level in blood was oleic acid (C18:1n9c) (20.4%). The two fatty acids with the highest rates in milk, palmitic acid and oleic acid, total 52.14% of all fatty acids (32.54%, 19.60% respectively).

The highest proportions of blood fatty acids were oleic acid, linoleic acid and palmitic acid (20.14%, 19.07%, 19.00% respectively) and the sum of these amounts constitutes 58.21% of all fatty acids. The greatest difference between milk and blood fatty acids of animals was observed in linoleic acid (2.65% and 19.07% in colostrum and blood respectively).

In the study, although 14 fatty acids were detected in the SFA group in milk, 12 fatty acids were detected in the blood. Butyric acid and undecanoic acid were not found in the blood (Table 2). In addition, it was seen in Figure 3 that the milk SFA content was higher than that of blood. The high milk SFA rate was due to the fact that palmitic acid rate was 32.54% in milk, but 19.00% in blood.

In addition, other fatty acids except behenic acid, stearic acid, margaric acid, lignoceric acid were detected in higher levels in milk. MUFA levels were detected in higher levels in blood (Figure 3). As a result of the analysis, 8 fatty acids from the MUFA group were detected in milk and 7 in blood.

Table 1 Fatty acids in colostrum and blood and saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) % ratios

Fatty acids	Carbon number	Molecular formula	Molecular weight (g/mol)	Colostrum (%)[1]	Blood (%)[1]	P-value
Caproic acid	C6:0	$C_6H_{12}O_2$	116.15	0.64±0.15	0.29±0.13	0.006
Caprylic acid	C8:0	$C_8H_{16}O_2$	144.21	0.41±0.08	0.28±0.07	0.027
Capric acid	C10:0	$C_{10}H_{20}O_2$	172.26	0.99±0.26	0.56±0.05	0.010
Laurik acid	C12:0	$C_{12}H_{24}O_2$	200.31	1.90±0.67	0.67±0.22	0.006
Myristic acid	C14:0	$C_{14}H_{28}O_2$	228.37	10.12±3.73	4.07±1.12	0.010
Pentadecanoic acid	C15:0	$C_{15}H_{30}O_2$	242.40	0.82±0.04	0.52±0.04	0.001
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.42	36.13±7.33	19.98±2.15	0.009
Margaric acid	C17:0	$C_{17}H_{34}O_2$	270.45	0.81±0.23	0.71±0.12	0.243
Stearic acid	C18:0	$C_{18}H_{36}O_2$	280.44	8.96±2.77	14.03±1.71	0.010
Arachidic acid	C20:0	$C_{20}H_{40}O_2$	312.53	0.51±0.15	0.44±0.10	0.234
Behenic acid	C22:0	$C_{22}H_{44}O_2$	340.58	0.06±0.01	0.11±0.02	0.001
Lignoceric acid	C24:0	$C_{24}H_{48}O_2$	368.64	0.17±0.01	0.44±0.06	0.002
Total SFA[2]				**61.52**	**42.10**	
Myristoleic acid	C14:1	$C_{14}H_{26}O_2$	226.36	0.84±0.39	0.39±0.14	0.051
Methyl pentadecanoate	C15:1	$C_{16}H_{30}O_2$	254.41	0.25±0.05	0.25±0.03	0.468
Palmitoleic acid	C16:1	$C_{16}H_{30}O_2$	254.41	1.93±0.34	1.55±0.34	0.082
Heptadecenoic acid	C17:1	$C_{17}H_{32}O_2$	268.44	0.21±0.12	0.40±0.02	0.021
Vaccenic acid	C18:1n7c	$C_{18}H_{34}O_2$	282.46	1.60±0.47	1.13±0.19	0.058
Oleic acid	C18:1n9c	$C_{18}H_{34}O_2$	282.46	22.97±6.77	22.28±2.72	0.429
Eicosanoic acid	C20:1	$C_{20}H_{40}O_2$	312.53	0.08±0.02	ND	
Nervonic acid	C24:1n9	$C_{24}H_{46}O_2$	366.62	0.05±0.01	ND	
Total MUFA[3]				**27.93**	**26.00**	
Linoleic acid	C18:2n6	$C_{18}H_{32}O_2$	280.44	3.68±0.25	20.37±4.67	0.006
Linolenic acid	C18:3n6	$C_{18}H_{30}O_2$	278.43	0.10±0.03	0.19±0.03	0.001
Alpha Linolenic acid	C18:3n3	$C_{18}H_{30}O_2$	278.43	0.30±0.07	1.18±0.41	0.006
Eicosadienoic acid	C20:2n6	$C_{20}H_{36}O_2$	308.50	0.05±0.01	0.07±0.01	0.060
Eicosatrienoic acit	C20:3n3	$C_{20}H_{34}O_2$	306.50	0.63±0.16	3.72±0.75	0.001
Dihomo-γ-linolenic acid	C20:3n6	$C_{20}H_{34}O_2$	306.48	0.09±0.02	0.34±0.05	0.001
Docosahexaenoc acid	C22:6n3	$C_{22}H_{32}O_2$	328.48	0.05±0.01	0.12±0.03	0.003
Total PUFA[4]				**4.90**	**25.99**	
MUFA/SFA				0.43	0.62	
PUFA/SFA				0.08	0.62	
PUFA/MUFA				0.18	1.00	
Total n6[5]				3.92	20.97	
Total n3[6]				0.63	5.02	
n6/n3				6.22	4.18	

[1] The ratios are shown as the mean ± standard deviation (SD).
[2] Total SFA: all saturated fatty acids (without any double bond. (4:0 to 24:0).
[3] Total MUFA: all monounsaturated fatty acids with a single double bond (14:1 to 24:1).
[4] Total PUFA: all polyunsaturated fatty acids.
[5] Total n-6 polyunsaturated fatty acids (PUFA): 18:2n6; 18:3n6; 20:2n6; 20:3n6.
[6] Total n-3 polyunsaturated fatty acids (PUFA): 18:3n3; 20:3n3, 22:6n3.
ND: not detected.

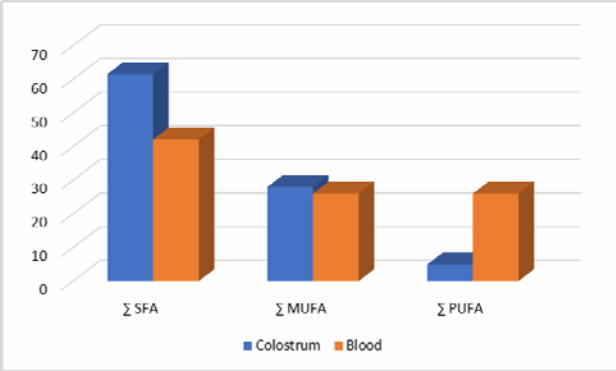

Figure 2 Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ratios of fatty acids in colostrum and blood

Eicosanoic acid was not detected in blood. Except for myristoleic acid, methyl pentadecanoate and eicosanoic acid, other MUFA contents were detected at high levels in the blood (Table 2). Figure 3 shows that the PUFA content was detected at high levels in the blood. The biggest difference between milk and blood PUFA contents was due to the fact that linoleic acid was detected at 19.07% in the blood, while it was 2.65% in milk.

When the colostrum and milk MUFA contents were examined in Figure 4, 7 MUFAs were determined in the blood of milked animals and 6 MUFAs were determined in the blood of colostrumed animals. Although erucic acid (C22:1) was determined in the blood of milked animals, it was not determined in the blood of animals from which colostrum was collected. When colostrum and milk PUFA ratios were examined, eicosadienoic acid (C20:2n6) was detected in colostrum but not in milk. In addition, when blood PUFA contents were examined, 7 PUFAs were detected in the blood of animals from which colostrum was taken and 6 PUFAs were detected in the blood of animals from which milk was taken. Although eicosatrienoic acid (C20:3n3) was found in the blood of animals from which colostrum was taken, it was not detected in the blood of animals from which milk was taken. After determining colostrum-blood and milk-blood fatty acids in the study, correlation analysis was performed to determine the relationships between them. Table 3 shows the correlation coefficients between colostrum and blood fatty acids and colostrum and blood SFA, MUFA and PUFA calculated for all cows in the study.

The correlation analysis conducted to determine the relationship between colostrum and blood fatty acids in the study showed that there was a highly statistically significant positive correlation ($P<0.01$) between colostrum and blood fatty acids ($r^2=0.768$) and colostrum-blood SFA ($r^2=0.894$), colostrum-blood MUFA ($r^2=0.932$) and colostrum-blood PUFA ($r^2=0.980$) contents in Table 3.

Regression graphs of statistically significant correlations between colostrum fatty acids and colostrum SFA, MUFA and PUFA contents and blood parameters were presented in Figure 4.

In the regression graphs obtained as a result of the correlation analysis in the study, it was observed that the changes in colostrum SFA, colostrum MUFA and colostrum PUFA were highly dependent on the changes in blood SFA, blood MUFA and blood PUFA ($R^2=0.800$, $R^2=0.868$, $R^2=0.961$, respectively) (Figure 5).

As a result of the correlation analysis conducted in the study, it was shown in Table 4 that there was a highly significant positive correlation ($P<0.01$) between milk and blood fatty acids ($r^2=0.749$) and milk-blood SFA ($r^2=0.891$), milk-blood MUFA ($r^2=0.957$) and milk-blood PUFA ($r^2=0.825$) values.

Regression graphs of statistically significant correlations between milk fatty acids and SFA, MUFA and PUFA levels and blood parameters were presented in Figure 6.

In the regression graphs obtained as a result of the correlation analysis in the study, it was observed that the changes in milk SFA, milk MUFA and milk PUFA were highly dependent on the changes in blood SFA, blood MUFA and blood PUFA ($R^2=0.793$, $R^2=0.916$, $R^2=0.680$, respectively).

Bovine colostrum supports the growth, health and welfare of young calves by providing essential bioactive compounds and many immune factors such as immunoglobulin to protect the calf from diseases (Fasse *et al.* 2021; Poonia and Shiva, 2022).

It was important to improve the knowledge on changes in colostrum and milk and blood FA profiles, to understand the physiology of dairy cows and the requirements of newborn calves, and to determine the correlation with blood fatty acid changes to find out whether the deficiency detected in blood is also reflected in colostrum and milk. Therefore, the present study was conducted to evaluate the colostrum-blood and milk-blood fatty acids and colostrum-blood and milk-blood SFA, MUFA and PUFA levels of cows and the correlation between them.

The milk FA profile represents a fingerprint of the nutritional and metabolic status of the cow (Zwierzchowski *et al.* 2024; Chen *et al.* 2025). Therefore, not only milk but also colostrum FAs can be considered as putative indicators of energy and metabolic status at both cow and calf levels. For these indicators, not only milk and colostrum but also blood fatty acid levels are important. To capture the complex relationships between FAs, researchers have found that analyzing simultaneous changes in FA groups is more efficient than analyzing changes in individual FAs (Palombo *et al.* 2020; Turini *et al.* 2020).

Table 2 Fatty acids in milk and blood and saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contents

Fatty acids	Carbon Number	Molecular formula	Molecular weight (g/mol)	Milk (%)[1]	Blood (%)[1]	P-value
Butyric acid	C4:0	$C_4H_8O_2$	88.11	1.91±1.13	ND[7]	
Caproic acid	C6:0	$C_6H_{12}O_2$	116.15	1.43±0.83	0.31±0.18	0.019
Caprylic acid	C8:0	$C_8H_{16}O_2$	144.21	0.99±0.17	0.15±0.06	0.001
Capric acid	C10:0	$C_{10}H_{20}O_2$	172.26	2.58±0.58	0.52±0.14	0.001
Undecanoic acid	C11:0	$C_{11}H_{22}O_2$	186.29	0.25±0.04	ND	
Laurik acid	C12:0	$C_{12}H_{24}O_2$	200.31	3.91±0.54	0.48±0.12	0.500
Myristic acid	C14:0	$C_{14}H_{28}O_2$	228.37	11.09±1.26	4.65±1.77	0.001
Pentadecanoic acid	C15:0	$C_{15}H_{30}O_2$	242.40	1.05±0.06	0.48±0.11	0.001
Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.42	32.54±1.41	19.00±5.27	0.006
Margaric acid	C17:0	$C_{17}H_{34}O_2$	270.45	0.58±0.03	0.79±0.05	0.001
Stearic acid	C18:0	$C_{18}H_{36}O_2$	280.44	11.57±2.12	14.76±1.92	0.034
Arachidic acid	C20:0	$C_{20}H_{40}O_2$	312.53	0.69±0.13	0.40±0.11	0.008
Behenic acid	C22:0	$C_{22}H_{44}O_2$	340.58	0.05±0.00	0.11±0.06	0.062
Lignoceric acid	C24:0	$C_{24}H_{48}O_2$	368.64	0.04±0.00	0.38±0.09	0.001
Total SFA[2]				**68.68**	**42.03**	
Myristoleic acid	C14:1	$C_{14}H_{26}O_2$	226.36	1.14±0.11	0.25±0.04	0.001
Methyl pentadecanoate	C15:1	$C_{16}H_{30}O_2$	254.41	0.37±0.06	0.24±0.04	0.004
Palmitoleic acid	C16:1	$C_{16}H_{30}O_2$	254.41	0.96±0.45	1.75±0.56	0.036
Heptadecenoic acid	C17:1	$C_{17}H_{32}O_2$	268.44	0.15±0.05	0.39±0.06	0.001
Vaccenic acid	C18:1n7c	$C_{18}H_{34}O_2$	282.46	0.58±0.11	1.02±0.02	0.001
Oleic acid	C18:1n9c	$C_{18}H_{34}O_2$	282.46	19.60±3.00	20.14±4.54	0.425
Eicosanoic acid	C20:1	$C_{20}H_{40}O_2$	312.53	0.09±0.03	ND	
Erucic acid	C22:1	$C_{22}H_{42}O_2$	338.6	0.20±0.02	2.77±0.10	0.001
Total MUFA[3]				**23.09**	**26.56**	
Linoleic acid	C18:2n6	$C_{18}H_{32}O_2$	280.44	2.65±0.73	19.07±8.61	0.016
Linolenic acid	C18:3n6	$C_{18}H_{30}O_2$	278.43	0.13±0.03	0.26±0.13	0.043
Alpha Linolenic acid	C18:3n3	$C_{18}H_{30}O_2$	278.43	0.21±0.01	0.83±0.61	0.067
Eicosadienoic acid	C20:2n6	$C_{20}H_{36}O_2$	308.50	ND	0.08±0.04	
Eicosatrienoic acit	C20:3n3	$C_{20}H_{34}O_2$	306.50	0.15±0.06	ND	
Dihomo-γ-linolenic acid	C20:3n6	$C_{20}H_{34}O_2$	306.48	0.03±0.00	0.30±0.21	0.001
Arachidonic acid	C20:4n6	$C_{20}H_{32}O_2$	304.5	0.06±0.00	ND	
Eicosapentaenoic acid (EPA)	C20:5n3	$C_{20}H_{30}O_2$	302.5	0.02±0.00	ND	
Docosahexaenoc acid	C22:6n3	$C_{22}H_{32}O_2$	328.48	0.05±0.00	0.28±0.14	0.010
Total PUFA[4]				**3.30**	**20.82**	
MUFA/SFA				0.34	0.63	
PUFA/SFA				0.05	0.50	
PUFA/MUFA				0.14	0.78	
Total n6[5]				2.87	19.71	
Total n3[6]				0.43	1.11	
n6/n3				6.67	17.76	

[1] The ratios are shown as the mean ± standard deviation (SD).
[2] Total SFA: all saturated fatty acids (without any double bond. (4:0 to 24:0).
[3] Total MUFA: all monounsaturated fatty acids with a single double bond (14:1 to 24:1).
[4] Total PUFA: all polyunsaturated fatty acids.
[5] Total n-6 polyunsaturated fatty acids (PUFA): 18:2n6; 18:3n6; 20:2n6; 20:3n6.
[6] Total n-3 polyunsaturated fatty acids (PUFA): 18:3n3; 20:3n3, 22:6n3.
ND: not detected.

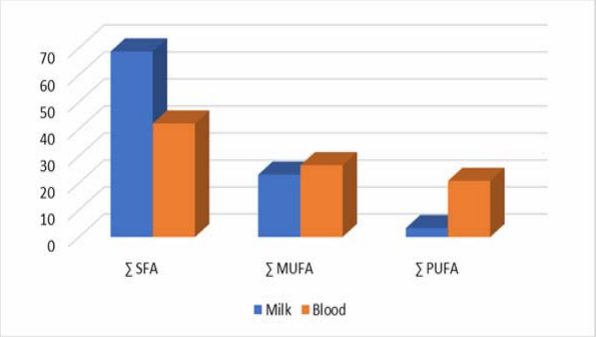

Figure 3 Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ratios of fatty acids in milk and blood

Statistical approaches using comparative methods such as correlation and regression analysis can be applied to explain the correlation network between FAs and to extract several latent, phenotypically independent variables that can define specific metabolic mechanisms (Boetto, 2024; Chetty and Blekhman, 2024). In our study, a correlation analysis was conducted to determine the relationship between colostrum and blood fatty acids, which are particularly important for calf nutrition and health. A statistically significant positive correlation ($P<0.01$) was found between colostrum and blood fatty acids and colostrum-blood SFA, colostrum-blood MUFA, and colostrum-blood PUFA contents. Furthermore, regression graphs in the study showed that changes in colostrum SFA, colostrum MUFA, and colostrum PUFA were highly dependent on changes in blood SFA, blood MUFA, and blood PUFA. Therefore, in addition to other research results in our study, estimating blood fatty acid ratios by looking at colostrum fatty acid ratios can be considered as indicators of various energy and metabolic status.

The analysis results of the study showed that colostrum is rich in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), but lower in polyunsaturated fatty acids (PUFA), and that the SFA content of colostrum is lower and the PUFA, content was higher compared to milk, which was consistent with the studies of other authors (O'Callaghan *et al.* 2020; Van *et al.* 2020; Uken *et al.* 2021; Wilms *et al.* 2022). In addition, the average milk SFA, MUFA and PUFA contents in the current study were consistent with the data reported by Van *et al.* (2020) for milk fats. Linoleic acid (LA) and alpha-linolenic acid (ALA) from the PUFA group in colostrum and milk were essential fatty acids classified as n-3 and n-6 FA, respectively (Uken *et al.* 2021). They were the precursors of n-3 and n-6 PUFA, which have metabolic regulation, cell membrane function and gene regulation functions in the body. However, they cannot be synthesized by mammals and therefore must be taken externally (O'Callaghan *et al.* 2020; Uken *et al.* 2021). The reason why LA and ALA were higher in colostrum than in milk in the study was due to the fact that they were necessary for the offspring to perform important tasks in their bodies, as stated by Uken *et al.* (2021). However, Wilms *et al.* (2022) and O'Callaghan *et al.* (2020) stated that colostrum MUFA content was detected at a higher rate in colostrum (Colostrum MUFA; 27.93; milk MUFA:23.09). In our study, the correlation analysis performed to determine the fatty acids not only in the colostrum but also in the blood of the animals and to determine the relationship between colostrum and blood SFA, MUFA and PUFA, and the high level of relationships between colostrum and blood fills the gap in other studies (O'Callaghan *et al.* 2020; Wilms *et al.* 2022). The concentration and composition of FAs change significantly under various physiological and pathological conditions. The differences in the analysis results in the study may depend on the analysis method, season, lactation number of animals, feeding habits, and environmental conditions (Qin *et al.* 2018; McDermott *et al.* 2024).

McDermott *et al.* (2024) reported that in the analysis of colostrum and milk fatty acids, MUFA C14:1 was higher in colostrum than in milk, C16:1, C17:1, C18:1n7c and C18:1n9c fatty acids were lower in colostrum, and PUFA C18:2n6, C18:3n3 and C20:3n6 fatty acids were higher in colostrum than in milk. However, in our study, MUFA levels in colostrum do not support the findings of McDermott *et al.* (2024). In their study, colostrum C14:1 was lower than in milk, C:16, C17:1 and C18:1n7c fatty acids were higher in milk. Colostrum PUFA (C18:2n6 C18:3n3 C20:3n6) ratios are consistent with the findings of the study by McDermott *et al.* (2024). The differences observed in MUFA levels in the current study may be due to the age of the animal, lactation stage, feeding method applied on the farm, analysis method and device differences (Qin *et al.* 2018).

The current study results were parallel to the study by Van *et al.* (2020). They reported that stearic acid (18:0), oleic acid (18:1n9c) and palmitic acid (16:0) were detected as the dominant fatty acids in milk and colostrum, and these fatty acids were also detected at high levels in the blood. In addition, Van *et al.* (2020) observed higher oleic acid (18:1n9c) concentrations in colostrum and stated that this fatty acid was primarily released from fat cells through lipolysis. In the current study, oleic acid (18:1n9c) was detected in higher amounts in colostrum than in milk, in parallel with the study of Van *et al.* (2020).

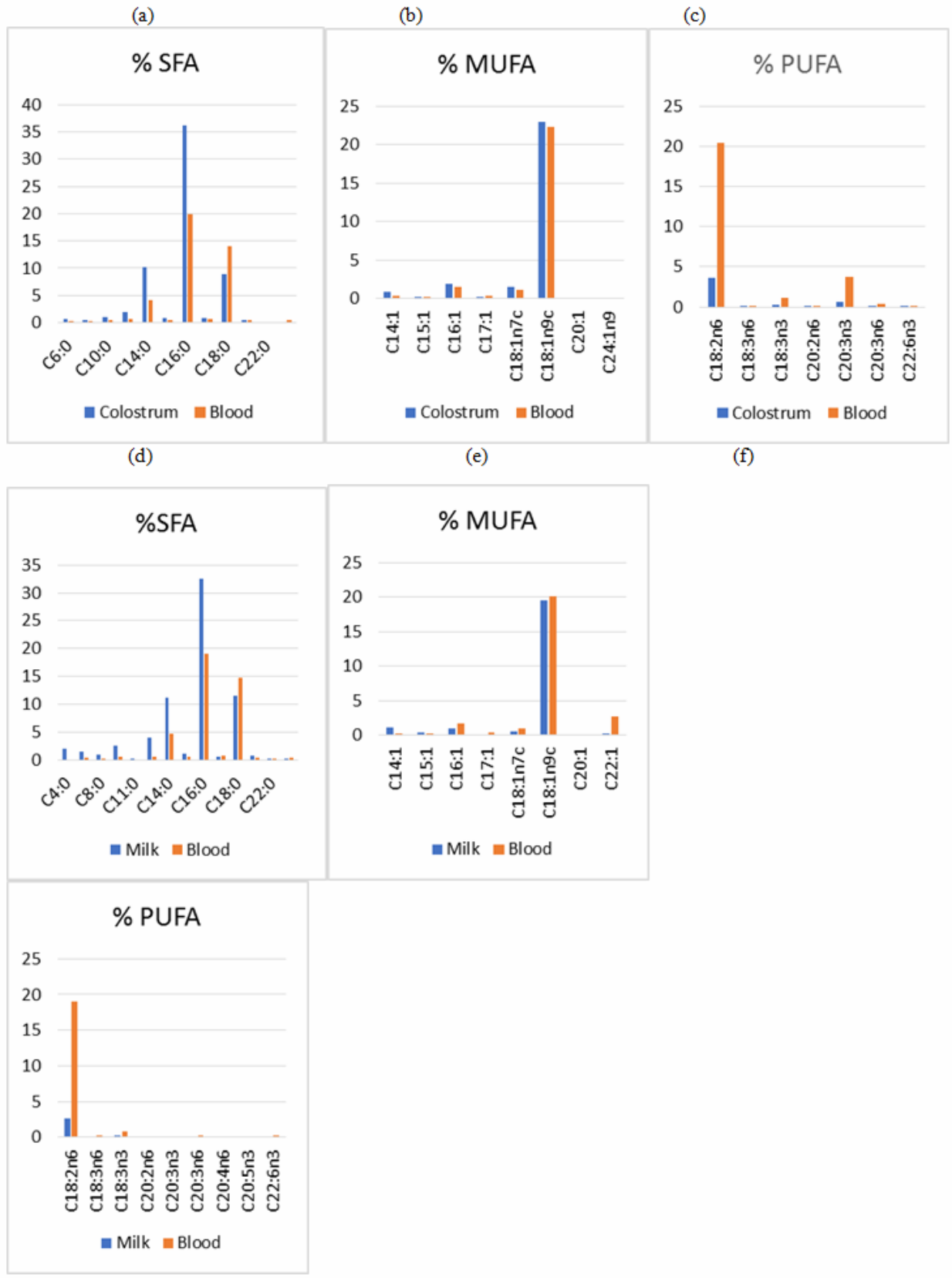

Figure 4 (a) colostrum-blood SFA; (b) colostrum-blood MUFA; (c) colostrum-blood PUFA; (d) milk-blood SFA; (e) milk-blood MUFA and (f) milk-blood PUFA % contents

Table 3 Correlation coefficients between colostrum and blood fatty acids and saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)

Correlation	Pearson correlation (r)	P-value
Colostrum-blood fatty acids	0.768**	0.000
Colostrum-blood SFA	0.894**	0.000
Colostrum-blood MUFA	0.932**	0.000
Colostrum-blood PUFA	0.980**	0.000

r: highly significant correlation.
** (P<0.01).

Therefore, the high amount of this fatty acid in colostrum compared to milk confirms that the cow secretes colostrum both during and after birth to feed her calf, and that the energy needed by the cow was secreted from fat cells through lipolysis and provides the necessary energy.

The intake of palmitic acid, which was from the SFA group of colostrum, by the newborn calf was of great importance because palmitic acid plays an important role in the growth of the calves and the production of energy required for the body (Ren *et al.* 2022; Ayala *et al.* 2024). The fact that the palmitic acid content of colostrum was higher than that of milk in the study shows that it was one of the essential fatty acids for the calves.

The result that colostrum palmitic acid was the fatty acid with the highest rate in the study was consistent with the findings of other authors (Wilms *et al.* 2022; McDermott *et al.* 2024). Although palmitic acid found in colostrum can be synthesized by mammary epithelial cells, it initially comes to the breast from blood plasma through body fat mobilization (Ayala *et al.* 2024).

In the study, palmitic acid being the SFA with the highest level in blood fatty acids explains this situation. In addition, as a result of the correlation analysis conducted in the study, the high level of significant positive correlation (r: 0.894; P<0.01) between colostrum-blood SFA values explains the reason for the high correlation between colostrum and blood due to the transfer of palmitic acid in the blood to the breast.

In the current study, in the correlation analysis of fatty acids in blood taken from colostrum and colostrum-receiving animals, it was determined that there was a highly significant positive correlation between colostrum-blood SFA, MUFA and PUFA contents. The lactation phase of the calf of the calf significantly affects the fatty acid composition of colostrum (Wilms *et al.* 2022).

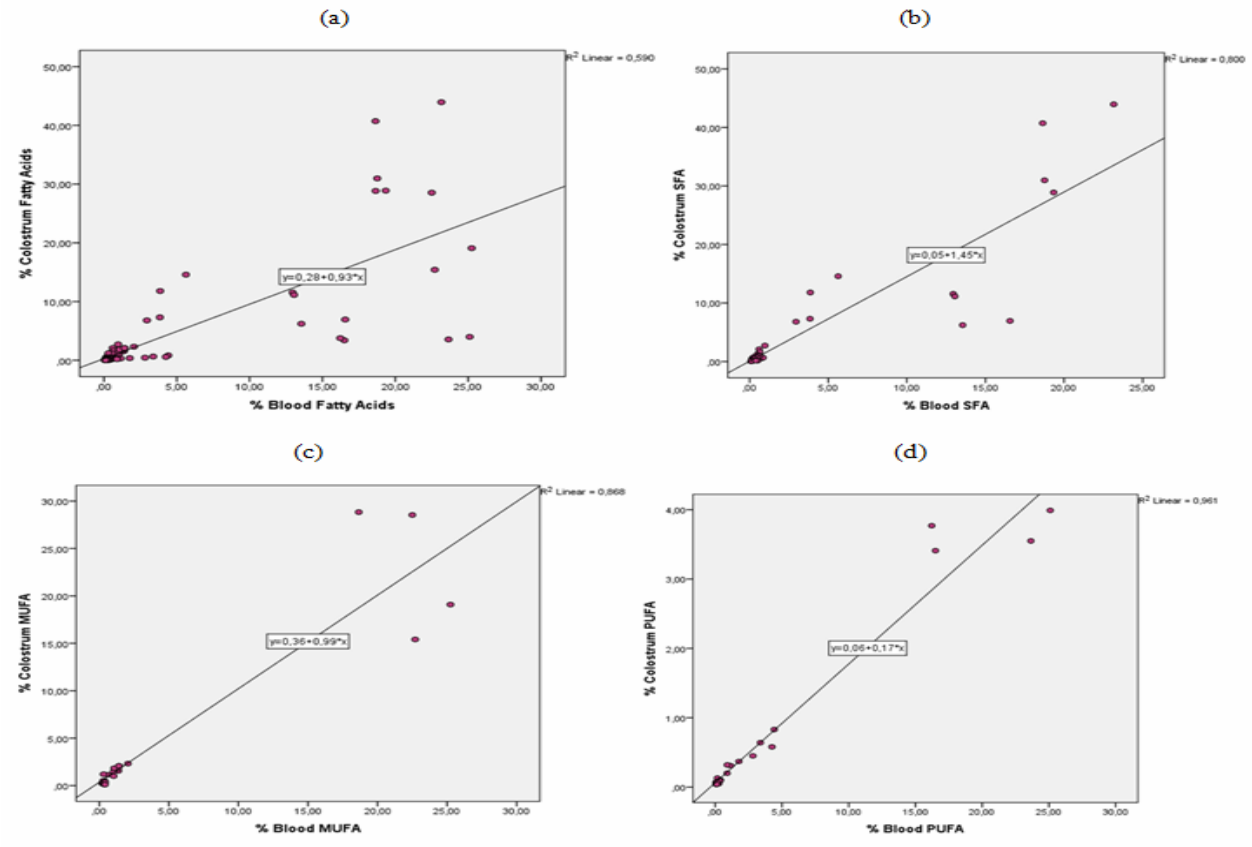

Figure 5 (a) relationship between colostrum and blood fatty acids; (b) relationship between colostrum and blood SFA contents; (c) relationship between colostrum and blood MUFA contents and (d) Regression graphs showing the relationships between colostrum and blood PUFA

Table 4 Correlation coefficients between milk and blood fatty acids and milk-blood saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)

Correlation	Pearson correlation (r)	P-value
Milk-blood fatty acids	0.749**	0.000
Milk-blood SFA	0.891**	0.000
Milk-blood MUFA	0.957**	0.000
Milk-blood PUFA	0.825**	0.000

r: highly significant correlation.
** (P<0.01).

In dairy cows, the energy required after birth in the first weeks after birth was compensated by the transfer of fatty acids from body reserves to the blood. This results in the release of preformed fatty acids in the body via lipolysis, and preformed fatty acids, especially long-chain fatty acids (≥C18), deri-ved from plasma, are incorporated into colostrum (Bionaz *et al.* 2020). Therefore, fatty acid concentrations in the blood were related to colostrum FA concentrations. The high level of significant positive correlation between colostrum-blood SFA, MUFA and PUFA values in the present study supports this view. Studies on this topic have compared fatty acids from colostrum and milk, but the relationship between colostrum-blood and milk-blood has been largely neglected (Andjelić *et al.* 2022; Wilms *et al.* 2022). Our study addresses this gap by determining the relationships between them through correlation.

Determining the physiological ranges of milk and blood fatty acids in lactating animals and the relationship between them was important for having a healthy herd. Andjelić *et al.* (2022) investigated the relationships between different blood and milk metabolic biomarkers in dairy cows at various stages of lactation by correlation analyses to determine the relationship between blood NEFA (non-esterified fatty acids) and milk metabolic parameters. They stated that blood NEFA levels, which were the best indicators of negative energy balance and lipomobilization during lactation, were significantly higher in early lactation cows compared to late lactation cows and showed a positive correlation between blood and milk concentrations. In the current study, the result that there was a high level of positive correlation (P<0.01) between milk and blood fatty acids of cows and that the change in blood was due to the change in milk fatty acids was consistent with the findings of other studies (Djoković *et al.* 2019; Andjelić *et al.* 2022). Determination of the ratios of blood and milk fatty acids and the high level of positive correlation between them indicate intensive mobilization of fat reserves in cows after calving. In conclusion, similar changes in blood and milk metabolite concentrations during lactation and milk-blood correlations confirm that milk has a great potential to predict blood metabolites and metabolic status of cows.

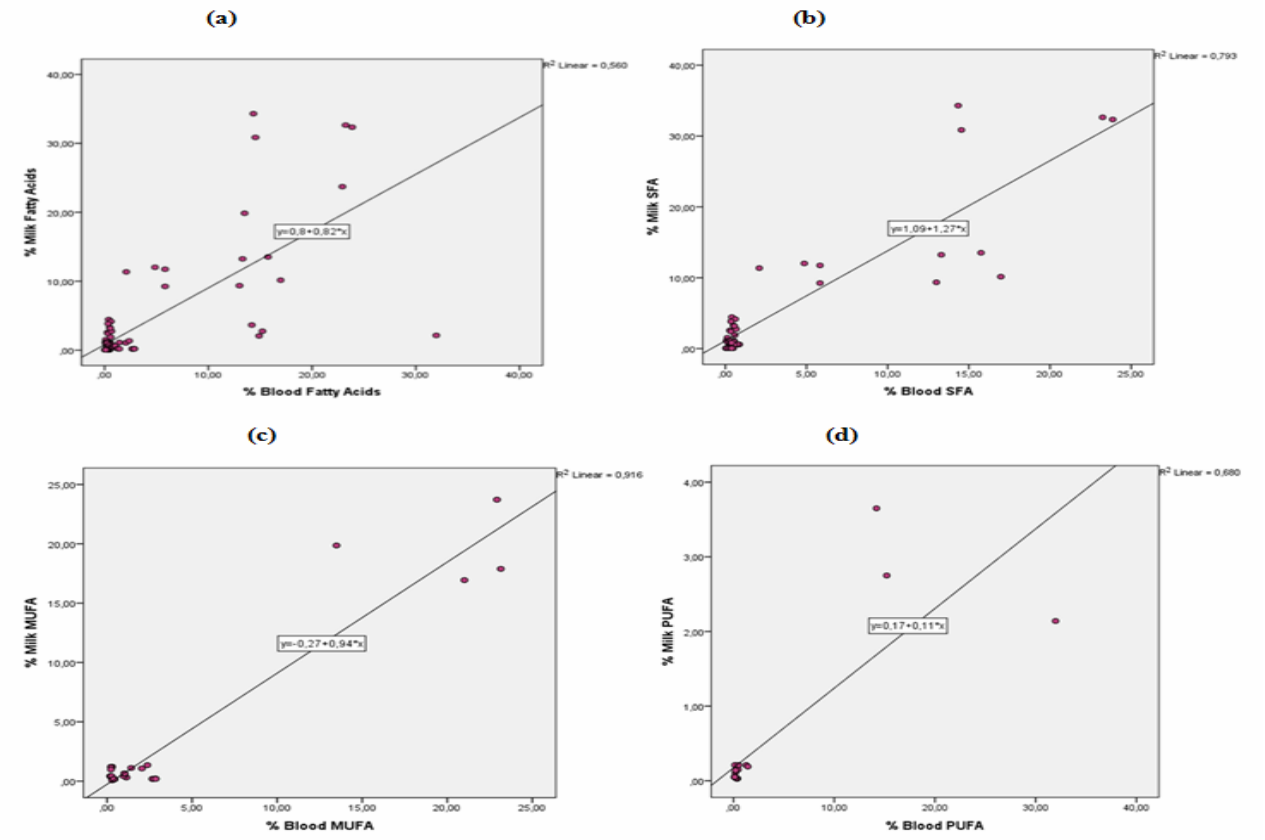

Figure 6 Regression graphs showing the relationships between (a) milk and blood fatty acids; (b) milk and blood SFA ratios; (c) milk and blood MUFA ratios and (d) milk and blood PUFA

It was important to determine the physiological ranges of biochemical parameters in a healthy herd. Therefore, in the study, it was important to compare the SFA, MUFA and PUFA values of colostrum, blood and milk fatty acids, as well as the comparison of n-6/n-3 polyunsaturated FAs. Because the increase in the ratio of n-6/n-3 polyunsaturated FAs in animals under stress and negative energy conditions may indicate biomembrane dysfunction, a violation of the implementation of membrane-related functions of cellular and subcellular structures, and may negatively affect dairy cows (Mylostyvyi *et al.* 2021). In the study, the n-6/n-3 ratio in the blood of milk-extracted animals was found to be higher than that of colostrum-extracted animals (17.76%; 4.18%, respectively). Revskij *et al*. (2019) stated that a high n-6/n-3 PUFA content or a high omega-3 index in plasma was associated with the emergence of metabolic diseases, inflammation and other disorders, and in our study, the low n-6/n-3 PUFA content in the blood of colostrum-extracted animals shows that it was important for calf nutrition and especially health. In addition, the low n-6/n-3 ratio in milk was considered an index of healthy balance in the body (Mylostyvyi *et al.* 2021). The fact that the colostrum n-6/n-3 ratio was found to be lower than milk (6.22%; 6.67%, respectively) supports the study of Mylostyvyi *et al.* (2021). In our study, determination of milk, blood and colostrum fatty acids as well as milk, colostrum and blood n-6/n-3 ratios provide important information for calf health and nutrition.

CONCLUSION

Newborn calf feeding management has a significant impact on rearing success and also impacts health and performance in later life. Therefore, adequate colostrum content is crucial for calf nutrition. A high positive correlation was found between colostrum-blood fatty acid and milk-blood fatty acid concentrations, and colostrum-blood and milk-blood SFA, MUFA, and PUFA levels. This high correlation suggests that changes in colostrum and milk are also highly dependent on changes in blood. These results suggest important findings regarding cow and calf health and nutrition. The high correlations between colostrum-blood and milk-blood confirm that colostrum and milk have great potential in predicting blood metabolites and metabolic status in cows.

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