IJAS Iranian Journal of Applied Animal Science

# The Comparison of Chemo Signal Compositions in Body Fluids of Holstein Cows from Different Estrus Periods

Research Article

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Received on: 3 Apr 2023
Revised on: 25 May 2023
Accepted on: 24 Jun 2023
Online Published on: Sep 2023

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Online version is available on: www.ijas.ir

## ABSTRACT

Artificial insemination is widely used in livestock breeding to control the calving interval and milk production in dairy cattle and to obtain one offspring per year. The correct timing of artificial insemination can be achieved by correct detection of estrus. Blood, feces and urine samples from estrus and diestrus periods have taken from Holstein cows which similar characteristics. Cows have synchronized for this purpose using ovsynch protocol. Fatty acid analyzes of the samples were made by gas chromatography (GC). The rates of all saturated fatty acids (∑SFA), all monounsaturated fatty acids with a single double bond (∑MUFA) and all polyunsaturated fatty acids (∑PUFA) were found to be different in estrus and diestrus periods. The largest difference between estrus and diestrus ∑SFA ratios was found in the urine (estrus: 14.22%, diestrus: 23.11%). The high rate of ∑SFA in urine during estrus was due to the ratios of palmitic acid and butyric acid. The highest ∑MUFA rate was detected in the urine during diestrus. The high rate of ∑MUFA in urine was due to the detection of oleic acid at a rate of 13.38% in estrus and 25.53% in diestrus. The largest difference between ∑PUFA rates was detected in feces. Linolenic acid was detected in feces at a rate of 0.58% in estrus and 3.44% in diestrus. The reason for the difference between urinary ∑PUFA rates was the detection of eicosadienoic acid at a rate of 8.66% in estrus and 1.30% in diestrus. These compounds can be used as biomarkers for sensored biotechnological devices due to their chemo signal properties in estrus detection.

KEY WORDS chemo signal, dairy cow, diestrus, estrus, fatty acid, gas chromatography.

## INTRODUCTION

The estrus cycle in dairy cattle is crucial for reproductive performance and conscious monitoring of the estrus cycle by farmers or business owners is essential for a good breeding management program (Kaproth and Foote, 2011; Nebel *et al*. 2011). Artificial insemination is widely used in livestock breeding to control the calving interval and milk production in dairy cattle and to obtain one offspring per year. The correct timing of artificial insemination can be achieved by correct detection of estrus (Kaproth and Foote, 2011; Nebel *et al*. 2011). However, due to the quiet or weak signs of estrus in some cows, determining exactly when to inseminate is a challenge. In the researches, it was stated that the correct estrus detection of the enterprises and accordingly the artificial insemination and fertility rate ranged between 60% and 73% (Hastuti, 2008; Tadesse *et al*. 2022). The low rate of estrus detection has led to the development of more effective new estrus methods. Increasing the success rate in estrus detection will significantly increase the profitability of dairy cattle breeders. In animals, the endocrine state regulates behavioral expressions, estrus stages, and the physical and biochemical components of bodily fluids. For this reason, hormonal changes occurring in the

body at certain periods cause animals to behave differently and to changes in the components in their body fluids (Denver *et al*. 2009). Fatty acids, which have important roles for living organisms, are one of the components that change in body fluids in certain periods. The roles of fatty acids in the organism are closely related to the structure of these biologically active molecules, which have different carbon chain lengths and degrees of saturation. Fatty acids (FA) are precursors of many biologically active substances (prostaglandin, leukotriene, thromboxane and others) involved in the regulation of metabolic processes in humans and animals. The concentration and composition of fatty acids vary considerably under various physiological and pathological conditions. Many researchers emphasize the importance of studying certain types of FA as biomarkers for the early detection of pathological states in the body (Hammami *et al*. 2015; Didenko *et al*. 2017). Determination of contents in various biological substrates, including blood plasma, can be an important diagnostic tool for detecting pathological conditions (Caron *et al*. 2018). Zebari *et al*. (2019) reported that there were changes in the concentrations of some milk fatty acids during the estrus and diestrus periods of lactating dairy cows. It has been determined that hormonal changes that occur in the body at certain periods cause different behaviors of animals and changes in the components in body fluids (Denver *et al*. 2009). Changes in fatty acids found in body fluids such as blood, milk, urine and feces of animals, especially before and during estrus, can create important findings for the detection of estrus. It is important for both breeders and farmers to investigate whether the determination of fatty acid changes in metabolism, especially during estrus, with body fluids, can be a reliable biological indicator for early detection of estrus. Because determining the right time for artificial insemination will enable the business to make profit and increase productivity in production in order to obtain one calf per year. In this study, it was aimed to investigate the fatty acid changes in blood, milk, urine and feces of cows in estrus and diestrus periods. For this reason, it was stated that these fatty acids, which were determined at different rates in different periods by gas chromatography, can be a reliable biological indicator for the detection of estrus.

## MATERIALS AND METHODS

### Ethical approval

This study was approved by Cukurova University Animal Experiments Local Ethics Board (Approval no:7 26.02.2018/2).

### Animals

The present study was conducted at research and experimental farm located at Faculty of Agriculture, Çukurova University, Adana, Turkey. In this study, healthy Holstein cows with similar characteristics (Weight 600-650 kg, body condition score was between 2.5 and 3, 3-6 years old, gave birth at least once, has no reproductive problems, and was postpartum between 45-60 days) were selected from Cukurova University Faculty of Agriculture Dairy Cattle Research and Application Farm. It was determined by the enterprise veterinarian that the animals evaluated within the scope of the study did not have any disease table, through anamnesis information and routine clinical examinations.

Cows from which trial samples were taken were fed with a total mix ration (TMR) with a concentrate: roughage ratio of 60:40. TMR was composed of corn silage, alfalfa, wheat straw and concentrate (18% crude protein and 2650 kcal/metabolic energy (ME)/kg) and was given at 07.00 and 16.00 in the morning. Experimental animals were milked twice a day with an automatic milking system at the central milking parlor at 12-hour intervals.

### Synchronization process

In the study, the estrus of the animals were aggregated using the Ovsynch protocol (Haga *et al*. 2021). The first GnRH injection, in which the synchronization process was performed, was counted on the 1st day. Animals were sampled for the diestrus period between days 5 and 7 of synchrony. Samples were taken for the estrus period 10 days after synchronization (on the day of artificial insemination). Animals were observed visually to determine that they were in estrus. The exact onset of estrus was determined by the cows displaying signs of estrus-specific behavior.

### Collection of samples

Urine samples were taken by manual stimulation of the perineal regions of cows (Sankar and Archunan, 2008), but a catheter was used in cases where there was a problem in urine collection. 10 mL of urine was taken into a sterile tube. The feces samples of the cows were washed with clean water and dried with a paper towel, as Sankar and Archunan (2008) stated, and approximately 30 g was collected from the rectum. For the milk samples, after the milk accumulated on the teat in the morning was discarded, it was milked and taken into a 250 mL sample container and the samples were transported under suitable conditions to maintain the cold chain until the analysis. Blood samples were collected from the vena jugularis early in the morning (Klemm *et al*. 1994). Samples were prepared by mixing a 10 ml aliquot of blood with 10% sodium citrate in a 30 mL injection vial sealed with a Teflon-lined septum. Samples were snap frozen and stored at -80 ˚C until prepared for analysis by gas chromatography.

### Gas chromatography analysis of all samples

Bligh and Dyer (1959) method was used for lipid extraction from samples taken from animals. Methyl esters to be

formed from the samples were prepared by transmethylation using 2 M KOH in methanol and n-hexane; 10 mg of the 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 and centrifuged at 4000 rpm for 10 minutes, then transferred to the hexane layer for GC analysis. Samples collected in glass containers were sieved using filter paper (60-120 µm) and stored frozen at -20 ˚C (Kumar *et al*. 2000).

Samples were analyzed for fatty acid compositions on the GC Clarus 500 with a flame ionization detector and autosampler (Perkin Elmer USA) equipped with a silica capillary SGE column (30 m×0.32 mm, ID×0.25 µm, BP20 0.25 UM, USA). After the injector and detector temperatures were adjusted to 220 ˚C and 280 ˚C, respectively, the oven temperature was kept at 140 ˚C for 5 minutes. It was increased to 200 ˚C at a rate of 4 ˚C/min and then to 220 ˚C at a rate of 1 ˚C/min. Samples were sized to 1 µL and carrier gas operated at 16 psi, using a 1:100 split ratio as separation.

**Data analysis**

The average of the fatty acid concentrations in the three replication samples for both periods was taken. Descriptive statistics were used to describe the entire data set in terms of specific characteristics. Fatty acids % values determined in this context, Mean ± SEM (standard mean error) were summarized in the tables. The collected data were analyzed using Excel and SPSS 20.0 (SPSS, 2011) package program. In the SPSS program, it was analyzed whether the data were normally distributed or not. Kolmogorow-Smirnov test of normality was used to determine whether the data were suitable for normal distribution. In case a variable did not show a normal distribution, Log Transformation, one of the data transformation techniques, was applied. Transformed data of the two groups were compared. In addition, it was tried to determine whether there was any relationship between dependent variables and independent variables by correlation analysis with the SPSS package program. Scatter plots were also used to visually express the analysis results. The level of significance in the analyzes was $P < 0.05$.

## RESULTS AND DISCUSSION

Comparison of body fluids fatty acid compositions of different estrus periods of Holstein cows were given in detail in Table 1.When Table 1 was examined, a total of 28 fatty acids in feces, 29 in blood, 32 in milk and 22 in urine were detected in both periods. In Table 1, butyric acid, dihomo-γ-linolenic acid and docosadienoic acid were found in the feces during diestrus but were not detected during estrus. Caprylic acid was detected in milk and blood in both estrus and diestrus periods, but it was not detected in feces in both periods. Behenic acid and nervonic acid were detected in blood only during diestrus period (Table 1). Eicosadienoic acid, docosadienoic acid and nervonic acid were the fatty acids that were not detected in the estrus period and butyric acid were not detected in the diestrus period in milk (Table 1). Butyric acid and caprylic acid were not detected in the urine during diestrus period. In addition, although oleic acid was found in the urine at a rate of 13.38% in the estrus period, it was found at a rate of 25.53% in the diestrus period. Eicosadienoic acid was detected at a rate of 8.66% in the estrus period and 1.30% in the diestrus period in urine (Table 1). The high detection rate of this fatty acid in the urine during the estrus period can be a reliable biological indicator showing that the animal is not in the diestrus period.

When Figure 1 was examined, it was seen that the ratios of ∑SFA, ∑MUFA and ∑PUFA were different in feces, milk, urine and blood in both estrus and diestrus periods. As a result of the statistical analysis, only the difference between estrus and diestrus periods in feces was significant ($P<0.05$).

In Table 1, although the ∑SFA ratios were around 30% in feces and blood on average, it was detected at an average rate of 60% in milk. The high level of ∑SFA in milk was due to the fact that palmitic acid (C16:0) had a ratio of more than 30%. Compared to the diestrus period, the estrus ∑SFA ratio was higher in feces and milk, lower in blood and urine. In addition, when the ∑SFA rates detected in feces, milk, blood and urine were examined, it was seen that the most important difference between the two periods was in the urine (estrus 14.22%, diestrus 23.11%). The reason for the difference between the two periods was the high ratios of butyric acid (C4:0) and palmitic acid (C16:0) in diestrus (Table 1). Butyric acid was detected in urine only during diestrus. Palmitic acid (C16:0) was detected in urine at a low rate (5.68%) in estrus and high rate (9.63%) in diestrus. Both butyric acid and palmitic acid can be a reliable biological indicator showing that the animal is in diestrus. When Figure 1. D was examined, it was seen that the rate of ∑MUFA varies between 16% and 32%. The highest ∑MUFA ratio was detected in the urine of the animal during the diestrus period (31.23%). The high rate of ∑MUFA in urine was due to the detection of oleic acid (C18:1n9c) at a rate of 13.38% in estrus and 25.53% in diestrus.

Figure 2 showed that the lowest ∑PUFA ratio was detected in milk. The reason for this difference in ∑PUFA ratio was due to the in linoleic acid (C18:2n6c) ratio. Because linoleic acid was about 11% in feces, 31% in blood and 2 % in milk.

**Table 1** The rates of ∑ (total) SFA, ∑MUFA and ∑PUFA detected in feces, blood, milk and urine of Holstein cows during estrus and diestrus periods (%)

| | Feces | | Blood | | Milk | | Urine | |
|---|---|---|---|---|---|---|---|---|
| **Fatty Acids** | **Estrus*** | **Diestrus*** | **Estrus** | **Diestrus** | **Estrus** | **Diestrus** | **Estrus** | **Diestrus** |
| Butyric acid (C4:0) | | 4.53±0.19 | | | 0.96±0.03 | | | 3.06±0.41 |
| Caproic acid (C6:0) | 0.35±0.02 | 0.36±0.04 | 0.02±0.00 | 0.09±0.02 | 0.79±0.02 | 0.84±0.09 | 0.77±0.02 | 0.36±0.05 |
| Caprylic acid (C8:0) | | | 0.08±0.01 | 0.06±0.01 | 0.81±0.02 | 0.75±0.07 | | 0.66±0.07 |
| Capric acid (C10:0) | 0.17±0.03 | | 0.35±0.05 | 0.18±0.03 | 2.75±0.10 | 2.76±0.18 | | |
| Undecanoic acid (C11:0) | | | | | 0.07±0.00 | 0.14±0.03 | | |
| Laurik acid (C12:0) | 0.49±0.05 | 0.22±0.03 | 0.55±0.03 | 0.31±0.03 | 4.16±0.10 | 4.01±0.21 | | |
| Myristic acid (C14:0) | 2.85±0.30 | 1.48±0.27 | 2.19±0.08 | 1.15±0.05 | 12.02±0.60 | 12.97±0.19 | 0.87±0.03 | 1.53±0.23 |
| Pentadecanoic acid (C15:0) | 2.08±0.18 | 1.10±0.21 | 0.53±0.02 | 0.54±0.09 | 1.09±0.06 | 1.54±0.08 | 0.20±0.00 | 0.16±0.02 |
| Palmitic acid (C16:0) | 13.45±0.80 | 11.96±0.32 | 14.24±1.00 | 14.29±0.66 | 34.30±2.00 | 35.68±1.53 | 5.68±0.23 | 9.63±0.45 |
| Margaric acid (C17:0) | 0.73±0.10 | 0.54±0.05 | 0.74±0.02 | 1.32±0.11 | 0.59±0.04 | 0.64±0.04 | 0.12±0.01 | 0.15±0.02 |
| Stearic acid (C18:0) | 8.88±0.30 | 10.56±0.39 | 15.45±0.45 | 23.67±0.96 | 10.15±1.01 | 6.85±0.30 | 4.62±0.22 | 6.82±0.32 |
| Arachidic acid (C20:0) | 0.97±0.03 | 0.21±0.02 | 0.31±0.02 | 0.31±0.11 | 0.66±0.06 | 0.66±0.05 | 1.74±0.04 | 0.36±0.03 |
| Behenic acid (C22:0) | 3.86±0.30 | 1.85±0.21 | 0.03±0.00 | | 0.05±0.00 | 0.04±0.00 | 0.21±0.01 | 0.16±0.02 |
| Lignoceric acid (C24:0) | 0.12±0.01 | 0.38±0.03 | 0.03±0.00 | 0.15±0.03 | 0.04±0.00 | 0.02±0.00 | 0.01±0.00 | 0.22±0.02 |
| **∑ SFA** | **33.95** | **33.19** | **34.52** | **42.07** | **68.44** | **66.90** | **14.22** | **23.11** |
| Myristoleic acid (C14:1) | 2.53±0.20 | 1.56±0.20 | 0.30±0.01 | 0.38±0.06 | 1.17±0.10 | 0.56±0.07 | | |
| Methyl pentadecanoate (C15:1) | 1.35±0.10 | 0.48±0.04 | 0.19±0.01 | 0.33±0.04 | 0.34±0.02 | 0.21±0.01 | | |
| Palmitoleic acid (C16:1) | 1.67±0.20 | 1.08±0.17 | 1.15±0.10 | 0.38±0.07 | 1.11±0.10 | 0.42±0.03 | 1.04±0.02 | 2.07±0.06 |
| Heptadecenoic acid (C17:1) | 0.66±0.04 | | 0.09±0.00 | 0.12±0.03 | 0.07±0.00 | 0.07±0.00 | | |
| Vaccenic acid (C18:1n7) | 1.10±0.10 | | 0.28±0.02 | 0.5±0.18 | 0.37±0.03 | 0.87±0.06 | | |
| Oleic acid (C18:1n9) | 10.20±0.70 | 11.24±0.36 | 15.61±0.50 | 16.32±0.51 | 17.89±2.00 | 18.73±0.76 | 13.38±1.03 | 25.53±5.50 |
| Eicosanoic acid (C20:1n9) | 0.48±0.06 | 2.18±0.05 | 0.32±0.02 | 0.20±0.06 | 0.19±0.02 | 0.90±0.00 | 1.31±0.04 | 3.23±0.29 |
| Erucic acid (C22:1n9) | 0.16±0.02 | | 0.07±0.00 | 1.97±0.24 | 0.19±0.02 | 0.24±0.02 | 0.18±0.01 | 0.40±0.08 |
| Nervonic acid (C24:1n9) | | | 0.03±0.00 | | | 0.06±0.00 | | |
| **∑ MUFA** | **18.15** | **16.54** | **18.04** | **20.20** | **21.33** | **22.06** | **15.91** | **31.23** |
| Linoleic acid (C18:2n6) | 11.26±0.26 | 11.90±0.20 | 31.13±1.00 | 34.01±1.00 | 2.85±0.25 | 3.74±0.11 | 5.37±0.12 | 11.58±0.31 |
| Linolenic acid (C18:3n6) | 0.58±0.08 | 3.44±0.31 | 1.23±0.20 | 0.41±0.12 | 0.05±0.00 | 0.06±0.00 | 4.39±0.15 | 0.27±0.03 |
| Alpha Linolenic acid (C18:3n3) | 1.52±0.20 | 0.48±0.07 | 0.14±0.00 | 0.76±0.54 | 0.21±0.02 | 0.24±0.02 | 2.13±0.02 | 2.98±0.45 |
| Eicosadienoic acid (C20:2n6) | | | 0.17±0.01 | 0.13±0.05 | | 0.06±0.00 | 8.66±1.20 | 1.30±0.31 |
| Dihomo-γ-linolenic acid (C20:3n6) | | 1.47±0.17 | 0.05±0.00 | 0.14±0.06 | 0.02±0.00 | 0.03±0.00 | 0.44±0.01 | 2.02±0.10 |
| Arachidonic acid (C20:4n6) | 0.23±0.03 | 0.32±0.03 | 0.14±0.01 | 0.19±0.06 | 0.06±0.00 | 0.06±0.00 | | |
| Eicosapentaenoic acid (EPA) (C20:5n3) | 0.29±0.40 | | 0.32±0.03 | 0.15±0.01 | 0.04±0.00 | 0.03±0.00 | 0.54±0.02 | 1.12±0.11 |
| Docosahexaenoic acid (C22:6n3) | 0.60±0.01 | 0.58±0.04 | 0.48±0.03 | 0.18±0.03 | 0.05±0.00 | 0.04±0.00 | 1.52±0.12 | 3.86±0.17 |
| Docosadienoic acid (C22:2n6) | | 1.14±0.03 | | | | 0.08±0.01 | | |
| **∑ PUFA** | **14.48** | **19.33** | **33.66** | **35.97** | **3.28** | **4.34** | **23.05** | **23.13** |
| MUFA/SFA | 0.53 | 0.50 | 0.52 | 0.48 | 0.31 | 0.33 | 1.12 | 1.35 |
| PUFA/SFA | 0.43 | 0.58 | 0.98 | 0.86 | 0.05 | 0.06 | 1.62 | 1.00 |
| PUFA/MUFA | 0.80 | 1.17 | 0.87 | 1.78 | 0.15 | 0.0 | 1.45 | 0.74 |
| ∑n6 | 12.07 | 18.27 | 32.72 | 34.88 | 2.98 | 4.03 | 18.86 | 15.17 |
| ∑n3 | 2.41 | 1.06 | 0.94 | 1.09 | 0.30 | 0.31 | 4.19 | 7.96 |
| n6/n3 | 5.00 | 17.24 | 34.81 | 32.00 | 9.93 | 2.83 | 4.50 | 1.91 |

∑ SFA: all saturated fatty acids (without any double bond. 4:0 to 24:0); ∑ MUFA: all monounsaturated fatty acids with a single double bond (14:1 to 24:1); ∑ PUFA: all polyunsaturated fatty acids; ∑ n-6 polyunsaturated fatty acids (PUFA): 18:2n6; 18:3n6; 20:2n6; 20:3n6; 20:4n6 and ∑ n-3 polyunsaturated fatty acids (PUFA): 18:3n3; 20:5n3; 22:6n3. The means within the same column with at least one common letter, do not have significant difference (P>0.05).

The biggest difference between estrus and diestrus ∑PUFA ratios was detected in feces. The rates of ∑PUFA in feces were detected as 14.48% in the estrus period and 19.33% in the diestrus period. The reason for this difference was due to the high detection of linolenic acid (C18:3n6) during diestrus and the detection of docosadienoic acid (C22:2n6) and dihomo-γ-linolenic acid (C20:3n6) only during diestrus.

Although there was no significant difference between the two periods in the ∑PUFA ratio in urine, significant differences were found between the compounds. These compounds are eicosadienoic acid (C20:2n6) and linoleic acid (C18:2n6). Eicosadienoic acid (C20:2n6) was detected 1.30% in diestrus and 8.66% in estrus, while linoleic acid (C18:2n6) was detected with a rate of 5.73% in estrus and 11.58% in diestrus (Table 1).

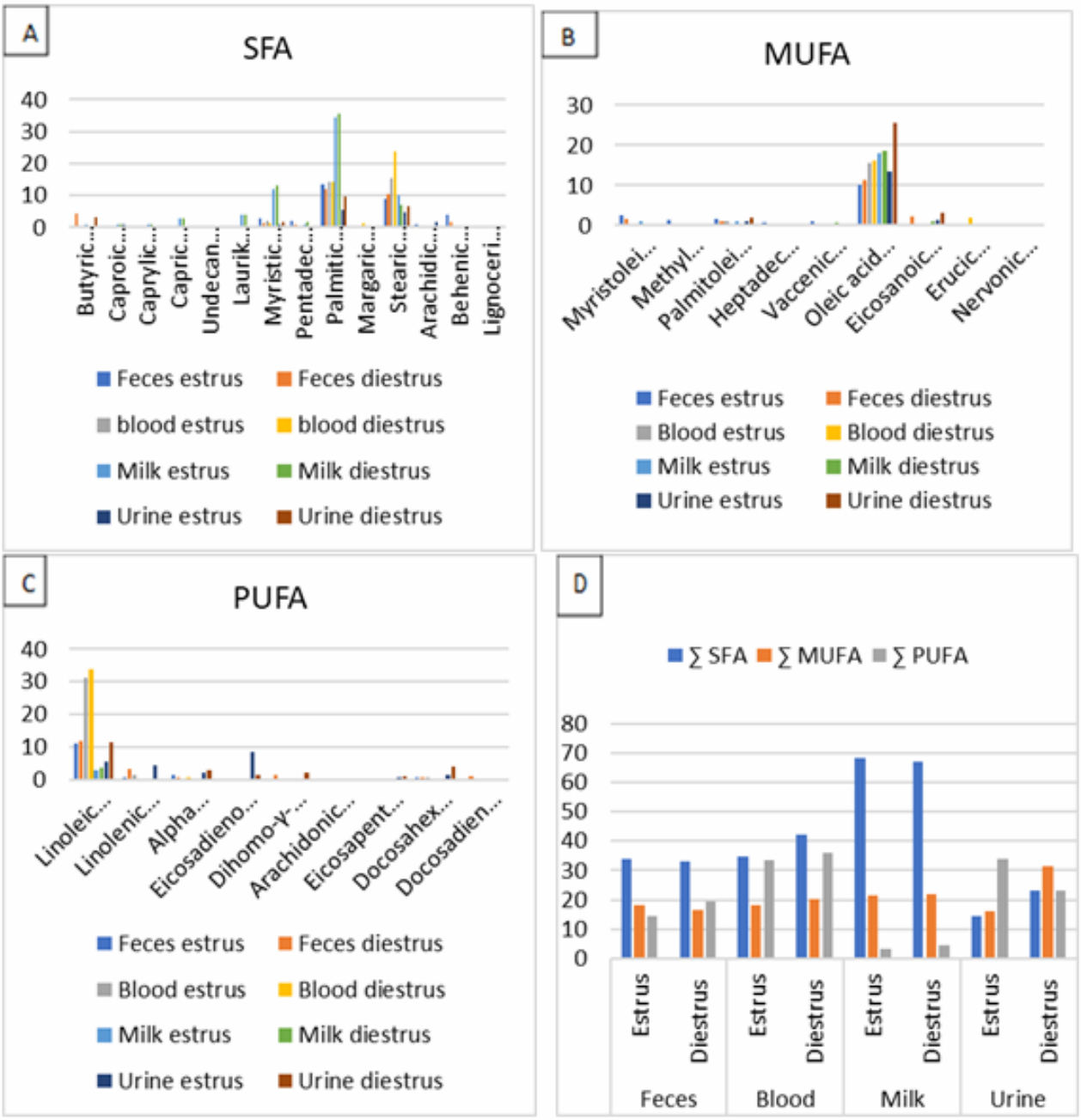


**Figure 1** SFA (A), MUFA (B), PUFA (C) and ∑SFA, ∑MUFA, ∑PUFA (D) ratios of feces, blood, milk and urine fatty acids

**The correlation between feces and urine fatty acids**

In our study, pearson correlation analysis was used to determine the relationship between feces and urine fatty acids detected in estrus period. The results obtained were shown in Table 2. The relationship between urinary and fecal fatty acids detected during estrus was significant. Since the Pearson Correlation level was determined as r= 0.739, there was a strong (0.739) and significant positive relationship ($P<0.05$) between the two variables.

A strong and significant relationship was found between feces fatty acids and urinary fatty acids during estrus. It was seen in the correlation analysis that when feces fatty acids increase positively, there was also a positive increase in urinary fatty acids (Table 2). The variance affecting the variables on each other was determined as 54.61%. In other words, approximately 55% of the fatty acids detected in the feces during the heat period may be due to urinary fatty acids. This relationship was seen in Figure 2.

**Table 2** Relationship between fatty acids in feces and urine

| Item | Feces | Urine |
|---|---|---|
| Pearson correlation (r) | - | 0.739** |
| Sig. (2-tailed) | - | 0.0000 |
| N | - | 18 |

** Correlation is significant at the 0.01 level (2-tailed).

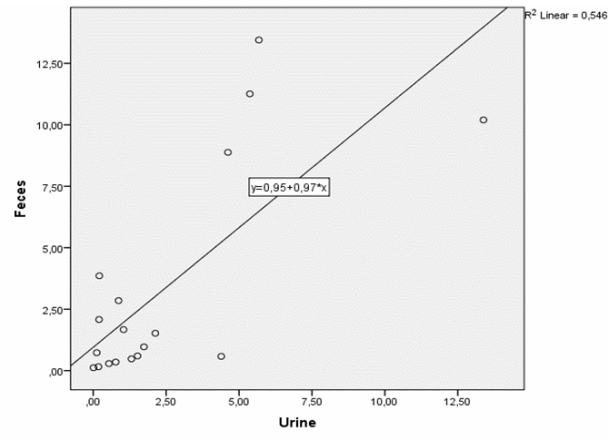


**Figure 2** Correlation analysis scatter plot of feces and urine fatty acids

Studies have identified estrus-specific compounds in feces, urine, milk, vaginal fluids, saliva, and serum (Archunan *et al*. 2014; Le Danvic *et al*. 2015; Nordéus *et al*. 2016). In the study, it was observed that the urine, feces, blood and milk fatty acid ratios differed in estrus and diestrus periods (Table 1). The detection of these fatty acids at different rates in the two periods Douglas *et al*. (2007) and Zebari *et al*. (2019) was in agreement with the results. In addition, when Table 1 was examined, the fact that there were significant differences in feces compounds during the estrus cycle of cattle supports the results of the researchers' study on this subject (Sankar and Archunan, 2008; Gnanamuthu and Rameshkumar, 2014; Mozūraitis *et al*. 2017). Gnanamuthu and Rameshkumar (2014) reported in their study that valeric acid, caproic acid, myristic acid, gadoleic acid and pelargonic acid were found in cow feces in the estrus period, but not in the diestrus period. When Table 1 was examined, only caproic acid and myristic acid were detected in feces. In addition, caproic acid was detected at low rates and myristic acid at higher rates during estrus. The difference in the number and ratios of compounds detected by these researchers during the estrus period may be due to factors such as sample collection, breed, feed, environment, lactation, seasonal changes, genetic variation, differences in analysis and bioassay methods (Klemm *et al*. 1994; Nordéus *et al*. 2016).

In the present study, the difference in fatty acids in body fluids, especially in urine, during both estrus and diestrus periods (Table 1) suggests that it may be a precursor to detect metabolic changes in the bodies of animals during these periods.

Because urine was considered a reliable indicator of an animal's physiological state, due to its function of mediating the elimination of metabolic wastes (Weiner *et al*. 2014). Since blood also contains many urinary compounds, it was not surprising that urine could transmit a great deal of information about the animal's internal physiological state to the outside world, thus providing a source of chemical signals of many types. In addition, in the correlation analysis performed to determine the relationship between the fatty acids detected in the body fluids of animals, it was stated that the changes in the blood depended on the changes in the milk, feces and urine, and the changes in the feces were highly dependent on the changes in the urine, milk and blood. In addition, it was stated that the changes in fatty acids in milk, blood and urine contributed significantly to estimating the fatty acids in the feces (Anitaş and Göncü, 2018).

The fact that in the urinary fatty acids eicosadienoic acid, linolenic acid, arachidic acid and caproic acid were detected at higher rates in the estrus period in Table 1 supported the results of the research on this subject (Rameshkumar and Archunan, 2006; Achiraman *et al*. 2011). The most important reason for determining the differences of these fatty acids in studies was due to the opinion that the increase in their ratio in the estrus period causes the formation of odor and may act as a pheromone (Rameshkumar and Archunan, 2006; Achiraman *et al*. 2011). Detection of these odor compounds (pheromones) in body fluids was important because they were compounds that attract bulls in body fluids such as urine, feces, sweat and vaginal discharge, which determine that cows were in estrus period (Rameshkumar and Archunan, 2006; Mozūraitis *et al*. 2017; Anitaş and Göncü, 2018).

When a bull was brought near a cow in estrus, the male usually exhibits various behaviors (Rajanarayanan and Archunan, 2004). This behavior was called flehmen and can be considered as one of the best indicators for the female in estrus (Hradecky *et al*. 1983). It has been determined that flehmen behavior was controlled by certain sex pheromones. It has been stated that the bulls' behavior against the feces and urine of the cows in heat period has an important place in the detection of estrus by these body fluids. Because bulls smell urine and feces and guess whether the animal was in estrus or not (Rajanarayanan and Archunan, 2004; Arhunan and Rameshkumar, 2012). The high correlation between fecal and urinary fatty acids content in cows during estrus period in Table 2 supports the view that the bull recognizes the estrus cow by smelling these two important body fluids (Sankar and Archunan, 2008; Archunan and Rameshkumar, 2012; Mozūraitis *et al*. 2017). The difference in blood, feces, urine and milk fatty acids in both estrus and diestrus periods (Table 1) supports

the conclusion that the animals use the lipid they need for ovarian development (Apparicio *et al*. 2012). In addition, the fact that some fatty acids, especially SFA ratios, changes in body fluids during estrus in this study also supports the view that it may be a viable mechanism for regulating body metabolism (Douglas *et al*. 2007). In proestrus and estrus, as a result of the growth of cells under the influence of estrogen, changes that indicate the state of estrus in the cow genital organs are formed. Since these hormonal changes in the body also change the body metabolism, it was important to determine the SFA, MUFA and PUFA changes in body fluids, in order to determine the chemical changes that may occur during the estrus cycle.

In the study on the importance of fatty acids for the body, linoleic acid (C18:2n6) and alpha linolenic acid (C18:3n3), which were in the PUFA group, were defined as two essential fatty acids necessary for growth, structural health of the skin and reproduction (Hossain *et al*. 2016). In Table 1, the reason why both linoleic acid and alpha linoleic acid were different in body fluids during estrus and diestrus may be due to the difference in fatty acid ratios used and excreted in the body due to hormonal changes occurring in the body during these periods (Denver *et al*. 2009).

The difference in PUFA ratios in the study during estrus and diestrus periods supports the view of Manafi (2011) that unsaturated fatty acid compositions in the blood of cows may be affected by ovarian hormones. It has been noted that PUFA are involved in important tasks such as granulosa cells derived from follicles, ovulation, corpus luteum function (Wathes *et al*. 2007; Sharma *et al*. 2020; Collodel *et al*. 2022). Eicosadienoic acid (20:2n6) was the n6 fatty acid in the PUFA group with important functions. Eicosadienoic acid was a direct precursor of dihomo-y-linolenic acid. While the rate of eicosadienoic acid in urine was 8.66% in estrus, it was 1.30% in diestrus (Table 1). Considering that this fatty acid will play an important role in reproduction like other PUFAs, its high rate only during estrus may indicate that the body uses this fatty acid in various metabolisms and disposes of it. For this reason, high levels of this fatty acid in the urine during estrus may be an indication that the animal was in estrus.

## CONCLUSION

The rate of ∑SFA was found to be low in blood and urine during estrus and high in diestrus. In addition, the ∑SFA ratio was found to be high in feces and milk in the estrus period and at lower rates in the diestrus period. ∑MUFA ratio was found to be higher in feces and lower in blood, milk and urine during estrus period. The rate of ∑PUFA in milk, urine, feces and blood was found to be low during estrus and high during diestrus. Linoleic acid (C18:2n6) and alpha linolenic acid (C18:3n3), which were necessary for the growth and reproduction of animals, were detected at higher rates in urine during diestrus period. Butyric acid was detected in milk only in heat period, and in feces and urine during diestrus period. Palmitic acid (C16:0) was detected in urine at a high rate in diestrus and at a low rate in estrus. In addition, although the rate of eicosadienoic acid in urine was low rate in diestrus, it was very high in estrus. The determination of these compounds, which are specific to the estrus period in body fluids, will be useful for more detailed studies on the use of new technologies in the detection of estrus.

## ACKNOWLEDGEMENT

We gratefully thank to Prof. Dr. Yeşim ÖZOĞUL for her valuable help and contributions in the Laboratory of Seafood Processing Technology, Faculty of Fisheries, Çukurova University.

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