

# Ratio of fatty acids in sweat, blood and urine in cattle

## Proporción de ácidos grasos en sudor, sangre y orina en ganado bovino

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### ABSTRACT

In cattle metabolism, fatty acids are basic biological components that meet the body's energy needs and are used in important metabolic processes. In this study, sweat, urine and blood samples were taken from cows and the fatty acids of the samples were determined by gas chromatography. Sweat samples contained fewer fatty acids than blood and urine (14 in sweat, 25 in blood and 19 in urine). In the correlation analysis, there was a moderately positive, statistically significant ( $P < 0.01$ ) relationship between sweat fatty acids and blood fatty acids. A statistically significant ( $r = 0.855$ ,  $P = 0.000$ ) high correlation was found between blood and urine fatty acids. Regression analysis, there was a significant degree of positive association in the blood fatty acids, and sweat and urine fatty acids could explain 81% of the fluctuation in the blood. It was determined that there was a moderate correlation in urine fatty acids and that it could explain 79% of the changes in sweat fatty acids. It was determined that the changes in blood fatty acids were due to the changes in sweat and urine fatty acids. Therefore, it was concluded that blood and urine fatty acids in body fluids can be estimated by looking at sweat fatty acid levels.

**Key words:** Fatty acids; gas chromatography; sweat

### RESUMEN

En el metabolismo del ganado, los ácidos grasos son componentes biológicos básicos que satisfacen las necesidades energéticas del organismo y se utilizan en importantes procesos metabólicos. En este estudio, se tomaron muestras de sudor, orina y sangre de vacas y se determinaron los ácidos grasos de las muestras mediante cromatografía de gases. Se encontraron menos ácidos grasos en muestras de sudor que en muestras de sangre y orina (14 en el sudor, 25 en la sangre y 19 en la orina). En el análisis de correlación, hubo una relación moderadamente positiva y estadísticamente significativa ( $P < 0,01$ ) entre los ácidos grasos del sudor y los ácidos grasos de la sangre. Se encontró una alta correlación estadísticamente significativa ( $r = 0,855$ ,  $P = 0,000$ ) entre los ácidos grasos en sangre y orina. En el análisis de regresión, existe una relación positiva significativa entre los ácidos grasos en la sangre. Como resultado del análisis, se vio que el cambio de ácidos grasos en el sudor y la orina podría explicar el 81% del cambio en la sangre. También se determinó que existía una correlación moderada en los ácidos grasos de la orina. El cambio en los ácidos grasos de la orina podría explicar el 79% de los cambios en los ácidos grasos del sudor. Se determinó que los cambios en los ácidos grasos de la sangre estaban relacionados con los cambios en los ácidos grasos del sudor y la orina. Por lo tanto, se concluyó que los ácidos grasos en sangre y orina en los fluidos corporales se pueden estimar observando los niveles de ácidos grasos en el sudor.

**Palabras clave:** Ácidos grasos; cromatografía de gases; sudor

## INTRODUCTION

Cattle sweat has been the subject of studies for different purposes in the world in recent years. Researchers have reported that chemical changes occur in the blood, urine or milk of cattle, depending on clinical diseases and problems in the body, starting years ago [1]. This awareness, combined with the advances in technology that enables practical measurements at cow and herd levels through the tests carried out on the farm, has rapidly gained importance among dairy farm managers, veterinarians and other herd consultants [2, 3]. In addition to the diagnostic methods that were constantly used due to the discomfort caused by taking blood with needles for diagnosis and treatment in bovine, studies have been focused on developing alternative methods. One of these alternative methods was to determine the compounds and their proportions by performing sweat analysis. It constitutes an important advantage because the sweat of animals can be easily collected from the body surfaces and even mixed with molecules in the air [4]. Sweat in body fluids was in the less researched biological sample group [5].

Each animal has its unique body structure, nutrition, circumstances, breed, age, and illness status, which might change depending on body metabolism, however the majority of information obtained about body metabolism functioning was based on results in serum/blood metabolism and urine and fecal metabolism. However, the number of detailed studies on the metabolism of sweat in animals and their functions in the body, and in which situations the sweat-forming substances change, was very limited [6]. Therefore, in order to understand the health and disease states of animals, it was necessary to understand sweat metabolism and its relationship with other body fluids. In addition, examining many variables such as sweat formation, sweat components, functional roles and physical properties will provide useful information for animal health [7]. One of these parameters is the fatty acid ratios determined in animal sweat. Fatty acids (FAs) are biological molecules that are primarily used as metabolic fuels and are involved in important metabolic processes. Akbar *et al.* [8] stated that fats, fatty acids and metabolic products formed after the use of fatty acids in the body have important roles in metabolism. Among these duties of fatty acids were to create resistance to the stresses and damages that may come from external factors, to provide the necessary energy for the body, and being the precursor of hormone-like eicosanoid compounds such as thromboxane, leukotrienes and prostaglandins [9]. However, since the fluid excreted from the sweat gland was obtained from interstitial fluid, which was ultimately bound to plasma; it contains important information regarding events that occur directly within the body. Therefore, compounds in sweat were generally expected to contain clinical biomarkers detected in the blood. According to Nunome *et al.* [10] the fatty acids in human sweat were converted into sweat by blood as a result of the breakdown of these fatty acids in adipose tissue, particularly during stressful conditions. It was determined that the concentration of fatty acids in sweat is related to the increase in fatty acids in plasma [10]. Lack of studies on sweat fatty acid content, especially in cattle, reveals the deficit in this regard. In addition, the fatty acid composition determined in the sweat of farm animals will provide important information about the metabolism of the body [11].

This study was carried out in order to determine the concentration fatty acids of sweat, blood and urine samples, which were the body fluids of animals, by Gas Chromatography device and to correlate the concentration of sweat fatty acids corresponding to the concentration of blood and urine fatty acids in terms of cattle breeding.

## MATERIALS AND METHODS

The present study was conducted at a research and experimental farm located at Faculty of Agriculture, Çukurova University, Adana, Türkiye. This study was approved by Cukurova University Animal Experiments Local Ethics Board (Approval no: 26.02.2018/2). For the study, sweat, blood and urine samples were obtained from 6 Holstein cows. Healthy cows with similar characteristics were used (weight 600-670 kg (Tartimsan, Cattle Livestock Scale, Türkiye), body condition according to score 2.5-3, age 3-5 years, at least one delivery, no reproductive issues, 45-60 days postpartum). Based on anamnesis data and clinical examinations, the enterprise veterinarian concluded that none of the trial animals had any diseases. Sweat (10 mL tubes), urine (10 mL tubes) and blood samples (30 mL Teflon-lined septum) were obtained in accordance with animal welfare regulations without harming the animals. There were 160 dairy cows on the farm, 50 of which were healthy and had similar characteristics. The number of animals used has been determined by considering that the smallest sample representing this population is 5% with a 5% confidence level. Therefore, it was sufficient to sample 6 cows, since the number of dairy cows to be used in the study was determined to be 10% of the total number of cows (=50/0.10).

The experimental animals were milked twice a day using an automated milking system (Sezer Milking Machines, Çanakkale, Türkiye) at the central milking center. Cows were fed with a total mixture ration (TMR) with a concentrate: roughage ratio of 60:40. TMR consists of concentrate fed, alfalfa, wheat straw and corn silage (18% crude protein and 2650 kcal/metabolic energy (ME)/kg) and was given at 07.30 and 16.00.

Urine, blood and sweat samples were taken from experimental animals at certain rates and at specified periods, and analyzed by Gas Chromatography (Perkin-Elmer Clarus 590 Gas Chromatograph GC, USA) to detect fatty acids.

### Data collection and method

Urine samples were collected into 10 mL tubes by manual stimulation of the perineal areas of cows. For sweat samples, the animal's nose area was washed with water, then dried with a paper towel and the sweat collected on the nose area was taken into tubes. Sweat sample was placed in 10 mL tubes (Aromel, Konya, Türkiye) for analysis [7]. Blood samples Ahmad Shafi *et al.* [12] were taken as they stated. Blood samples were collected early in the morning. Samples were prepared by mixing a 10 mL aliquot of blood with 10% sodium citrate in a 30 mL injection bottle capped with a Teflon-lined septum (Obstitech ApS, Denmark). Sweat, urine, and blood samples were quickly frozen and stored at -80°C (Uğur UED 360 D-S F, Türkiye) until prepared for analysis in a Gas Chromatography device.

### Gas chromatography analysis

The method used by Bligh and Dyer [13] in their studies was applied for the analysis of lipid extraction from samples taken from animals in certain amounts. Methyl esters were prepared by Trans Methylation using 2 M KOH in methanol and n-hexane according to the method reported by Pollard and Shachar-Hill [14]. 10 mg of oil obtained from the analysis was dissolved in 2 mL of hexane, then 4 mL of 2 M methanol KOH was added. The tube was then vortexed (Ika vortex 3, IKA Turkey Laboratory and Process Technologies Inc., Türkiye) for 2 min at room temperature to mix the liquid in it thoroughly. After centrifuging (IndiaMART Laboratory Centrifuge Machine, Haryana, India) at 3075 G

Units (Force-Up 12V 3075 G Units, İstanbul, Türkiye) for 10 min, it was taken into the hexane layer for GC analysis. The samples were analyzed for fatty acid compositions with an auto sampler (Perkin Elmer, Clarus 500 GC, USA) GC Clarus 500 equipped with a flame ionization detector and a silica capillary SGE column (30 m × 0.32 mm, ID × 0.25 μm, BP20 0.25 μM, USA). After the injector and detector temperature were set to 220°C and 280°C, respectively, the oven temperature was kept at 140°C for 5 min. The oven temperature was raised to 200°C at a rate of 4°C·min<sup>-1</sup> and then to 220°C at a rate of 1°C·min<sup>-1</sup>. Samples (standard helium) were sized to 1 μL and carrier gas operated at 16 psi. Partition ratio of 1: 100 was used as separation. GC analysis was done twice on each sample while identifying the fatty acids, and the results were presented as the mean value and standard deviation by calculating the GC area. Fatty acids in sweat, urine and blood samples were determined by GC and the obtained parameters were analyzed using descriptive and inferential statistical methods. With correlation analysis and multiple regression analysis, it was tried to determine whether there was any relationship between the variables, and if there was, the direction, significance levels and degree of the relationship.

### Statistical analysis

The statistical analyzes were made in the SPSS 16.0 package program by determining fatty acids from sweat, urine and blood samples taken from animals. Following the application of analysis of variance (ANOVA) to the collected data, multiple comparison analysis was done using the *P*<0.05 significant threshold. Multiple regression model was under the effect of one dependent variable and more than one independent variable. In statistical theory, this multiple regression relationship was generally expressed as follows [15]:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \varepsilon$$

In the above equation, the dependent variable was determined by a linear combination of *Y*: *X*<sub>1</sub>, *X*<sub>2</sub>, ..., *X*<sub>*k*</sub> independent variables [16]. In the equation, *k*: the number of independent variables, *α*: the constant term, and *β*: the coefficients for the independent variables were used.

In our study, the variables of fatty acids found in sweat, urine and blood were used, and each variable was considered separately as a dependent variable and its relationship was determined by performing multiple regression analysis.

### RESULTS AND DISCUSSION

The body fluids of animals such as sweat, blood and urine fatty acids were determined by Gas Chromatography and their ratio (%) is shown in FIG 1 and TABLE I.

The fatty acids detected according to the analysis results of samples were shown in detail in TABLE I (14 in sweat, 25 in blood and 19 in urine). Although margaric acid, alpha linolenic acid, eicosadienoic acid, dihomogamma-linolenic acid, behenic acid and erucic acid were detected in blood and urine, they were not detected in sweat. Despite the fact that methyl pentadecanoate, heptadecenoic acid, vaccenic acid, linolenic acid, and lignoceric acid were found in blood, they were not found in sweat or urine. In addition, as a result of the analysis, the highest fatty acid content in animals was 24% palmitic acid and 17% myristic acid in sweat; 19% palmitic acid, 20% linoleic acid and 21% oleic acid in blood; In the urine, it was seen that 19% oleic acid and 14% linoleic acid. When the findings obtained in this study were examined, 14 fatty acids were detected in sweat, 25 in blood and 19 in urine, and it was seen that less fatty acids were detected in sweat compared to blood and urine (TABLE I).

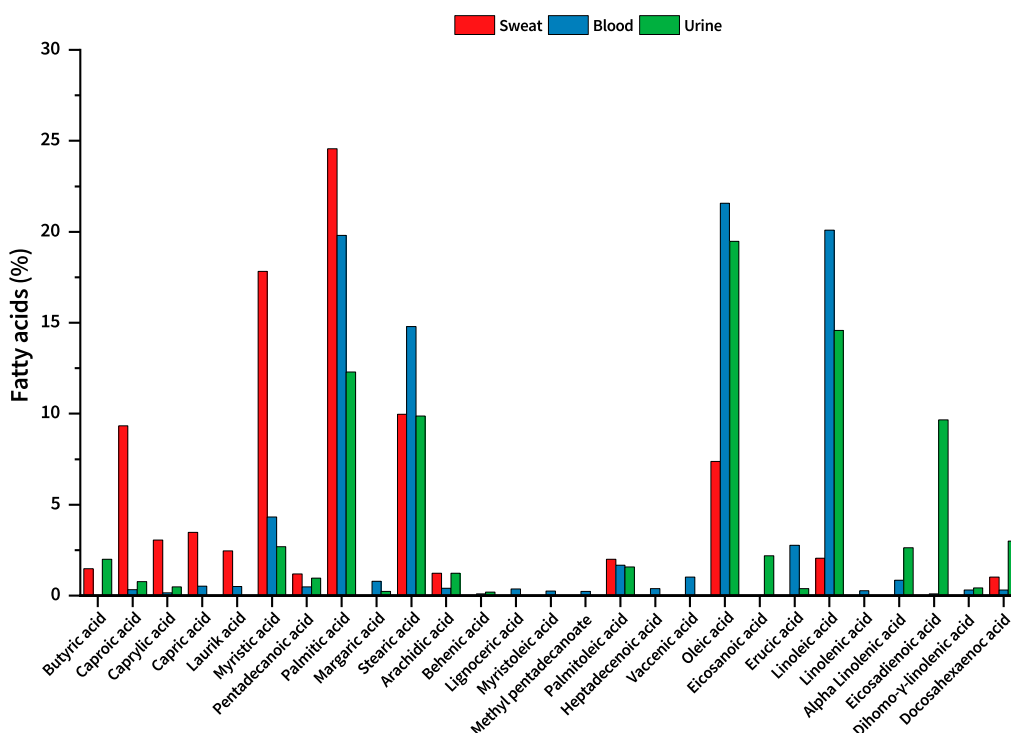


FIGURE 1. Comparison of sweat, blood and urine fatty acids of cows

**TABLE I**  
**Saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in sweat, blood and urine**

Fatty acids (%)	Carbon Number	Molecular formula	Molecular weight (g·mol <sup>-1</sup> )	Sweat (%)*	Blood (%)*	Urine (%)*
Butyric acid	C4:0	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	88.11	1.48 ± 0.42	ND**	2.00 ± 0.50
Caproic acid	C6:0	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116.15	9.34 ± 2.41	0.33 ± 0.15	0.77 ± 0.30
Caprylic acid	C8:0	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144.21	3.05 ± 1.33	0.15 ± 0.05	0.48 ± 0.08
Capric acid	C10:0	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172.26	3.48 ± 1.17	0.51 ± 0.11	ND
Lauric acid	C12:0	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.31	2.45 ± 0.65	0.49 ± 0.10	ND
Myristic acid	C14:0	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228.37	17.82 ± 2.97	4.33 ± 1.65	2.68 ± 0.83
Pentadecanoic acid	C15:0	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242.40	1.20 ± 0.13	0.48 ± 0.11	0.96 ± 0.14
Palmitic acid	C16:0	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42	24.57 ± 3.07	19.80 ± 4.28	12.29 ± 2.68
Margaric acid	C17:0	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45	ND	0.78 ± 0.05	0.24 ± 0.12
Stearic acid	C18:0	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	280.44	9.97 ± 1.72	14.78 ± 1.49	9.88 ± 2.10
Arachidic acid	C20:0	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.53	1.23 ± 0.05	0.40 ± 0.09	1.23 ± 0.38
Behenic acid	C22:0	C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>	340.58	ND	0.10 ± 0.05	0.20 ± 0.04
Lignoceric acid	C24:0	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	368.64	ND	0.37 ± 0.09	ND
<b>ΣSFA</b>				<b>74.59</b>	<b>42.52</b>	<b>30.73</b>
Myristoleic acid	C14:1	C <sub>14</sub> H <sub>26</sub> O <sub>2</sub>	226.36	ND	0.25 ± 0.04	ND
Methyl pentadecanoate	C15:1	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254.41	ND	0.24 ± 0.03	ND
Palmitoleic acid	C16:1	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254.41	2.00 ± 0.51	1.67 ± 0.46	1.57 ± 0.43
Heptadecenoic acid	C17:1	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268.44	ND	0.38 ± 0.05	ND
Vaccenic acid	C18:1n7c	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46	ND	1.02 ± 0.02	ND
Oleic acid	C18:1n9c	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46	7.38 ± 1.54	21.57 ± 4.15	19.47 ± 6.34
Eicosanoic acid	C20:1	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312.5304	ND	ND	2.18 ± 0.78
Erucic acid	C22:1	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338.60	ND	2.77 ± 0.08	0.38 ± 0.16
<b>ΣMUFA</b>				<b>9.38</b>	<b>27.90</b>	<b>23.60</b>
Linoleic acid	C18:2n6	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.44	2.06 ± 0.40	20.09 ± 7.36	14.57 ± 3.35
Linolenic acid	C18:3n6	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.43	ND	0.27 ± 0.11	
Alpha Linolenic acid	C18:3n3	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.43	ND	0.84 ± 0.48	2.63 ± 0.33
Eicosadienoic acid	C20:2n6	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308.50	ND	0.10 ± 0.06	9.67 ± 1.83
Dihomo-γ-linolenic acid	C20:3n6	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306.48	ND	0.30 ± 0.16	0.43 ± 0.02
Docosahexaenoic acid	C22:6n3	C <sub>22</sub> H <sub>32</sub> O <sub>2</sub>	328.48	1.02 ± 0.59	0.31 ± 0.13	2.99 ± 0.44
<b>ΣPUFA</b>				<b>3.08</b>	<b>21.91</b>	<b>30.29</b>
MUFA:SFA-1				0.13	0.66	0.77
PUFA:SFA-1				0.04	0.52	0.99
PUFA/MUFA				0.33	0.79	1.28
Σn6				2.06	20.76	24.67
Σn3				1.02	1.15	5.62
n6/n3				2.02	18.05	4.39

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

In FIG 2, comparison of sweat, blood and urine fatty acids (%) of cows; it was seen that saturated fatty acids values were high in sweat, low in urine, monounsaturated fatty acids values were high in blood, low in sweat, and polyunsaturated fatty acids values were high in urine and low in sweat.

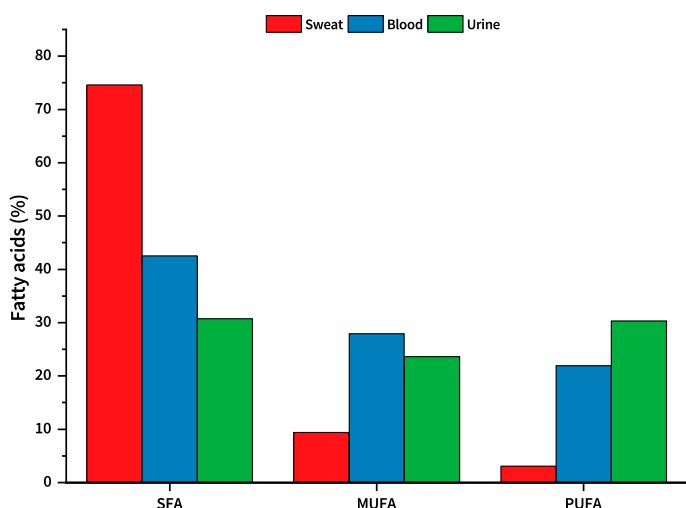


FIGURE 2. Comparison of sweat, blood and urine fatty acids SFA, MUFA and PUFA (%) of cow

The TABLE II shows the results of the correlation test between sweat fatty acids and blood and urine fatty acids of animals. The correlation between sweat, blood, and urine fatty acids was found to be statistically significant ( $P < 0.01$ ).

	n: Compound number	r	P-value
Sweat-blood	28	0.384**	0.008
Sweat-urine	28	0.236	0.133
Blood-urine	28	0.855**	0.000

\*\* : Correlation (r) is significant at the 0.01 level (2-tailed)

When the values in TABLE III are examined, the  $R^2$  value in the dependent variable, sweat, was calculated as 0.143 and the adjusted  $R^2 = 0.094$  and a positive relationship were found. The independent variables, urine and blood, explain 0.94% of the variation in sweat, which was the dependent variable. When blood was chosen as the dependent variable, the adjusted  $R^2 = 0.813$  was obtained, and it was said that there was a high level of positive correlation and that the independent variables, sweat and urine, could explain 81% of the variance in the dependent variable, blood. Furthermore, the adjusted  $R = 0.788$  in urine, the dependent variable, was found to be moderately correlated and could explain 79% of the variability in blood and sweat, the independent variables.

Dependent Variable	Independent Variable	R	$R^2$	Adjusted $R^2$	P-value
Sweat	Blood	0.379	0.143	0.094	0.067
	Urine				
Blood	Sweat	0.902	0.813	0.802	0.000
	Urine				
Urine	Sweat	0.894	0.799	0.788	0.000
	Blood				

TABLE IV displays the data values derived from the fatty acid regression analysis. When the dependent variable sweat was chosen, the constant term coefficient was calculated as 6,620 and the P value was stated as 0.001, and the constant term was found to be important in estimating the amount of sweat fatty acid content ( $P < 0.05$ ).

Dependent Variable	- Sweat		- Blood		- Urine			
	B	P	B	P	B	P		
Constant term	6.620	0.001	-550	0.630	1.496	0.84		
Blood	0.585	0.045	Urine	1.150	0.000	Sweat	-0.093	0.209
Urine	-0.483	0.209	Sweat	0.188	0.045	Blood	0.688	0.000

The lack of studies on fatty acid analysis in cow sweat does not allow comparison of these data. However, Klous *et al.* [17] stated in their study with human sweat that eccrine and apocrine sweat glands have different functions. They stated that sweat was produced in the secretory cells in these glands, that the necessary components of the sweat are reabsorbed as the produced sweat passes through the excretory channels, and the remaining liquid was secreted to the skin surface as sweat. When the values in TABLE I were examined, it was understood that the fatty acid content in cattle sweat was lower than the fatty acid content in blood and urine, and this finding was consistent with the study findings of Klous *et al.* [17].

The fatty acids with the greatest rate in the blood, according to TABLE I, were oleic acid (21.57%), linoleic acid (20.09%), palmitic acid (19.80%), and stearic acid (14.78%). Selionova *et al.* [18] stated that the compounds with the highest fatty acid ratio in the blood of cows were oleic acid 29.63%, palmitic acid 22.68%, stearic acid 20.33% and linoleic acid 18.34%. TABLE I shows that the proportions of other fatty acids, except linoleic acid, are lower than the findings of Selionova *et al.* [18].

In studies on fatty acids, they defined that linoleic acid (C18: 2 n6) and alpha linolenic acid (C18: 3 n3) were two essential fatty acids required for growth, structural health of the skin and reproduction in animals.

The researchers also stated that fatty acids are one of the most important structural components that make up cell membranes, and when fatty acids in cell membranes are included in phospholipids, they

are involved in processes such as permeability, flexibility, fluidity and activity of membrane-bound enzymes and signals occurring in the cell [19, 20]. Studies have shown that these fatty acids are found in the body in sufficient amounts; it was stated that it positively affects the functioning of red blood cells, immune cells [21], atherosclerotic plaques [22], heart tissue [23] and other cells in the body. When Table 1 is examined, linoleic acid, one of the important fatty acids, was found to be 2.06% in sweat, 20.09% in blood and 14.57% in urine. It was seen that linoleic acid is present in the blood at a rate of 0.27%, and this fatty acid was not detected in sweat and urine. The absence of linolenic acid in sweat and urine can be interpreted as an indicator of its significant level of use in body metabolism.

Studies have reported that polyunsaturated fatty acids (PUFA) positively affect the functioning of the reproductive system in animals [24]. In addition, some researchers have stated that PUFA supplementation to feeds increases the number of precursors for the synthesis of steroid hormones (estradiol, progesterone) and prostaglandins (PGF2 $\alpha$ ) and decreases mortality [25, 26]. Therefore, detecting changes in the composition of PUFA in body fluids and interpreting whether they were low for the body was important for body health. When TABLE I and FIG 2 was examined, it can be thought that the PUFA ratio was very high in urine and very low in sweat, that the body may form a defense mechanism to prevent PUFA deficiency.

Didara *et al.* [24] found SFA, MUFA, PUFA and n-6/n-3 values in the plasma fatty acid analysis of animals as 32.82, 13.11, 51.73 and 16.55%, respectively. In the current study, SFA and MUFA rates were determined to be higher than the rates determined by Klous *et al.* [17], and PUFA and n-6/n-3 rates were found to be lower. Selionova *et al.* [18] found the SFA rate in cow blood to be 46.07%, the MUFA rate to be 31.56%, and the PUFA rate to be 25.44%. The SFA, MUFA and PUFA ratios of the current study were compared with those of Selionova *et al.* [18]. It was detected at a lower rate than the findings of [18].

Looking at the results in TABLE II, it was clear that there was a weakly positive, statistically ( $P < 0.01$ ) significant connection ( $r = 0.384$ ,  $P = 0.008$ ) between the fatty acids found in sweat and blood. Furthermore, a significantly positive statistically significant connection ( $r = 0.855$ ,  $P = 0.000$ ) was discovered between blood and urine. The relation between sweat and urine was determined to be non-statistically significant ( $P < 0.01$ ) ( $r = 0.236$ ,  $P = 0.133$ ). In contrast to the findings in TABLE II, Nunome *et al.* [27] found in their study on human sweat fatty acids and blood fatty acids that there was a good association between sweat and blood fatty acids.

Anitaş and Göncü [28] reported a statistically significant ( $P < 0.01$ ) connection between fatty acids as a consequence of the correlation study they ran to establish the link between the fatty acid levels of milk, blood, urine, and feces. They also noted that there was a strong positive association between urine and blood, which was statistically significant ( $P < 0.01$ ). TABLE II revealed a high level of positive correlation between blood and urine, comparable to the findings of Anitaş and Göncü [28] and this connection was statistically significant ( $r = 0.855$ ,  $P = 0.000$ ).

Anitaş and Göncü [28] determined the dependent variable, the blood-corrected  $R^2$  rate, to be 0.835 and found a considerable level of positive relationship with other body fluids. They also reported that the independent variables milk, feces, and urine fatty acids could satisfy 83% of the change in the dependent variable blood fatty acids. They also observed that the dependent variable, the adjusted  $R^2$  in urine, was 0.613, indicating a moderate association and that

the independent variables milk, blood, and feces could account for 61% of the changes in urine fatty acids, which was the dependent variable. When TABLE III was examined, it was seen that in the multiple regression analysis, changes in blood were highly dependent on changes in sweat and urine, and changes in urine, changes in sweat and blood. Therefore, it could be said that blood can be predicted by looking at sweat and urine from body fluid samples to be made.

In the regression model of blood, the constant term coefficient was calculated as 0.585 and the  $P = 0.045$  value was found to be statistically significant ( $P < 0.05$ ). Since the regression model coefficient was positive, the rate of change in fatty acids in the blood has a directly proportional relationship with sweat. Fatty acid ratios detected in the blood have an important place in estimating the fatty acid ratios in sweat, which was a dependent variable.

The urine regression analysis was determined as  $-0.483$  and  $P = 0.209$ , and it was found that the rate of change had an inversely proportional relationship with sweat, and the urine was statistically insignificant ( $P < 0.05$ ) in the prediction of sweat and did not help a lot with estimation.

Regression model coefficient of blood when blood fatty acids were in the urine,  $B = 1.150$  and  $P = 0.00$ , in sweat,  $B = 0.188$  and  $P = 0.045$ , and the rate of fatty acids in the blood was directly proportional to urine and sweat, and the relationship was statistically significant ( $P = 0.045$ ,  $P < 0.05$ ). In other words, it was seen that the fatty acids found in urine and sweat significantly contribute to the model in predicting the blood fatty acids ratio, which was the dependent variable (TABLE IV).

If urine was used as the dependent variable, the association between sweat and blood appeared to be negligible ( $P < 0.05$ ). The regression analysis of blood fatty acids yielded  $B = 0.688$  and  $P = 0.000$ , indicating that the ratio of fatty acids in the blood was directly related to the ratio of fatty acids in the urine. The association between urine and blood fatty acids was determined to be significant ( $P < 0.05$ ) as a consequence of the investigation. In other words, fatty acids in urine considerably help the model predict the blood fatty acids ratio, which was the dependent variable. The analytical results reported in TABLE IV were found to be identical to the study results of Anitaş and Göncü [28].

## CONCLUSION

As a result of this study conducted to examine the relationships between bovine sweat fatty acids and blood and urine, a smaller number and proportion of fatty acids (14 in sweat, 25 in blood and 19 in urine) were detected in sweat compared to blood and urine. As a result of the correlation analysis, a significant positive relationship was found between sweat fatty acids and blood. It was also determined that there was a statistically high level of relationship between blood and urine. According to the results of the regression analysis, a significant positive relationship was found in blood. As a result of this relationship, it was determined that the independent variables sweat and urine could explain 81% of the variance in the dependent variable blood. In addition, it was concluded that there is a moderate correlation in the dependent variable, urine, and it can explain 79% of the variations in the independent variable, blood and sweat. The content of fatty acids in the blood can be estimated using correlation and regression analysis, but it is advisable to conduct a statistical study on a larger number of samples, and for this it is necessary to establish a formula with a high degree of precision.

**Ethical statement**

This study was approved by the Cukurova University Animal Experiments Local Ethics Board.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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