A novel approach for evaluating of goat milk quality: canonical correlation analysis between major milk composition parameters and fatty acid components in Damascus goats

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SUMMARY

The aim of this study was to investigate the effects of major milk composition parameters (lactose, protein and fat) and somatic cell score (SCS) on the variation of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), omega 6 (ω6), omega 3 (ω3), odour index (OI) and nutritive value (NV) of goat milk in the first month of the lactation. For this aim, 120 milk samples were collected in different times post-partum (0, 4, 7, 14 and 28 days) from 24 Damascus goats. Composition parameters and fatty acid components of milk samples were determined. Pearson correlation coefficient and canonical correlation analysis (CCA) were used to evaluate the relationships between major milk parameters and fatty acids components. In addition to positive correlations between major milk parameters, significant and variable correlations were found between major milk composition parameters and fatty acid components according to the Pearson Correlation coefficient. Most of the fatty acids components were also correlated with each other. However, only the first of four calculated pairs of canonical variables was found to be significant, strongly (r_c= 0.857, P<0.001). According to the calculations of canonical loading and cross-loading results, protein and lactose from the set of independent variables and OI and NV from the set of dependent variables were found to be the most important variables. On the other hand, redundancy indexes results showed that the fatty acid contents may be explained by the milk components in goat milk. CCA results has revealed that multidimensional evaluation of measurements in dependent and independent variable sets can provide to researchers with important and innovative information for assessment of milk quality. Considering the multiple relationships with a multivariate approach, strong and significant relations were found for the first time in terms of major milk quality parameters in goats.

KEY WORDS

Canonical correlation; Damascus goat; Goat milk; Milk composition; Milk fatty acid profile.

INTRODUCTION

Animals' products are the main components of human diets. Goat, one of the oldest domesticated animal, is an important source of animal food, particularly for milk and dairy products[1]. Furthermore, goat milk is commonly chosen by people with digestive system problems or allergies. It is greater in carbohydrate, fat and protein percentages compared to cow's milk. Moreover, it is richer in short and medium-length chain fatty acids[2]. Composition and fatty acid content of milk influence significantly the nutritional value and quality of milk[3]. Furthermore, they are important for monitoring the mammary gland's status and the health of goat kids. For these reasons, they are important features in animal management systems[2]. In addition, relationships between composition and fatty acids components of milk have critical importance for livestock. In this way, it has been aimed to determine the relationship between the fatty acid components of milk, which is more difficult to determine due to application difficulties and expensiveness, and the composition parameters that are easily determined[4]. The simple correlation coefficient is generally used to determine these relationships[5,6]. Although correlation analysis evaluates the strength and direction of the relationships between measurements, it remains insufficient to detect latent or indirect relations[7]. The multivariate statistical methods of canonical correlation analysis (CCA) can be used to successfully explain these relationships. CCA explains the relationship by calculating linear combinations between two data sets. In addition, CCA can produce structural and spatial inferences by handling two data sets simultaneously[8].

The aim of this study was to investigate the effects of major milk composition parameters (somatic cell count, lactose, protein and fat) on the variation in milk fatty acids profile in the first month of the lactation stage (early lactation stage, ELS) with CCA in goat milk.

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MATERIALS AND METHODS

Study design, animals and sample collection

All procedures and practices complied with The Regulation on the Studying Procedures and Principles of Animal Experiments of Ethics Committees of the Ministry of Agriculture and Forestry (2014, Turkey). The study was conducted in a farm located at 36° 21′ 52.6″ N and 36° 15′ 14.6″ E at an altitude of 82 m above sea level in Hatay, Turkey. The enrolled goats aged 3-4 years were in their 2^{nd}-3^{rd} lactation periods. In addition, all enrolled goats presented a singleton birth. The average of lactation period was maintained approximately 215 days. For this study, milk samples were collected from 24 healthy Damascus goats randomly selected from 200 goats for five times after parturition (0^{th}, 4^{th}, 7^{th}, 14^{th}, and 28^{th} days). The health status was evaluated by veterinarians. Animals were fed with 1.2 kg/goat concentrate feed (88.90% dry matter, 16.50% crude protein, and 2649 kcal/kg total ME) and 1.0 kg/goat wheat straw. 50 ml of milk for each animal were collected to sterile falcon tubes at morning milk and brought to laboratory in 30 min in the cold box (4 °C) at all experimental times. The samples were homogenized by manual mixing and divided into two aliquots. A total of 120 milk samples were collected. Before sampling, goats' udders were washed with alcohol-based disinfectant and cleaned with water and sterile cotton gauze swabs.

Determination of milk composition

All measurements were performed in the laboratory of genetics department (Faculty of Veterinary Medicine, Hatay Mustafa Kemal University). The compositional parameters of milk (fat, protein and lactose) samples were measured with milk analyzer (Milkotester Master Classic LM2-P1, BULGARIA) while somatic cell count (SCC) was determined with somatic cell counter (Lactoscan SCC 6010, BULGARIA). Obtained SCC data were normalized and transformed into Somatic Cell Score (SCS=log2(SCC/100.000) + 3)[9]. Somatic cell count and composition of milk were determined in validated devices.

Cream layer collection and fatty acids evaluation

All measurements for fatty acids were performed in an accredited laboratory (Technology and Research and Development Center, Hatay Mustafa Kemal University). Milk samples were centrifuged (NF800R, NUVE, TURKEY) at +4 °C at 1800 xg for 15 min for cream layer collection. Thereafter, the samples were kept at -20 °C for 15 min and the cream layers were collected into 1.5 mL volume sterile tubes. After, about 500 µL cream layers was added 2 mL 2N methanolic and waited for 4 min at room temperature. Following the incubation, 4 ml n-Heptane was added and samples were incubated at room temperature for 2 min. The samples were then centrifuged for 5 min at 200 xg and the aqua phases containing methyl esters were transferred to 1.5 mL vials.

Individual fatty acids of milk samples were identified using a gas chromatograph (GC-2025, Shimadzu, JAPAN) equipped with flame ionization detector. Separation was performed with a Restek Rt-2560 column (100 m length, 0.25 mm internal diameter x 0. 20 µm film thickness). After injection at 100 °C (held for 2 min), the oven temperature was raised to 250 °C at a rate 4 °C/min and finally held constant for 15 min. The flame ionization and injector temperatures were set at 250 °C with the following gas flow: carrier gas hydrogen: 40 mL/min, instrument air: 400 mL/min. The hydrogen flow rate was 1.2 mL/min, the split ratio was 1:50 and injection volume was set to 1 L. FAME peaks were identified by comparing their retention times with those of Restek FAME Mix Standard. While ω6 and ω3 percentages were calculated using the fatty acids in the formulas as C18:2 trans+C18:2 cis+C18:3 ω6+C20:2 ω6+C20:4 ω6+C22:2 ω6 and C18:3 ω3+C20:3 ω3+C20:5 ω3+C22:6 ω3, respectively, OI percentage and NV were calculated using the fatty acids in the formulas as C4:0+C6:0+C8:0+C10:0 and (C18:0+C18:1 ω9)/C16:0, respectively (Table 1).

Table 1 - Fatty acid related parameters.

Parameters	Formulas of parameters*
SFA	C4:0+C6:0+C8:0+C10:0+C12:0+C14:0+C15:0+ C16:0+C17:0+C18:0+C20:0+C22:0
MUFA	C14:1 ω5+C15:1 ω7+C16:1 ω7+C17:1 ω7+C18:1 ω9+C20:1 ω9+C24:1 ω9
ω6	C18:2 trans+C18:2 cis+C18:3 ω6+C20:2 ω6+C20:4 ω6+C22:2 ω6
ω3	C18:3 ω3+C20:3 ω3+C20:5 ω3+C22:6 ω3
PUFA	ω6+ω3
UFA	MUFA+PUFA
OI	C4:0+C6:0+C8:0+C10:0
NV	(C18:0+C18:1 ω9)/C16:0

SFA: Saturated fatty acids; **MUFA**: Monounsaturated fatty acids; **PUFA**: Polyunsaturated fatty acids; **UFA**: Unsaturated fatty acids; **ω6**: Omega 6; **ω3**: Omega 3; **OI**: Odour index; **NV**: Nutritive value; *: [2, 28].

Statistical analysis

Descriptive statistics, Pearson correlation coefficient and CCA were performed using the IBM SPSS Statistics software Version 23.0. Descriptive statistics of variables were calculated before statistical analysis. The variables included in the study were checked in terms of normality assumption by Kolmogorov-Smirnov test and in terms of the homogeneity of variance assumption by Levene test. The relationship between major milk composition parameters (SCS, fat, protein and lactose) and fatty acid components (SFA, MUFA, PUFA, UFA, ω6, ω3, OI, NV) was determined using the Pearson correlation coefficient. Before canonical correlation analysis, variables were evaluated in terms of multicollinearity problem. Accordingly, MUFA and UFA were excluded from the study due to their high Variance Inflation Factor (VIF) values (VIF>10). Then, the CCA was used to evaluate the relationships between the independent set (X) of the milk composition parameters (SCS, milk fat, protein and lactose) and the dependent set (Y) of the fatty acids' components (SFA, PUFA, ω6, ω3, OI and NV). With this analysis, the relations between variables (milk composition parameters) in a set and variables (fatty acid components) in another set can be examined.

The F values of the canonical correlation coefficients were examined using Wilk's Lambda values from the significance tests. According to CCA, canonical variables were created with linear combinations of these variables. Canonical variable pairs (U and V) were calculated. How much of the variance in one data set is explained by the other data set was calculated with the redundancy index. Significance level was accepted as $P<0.05$.

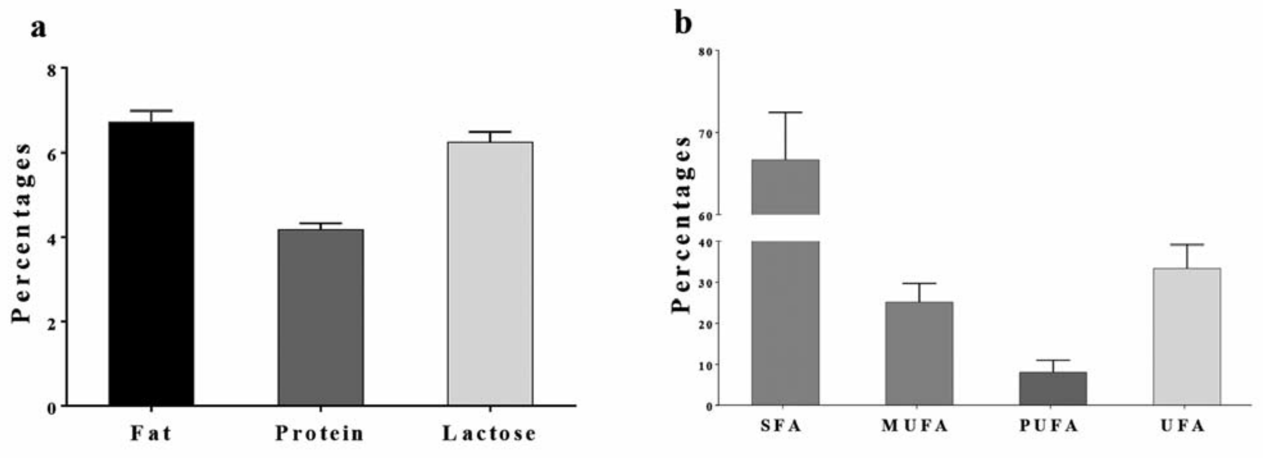

Figure 1 - The major composition parameters **(a)** and fatty acid components **(b)** (Mean ± SEM).

Figure 1a: major composition parameters; **Figure 1b:** fatty acid components; **SFA**: Saturated Fatty Acids, **MUFA**: Monounsaturated fatty acids, **PUFA**: Polyunsaturated fatty acids, **UFA**: Unsaturated fatty acids.

RESULTS

The major milk composition parameters and fatty acids components were summarized in Figure 1. SCS average was 11.83 ± 0.26 (SCC: 1873 x 10^3/mL). The sum of n6 and n3 values (%) were 6.66 and 1.56, respectively. The OI (%) and NV were also determined as 9.52 ± 0.44 and 1.66 ± 0.03, respectively.

Relations of milk composition parameters and fatty acid components were determined by Pearson correlation coefficient. Positive correlations were found between SCS and fat, protein and lactose. In addition, lactose and protein were positively correlated. While SFA and OI had a positive relation, a negative relation was found with PUFA, ω6, ω3 and NV. Positive correlations were determined between PUFA and ω6, ω3 and NV variables and between ω6 and ω3 and NV variables. A negative correlation was determined between OI and NV. PUFA, ω6, ω3 and OI in the dependent set Y were negatively correlated with milk fat. On the other hand, positive correlations were found between MUFA and protein and lactose. Finally, negative correlations were found between OI and SCS, fat, protein and lactose parameters (Figure 2).

According to CCA results, four different pairs of canonical variables and four different canonical correlation coefficients were obtained from the set X and set Y (Table 2). It was determined that only the first canonical correlation coefficient (0.857) calculated from the first canonical variable pair was significant (P<0.001). This result showed that there were significant relationships between linear combinations of major milk com-

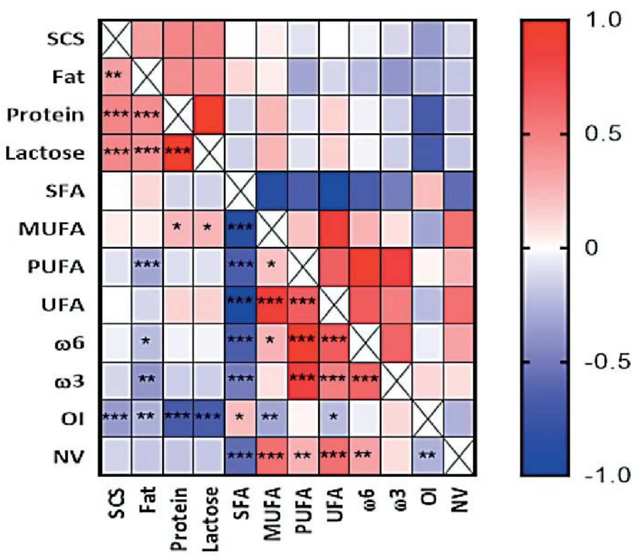

Figure 2 - Heatmap showing Pearson correlations between major composition parameters and fatty acids components.

SCS: Somatic cell score, **SFA**: Saturated fatty acids, **MUFA**: Monounsaturated fatty acids, **PUFA**: Polyunsaturated fatty acids, **UFA**: Unsaturated fatty acids, **ω6**: Omega 6, **ω3**: Omega 3, **OI**: Odour Index, **NV**: Nutritive Value; *P < 0.05; **P < 0.01; ***P < 0.001.

Table 2 - Results of canonical correlation analysis.

Canonical variables	Canonical correlation	Canonical R^2	Eigenvalue	F test	Wilks' Lambda	P
1	0.857	0.735	2.770	6.74	0.220	< 0.001
2	0.352	0.124	0.142	1.11	0.830	0.353
3	0.165	0.027	0.028	0.587	0.948	0.787
4	0.159	0.025	0.026	0.763	0.975	0.518

position parameters and fatty acids components. Canonical correlation coefficients calculated with other canonical variable pairs were found to be non-significant.

The linear components of the first canonical variable (U and V) were presented in Table 3. According to the results, the model was obtained through with standardized canonical coefficients as U_1 = - 0.052 (SCS) - 0.061 (Fat) + 1.638 (Lactose) - 2.567 (Protein) and V_1 = 0.449 (SFA) + 2.407 (PUFA) - 1.639 (ω6) - 0.726 (ω3) + 0.851 (OI) + 0.695 (NV).

Table 3 - Standardized Coefficients of the First Canonical Variables for set X and set Y.

Independent variables (X)	U_1	Dependent variables (Y)	V_1
SCS	-0.052	SFA	0.449
Fat	-0.061	PUFA	2.407
Lactose	1.638	ω6	-1.639
Protein	-2.567	ω3	-0.726
	OI	0.851	
	NV	0.695	

SCS: Somatic cell score, **SFA**: Saturated fatty acids, **PUFA**: Polyunsaturated fatty acids, **ω6**: Omega 6, **ω3**: Omega 3, **OI**: Odour Index, **NV**: Nutritive Value.

Table 4 - Canonical loading and cross-loading for the first canonical variables.

	Canonical loadings	Canonical cross-loadings
Independent variables (X)		
SCS	-0.531	-0.455
Fat	-0.493	-0.422
Lactose	-0.969	-0.831
Protein	-0.985	-0.845
Dependent variables (Y)		
SFA	0.119	0.102
PUFA	0.151	0.129
ω6	0.069	0.059
ω3	0.226	0.194
OI	0.815	0.698
NV	0.242	0.207

SCS: Somatic cell score, **SFA**: Saturated fatty acids, **PUFA**: Polyunsaturated fatty acids, **ω6**: Omega 6, **ω3**: Omega 3, **OI**: Odour Index, **NV**: Nutritive Value.

Table 5 - Result of redundancy analysis.

Canonical variables	Variable Set	Explained by canonical variables	Explained variation
1	X	U	60.9
1	X	V	44.8
1	Y	U	10.0
1	Y	V	13.6

Canonical loadings and canonical cross-loadings between the canonical variables and the original variables within their own sets were presented in Table 4. It was determined that the largest contribution to the canonical variable U_1 was provided by protein with the largest canonical loading (-0.985), followed by lactose (-0.969), SCS (-0.531) and fat (-0.493), respectively. In addition, OI (0.815) made the heaviest contribution to the canonical variable V_1. In canonical cross-loadings, protein and OI also displayed higher weights than other variables.

The total variances explained by canonical variables were determined by redundancy analysis (Table 5). 60.9% of the total variation in the X variable set was explained by U_1 and 44.8% by V_1. Otherwise, 10.0% of the total variation in the Y variable set was explained by U_1 and 13.6% by V_1.

DISCUSSION

The lactation stage in goats varies depending on many factors such as breeds, season, and management. However, it is possible to separate lactation into three stage: early, mid and late lactation[1, 10]. SCS strongly reflects mammary physiology and health of animal. According to obtained results, it has been found to be high as expected. Because, colostrum and transition milk have quietly high SCC[2]. Different studies reported a higher SCS value in goat milk during ELS, with different levels according to breed[2, 6, 11, 12]. Particularly, Damascus goats showed variable SCS in ELS between 31 to 9089 x 10^3 / ml[13, 14]. Since SCC in goats is variable and related with mammary infections, Koop et al. (2010) reported that bacteriological examination or milk yield measurements were necessary for the detection of healthy goats that were not subclinical mastitis. Even if the yield or bacterial status of milk are valuable markers for subclinical mastitis, milk and colostrum were presented to kids ad libitum for nutrition in ELS and therefore milk yield could not be measured in this study.

In addition, although the animals were determined to be physiologically healthy, it was thought that the lack of bacterial examination in the milk was a factor to be taken into account. According to Pearson correlation coefficient, SCS has positively correlated with major milk composition parameters (fat, protein and lactose). Studies have been showed that there are controversial relationships between SCS and major milk composition parameters depending on factors such as birth season and stage of lactation[15, 16].

In our study, the major composition parameters of goat milk during ELS showed differences compared to animals reported in different studies[12, 17]. However, significant interactions were detected between major composition parameters of milk (fat, protein and lactose) as reported[5, 18]. While El-Tarabany et al. (2019) found that lactose presented a negative relationship with protein, and no relationship with fat, positive correlations were found between lactose, protein, and fat in our study. Although these differences are mainly due to the breed type, they are significantly influenced by environmental factors such as season and ration[6].

Fatty acids components of milk are remarkable for milk quality as well as major components that are the basic elements of milk composition[19]. Recently, the increased concern of food quality led to focus on fatty acids components of milk[20]. In this study, significant and remarkable correlations were found between fatty acids components as other studies[6, 21]. These correlations showed that the quality of milk should be assessed with SFA, MUFA and UFA contents of milk. NV and OI indices (Table 1) used to evaluate fatty acids are very important due to their nutritional value and composition of desirable fatty acids (such as C4:0, C18:0, and C18:1 ω9) because of human health[22, 23]. Different studies in goat milk showed short chain fatty acids and C16:0 are lower, and C18:0 and C18:1 are higher in ELS compared to other stages[10, 22]. Moreover, C16:0 which is one of the fatty acids used in calculation of NV increase in metabolic disorders such as subclinical ketosis. This condition adversely affects milk quality by decreasing in NV[23]. It has been clearly understood that the negative relations of NV and OI has potent effect on the milk quality in ELS, although the fatty acids (shorter and longer fatty acids) used in the calculation of these parameters have been different.

Variable interactions were found between major composition parameters and fatty acids. PUFA, ω6, and ω3, were negatively correlated with milk fat. It was reported in ruminants that the relationships between milk fat and these fatty acid parameters were similar[24]. In another study, PUFA, ω6 and ω3 were found to be high in animals with low milk fat[25]. This situation confirms that milk fat presented negative relationships with PUFA, ω6 and ω3 in our study. OI showed a negative correlation with all major component parameters and SCS as expected. High SCS in ELS decreases the synthesis of short chain fatty acids by affecting secretory activity in the udder[2, 26].

On the other hand, MUFA was positively correlated with protein and lactose. Complex molecular mechanisms play a role in the formation of quantitative characters such as milk yield and milk quality in ruminants. It is thought that these relationships in ELS may be regulated by complex and interrelated mechanisms[2, 6].

However, a univariate statistical approach is insufficient to consider multiple relationships between these variables. On the other hand, CCA allows examining the effects between different variables' groups while preserving the relationships between the original variables in each group[27]. Considering the multiple relationships with a multivariate approach used in this study, strong and significant relationships were found between major composition parameters and fatty acids components in terms of quality parameters in goat milk during ELS.

Lactose, protein and OI had the heaviest weight in the analysis of data sets through CCA. Lactose and protein are necessary for the feeding the newborns, because the energy source can be digested faster than fat[28]. It was reported that OI tended to decrease from the beginning to mid lactation stage, and to increase in the last lactation stage[1, 10]. The short-chain fatty acids are related to the nutrition and growing of the offspring due to the immaturity of digestive systems and immunity[2]. The NV was constituted by the ratio between C18:0 and C18:1 with C16:0 fatty acids. The C18:0 and C18:1 concentrations increased in milk during ELS, while C16:0 concentration decreased respectively. Despite NV did not show an important weight, it is one of the main factors for the quality of milk[2, 29]. According to CCA, protein, lactose, OI, and NV of milk might be said to be the most influential variables in terms of both dependent (fatty acids components) and independent (major composition parameters) variables. However, canonical loadings should be considered as a set, since they have been obtained through linear combinations of variables.

As known, the quality parameters of goat milk in ELS can be explained by the effects of many dependent and independent variables. Therefore, both Pearson correlation analysis that examines cases in one dimension and CCA, that examines cases in multidimensional were preferred in this study. The relationships between major composition parameters and fatty acids components in goat milk in ELS were evaluated separately with Pearson correlation coefficient, while the effects of all parameters in independent and dependent variable sets were examined with CCA.

In addition, the parameters that contributed the most to the correlation between data sets and how much the dependent variables could be explained by the independent variables were determined by CCA in this study.

In general, it has been shown with explained variance ratios calculated with the redundancy index (Table 5) in our study that the change in the fatty acid profile in ELS may be explained by some of the quality parameters of milk. In addition, researchers have stated that the explained variation in parameters are important[7, 18].

Although there is no acceptable general guideline for the redundancy index that is used in the calculation of the explained variance ratios, it could provide important information to the researcher even if the variations are low[30].

CONCLUSION

CCA might be considered as a convenient method to evaluate the relationships between data sets and to identify influencing factors. Negative relationships were obtained between major milk composition parameters (independent variable set) and milk fatty acids components (dependent variable set) in goat's milk during ELS. Goat milk that is a quality food due to its macronutrients (protein, carbohydrate and fat) and essential fatty acids in human nutrition is important in terms of nutritional value and health. Although the first 28-days milk quality parameters of Damascus goats seem to be acceptable in terms of human nutrition, it has deduced that milk is actually more necessary for the goat kids because of the health and development.

In conclusion, information about independent data set may estimate milk fatty acids, considering that the analysis of the dependent data set is more difficult and expensive. To the best of our knowledge, this is the first study that attempts to reveal the relationship between major milk composition parameters and fatty acid components with CCA in ELS milk of Damascus goats. The outputs of this study clearly indicate that more studies are needed to examine the relationships between milk composition and fatty acids components in different breeds of goats and even different livestock animals.

DECLARATION OF CONFLICTING INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) with the 119O716 project number.

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