Vet Hekim Der Derg 94 (2): 119-126, 2023

VETERİNER HEKİMLER DERNEĞİ DERGİSİ

JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası /*Journal homepage:*
http://dergipark.org.tr/vetheder

OPEN ACCESS

10.33188/ vetheder.1206837

Araştırma Makalesi / Research Article

Determination of milk fatty acids and some phenotypic characters affecting total milk fat in dairy cows with multiple linear regression

Ufuk KAYA [1,a*], ***Hüseyin ÖZKAN*** [2,b], ***Murat Onur YAZLIK*** [3,c], ***Güven GÜNGÖR*** [4,d], ***Baran ÇAMDEVİREN*** [5,e], ***İrem KARAASLAN*** [6,f], ***Sevda DALKIRAN*** [5,g], ***Hasan Hüseyin KEÇELİ*** [2,h], ***Aytaç AKÇAY*** [7,i], ***Akın YAKAN*** [2,j]

[1]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Biostatistics, Hatay, Turkey [2]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Genetics, Hatay, Turkey [3]Ankara University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey [4]Erciyes University, Faculty of Veterinary Medicine, Department of Biometrics, Kayseri, Turkey [5]Hatay Mustafa Kemal University, Institute of Health Sciences, Department of Molecular Biochemistry and Genetics, Hatay, Turkey [6]Hatay Mustafa Kemal University, Technology and Research & Development Center (MARGEM), Hatay, Turkey [7]Ankara University, Faculty of Veterinary Medicine, Department of Biostatistics, Ankara, Turkey

0000-0002-4805-0993 [a]; 0000-0001-5753-8985 [b]; 0000-0002-0039-5597 [c]; 0000-0003-3695-9443 [d];0000-0003-1508-7869 [e];0000-0002-7485-192X [f]; 0000-0002-5704-5774 [g]; 0000-0002-4017-8765 [h]; 0000-0001-6263-5181 [i]; 0000-0002-9248-828X [j]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
18 Kasım 22
18 November 22
Revizyon/Revised:
25 Mart 23
25 March 23
Kabul / Accepted:
28 Mart 23
28 March 23
Keywords:
Dairy cattle
Milk fat
Milk fatty acids
Pregnancy
Regression
Anahtar Sözcükler:
Süt sığırı
Süt yağı
Süt yağ asitleri
Gebelik
Regresyon

ABSTRACT:

This study aimed to determine the effect of milk fatty acid composition, breed, and pregnancy status on total milk fat in dairy cows. The study was conducted with a total of 400 milk samples collected form healthy Holstein and Simmental cows. Milk samples were collected for total milk fat and fatty acid analysis. To investigate the effects of milk fatty acids, breed and pregnancy status on total milk fat, multiple linear regression analysis was performed. As a result of the analysis, breed, pregnancy status, C11:0, C14:0, C18:0, C18:1 ω9 and C18:3 ω6 were found to be statistically significant ($p<0.05$), while the C18:3 ω3 was nearly significant ($p=0.069$) on total milk fat. Also, Holstein cows, C11:0 and C18:0 were found to have a positive effect, while pregnant cows, C14:0, C18:1 ω9, C18:3 ω3, and C18:3 ω6 had a negative effect on total milk fat. The multiple explanatory coefficient of the regression model (R2) was 0.238. In the light of all these findings, it was thought that the established regression model was sufficient to determine the variables that affect total milk fat. Moreover, it is suggested that the regression model can be improved further by adding different variables in future studies.

Sütçü ineklerde toplam süt yağı üzerine etkisi olan yağ asitleri ve bazı fenotipik karakterlerin çoklu doğrusal regresyon ile belirlenmesi

ÖZET:

Bu çalışma, süt ineklerinde süt yağ asitleri, ırk ve gebelik durumunun toplam süt yağına etkisini incelemeyi amaçlamıştır. Çalışma sağlıklı Holştayn ve Simental ineklerden toplanan toplam 400 adet süt örneği ile yürütülmüştür. Süt örnekleri, toplam süt yağı ve yağ asidi analizi için toplanmıştır. Süt yağ asitlerinin, ırkın ve gebelik durumunun toplam süt yağı üzerindeki etkilerini araştırmak için çoklu doğrusal regresyon analizi yapılmıştır. Analiz sonucunda toplam süt yağı üzerine ırk, gebelik durumu, C11:0, C14:0, C18:0, C18:1 ω9 ve C18:3 ω6 istatistiksel olarak anlamlı bulunurken ($p<0,05$), C18:3 ω3 neredeyse anlamlıydı ($p=0,069$). Ayrıca, toplam süt yağı üzerine Holştayn inekler, C11:0 ve C18:0 pozitif bir etkiye sahip bulunmuşken, gebe inekler, C14:0, C18:1 ω9, C18:3 ω3 ve C18:3 ω6 negatif bir etkiye sahipti. Regresyon modelinin çoklu açıklayıcılık katsayısı (R2) 0,238'dir. Tüm bu bulgular ışığında kurulan regresyon modelinin toplam süt yağını etkileyen değişkenleri tespit etmede yeterli olduğu düşünülmüştür. Ayrıca ileriki çalışmalarda farklı değişkenler eklenerek regresyon modelinin daha da geliştirilebileceği önerilmektedir.

How to cite this article: Kaya U, Özkan H, Yazlık MO, Güngör G et al. Determination of milk fatty acids and some phenotypic characters affecting total milk fat in dairy cows with multiple linear regression. Vet Hekim Der Derg 94(2):119-126, 2023. DOI: 10.33188/ vetheder.1206837

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* u.kaya@mku.edu.tr

1. Introduction

Milk is an important animal product because of its nutritional values and positive effects on health. More than 90% of milk production in Turkey is obtained from dairy cattle and mostly Holstein and Simmental breeds (1). Holstein cows, which are susceptible to diseases but have a high milk yield, are known as the most widely raised breed in the world (2,3). Simmental cows, which are highly adaptable, resistance to diseases and frequently preferred in breeding studies, are a combined yield-oriented breed (2-4). In dairy cattle enterprises, milk revenues should cover feed expenses. Thus, milk revenue is crucial factor for profitability. Milk composition and milk quality are effective factors on milk revenue. Also, milk composition and milk quality has become an important factor with the advancement of technology. Fatty acid profile and fat percentages of milk have increasingly evaluated as quality indicators in terms of human health as well as animal husbandry and dairy industry (5,6). It is known that fatty acids have benefits such as preventing coronary heart diseases and reducing cholesterol in terms of human health (7). In addition, it has known that these indicators are affected by environmental factors and phenotypic characters.

Multivariate statistical methods are frequently applied to the data obtained from studies in the field of animal husbandry (8). Multiple linear regression, which is one of multivariate statistical methods, is a method that makes predictions between a dependent variable and multiple independent variables by establishing a relationship with mathematical models. In addition, predictions can be made using the mathematical model created with the analysis (9).

It was aimed to investigate the fatty acids in milk that are affected on total milk fat of cows with multiple linear regression analysis in this study. Moreover, the effects of breed (Holstein-Simmental cows) and pregnancy status (pregnant-non-pregnant) were also investigated.

2. Material and Methods

Milk samples were collected in accordance with the “Regulation on Studying Procedures and Principles of Animal Experiments of Ethics Committees” of the Ministry of Agriculture and Forestry (2014, Republic of Turkey) and the regulations of Animal Experiments Local Ethics Committees of Hatay Mustafa Kemal University. In addition, required approvals were obtained from the enterprise.

The study material consisted of milk samples that were collected from Holstein (n=269) and Simmental (n=131) cows in a private dairy enterprise in Kayseri, Turkey. In addition, the pregnancy status of these animals (pregnant n=128 and non-pregnant n=272) were also recorded from the enterprise record system (AfiMilk, Afkim, Israel). The cows included in the study were housed in freestalls and fed TMR with 57.8% dry matter and 17.8% crude protein under similar environmental conditions. Moreover, the animals were healthy according to the results of the California Mastitis Test (CMT).

Milk samples were collected into two sterile falcons with a total of approximately 100 ml during morning milking. After the milk collection process, one falcon of the milk samples from each animal was used for measurement of the total milk fat by milk analyzer (Milkotester Master Classic LM2, Bulgaria) in the laboratory of the enterprise. The second falcon of the milk samples was transferred to the laboratory in cold chain for the fatty acid analysis. The milk samples brought to the laboratory were centrifuged at + 4°C 1800 xg for 15 minutes to collect the cream layers. After centrifugation, the cream layers were collected after the samples were kept at -20 °C for 15 minutes. Afterwards, fatty acid extraction was performed with 2 ml of methanolic KOH, and milk fatty acids were analyzed by gas chromatography device (Shimadzu GC-2025, Japan). Total milk fat measurement, extraction process and fatty acid analysis were performed according to the methods reported by Özkan et al. (10) and Özkan et al. (11). The milk fatty acid composition of the samples was expressed as percentage (%).

Statistical analysis

Before the study, power analysis was performed to determine the sample size. As a result of the power analysis, a total of 340 milk samples were found to be appropriate, with type 1 error probability (α) = 0.05, power (1-β) = 0.80 and f (effect size) = 0.17. The variables were primarily evaluated in terms of parametric test assumptions. As a result, it was determined that the parametric test and multiple linear regression assumptions were met. The relationships between the total fat in milk (%) (the dependent variable) and breed, pregnancy status and fatty acids (independent variables) were examined using multiple linear regression analysis. Backwards stepwise method was used in the regression analysis. The autocorrelation of the residuals was evaluated using the Durbin-Watson test, and the multicollinearity of the independent variables was evaluated using the Tolerance and VIF values. Descriptive statistics were calculated (mean, standard error). G*Power Version 3.1.9.2 was used for power analysis and Stata 12/MP4 statistical program was used for statistical analysis. Differences with $p < 0.05$ were considered statistically significant, and those with $0.05 < p < 0.10$ were considered tendencies.

3. Results

Descriptive statistics for total milk fat and fatty acid composition were represented in Table 1.

Table 1: Descriptive statistics for fat and fatty acid profile of total milk

Tablo 1: *Toplam süt yağı ve yağ aside profili için tanımlayıcı istatistikler*

Variables (%)	Mean	SE	Variables (%)	Mean	SE
Milk fat	4.753	0.090	C18:2 ω6	2.128	0.033
C4:0	0.964	0.069	C18:3 ω3	0.070	0.006
C6:0	2.295	0.115	C18:3 ω6	0.285	0.006
C8:0	2.374	0.088	C20:0	0.070	0.007
C10:0	6.329	0.173	C20:1 ω7	0.029	0.003
C11:0	0.403	0.012	C20:2 ω6	0.041	0.004
C12:0	6.886	0.118	C20:3 ω6	0.044	0.004
C13:0	0.245	0.005	C20:4 ω6	0.103	0.007
C14:0	16.819	0.114	C20:3 ω3	0.049	0.005
C14:1 ω5	1.852	0.035	C20:5 ω3	0.054	0.005
C15:0	1.348	0.018	C21:0	0.185	0.005
C15:1 ω5	0.202	0.008	C22:0	0.053	0.005
C16:0	38.360	0.344	C22:1 ω9	0.057	0.004
C16:1 ω7	2.087	0.036	C22:2 ω6	0.033	0.003
C17:0	0.354	0.006	C22:6 ω3	0.042	0.005
C17:1 ω8	0.201	0.005	C23:0	0.072	0.005
C18:0	3.384	0.065	C24:0	0.043	0.005
C18:1 ω9	12.487	0.179	C24:1 ω9	0.055	0.006

SE: standard error

Before performing to the multiple linear regression analysis, the assumptions of the regression analysis were checked. For this purpose, the autocorrelation of the residuals was examined by Durbin-Watson test, and the multicollinearity problem was examined by VIF and tolerance values. As a result of the Durbin-Watson test, it was determined that the residuals were not related to each other. As a result of the analysis that performed to determine the multicollinearity problem, C4:0, C6:0, C8:0, C10:0 and C12:0 fatty acids were not included in the multiple linear regression model since their VIF values were >10. The regression model created with the remaining fatty acids, breed and pregnancy status was shown in Table 2. In addition, as a result of the multiple linear regression analysis, the variables that were not statistically significant were shown in Table 3.

Table 2: Result of multiple linear regression model

Tablo 2: *Çoklu doğrusal regresyon model sonucu*

Variables	β	SE	t	p
Constant	7.638	1.375	5.554	<0.001
Breed	0.944	0.188	5.014	<0.001
Pregnancy Status	-1.164	0.187	-6.222	<0.001
C11:0	0.933	0.404	2.308	0.022
C14:0	-0.138	0.058	-2.390	0.017
C18:0	0.375	0.104	3.616	<0.001
C18:1 ω9	-0.100	0.045	-2.228	0.027
C18:3 ω3	-1.431	0.786	-1.822	0.069
C18:3 ω6	-3.257	0.94	-3.466	0.001

R^2=0.238 (F=12.671 ; p<0.001), β: regression coefficient, SE: standard error, t: t statistic

Breed (p<0.001), pregnancy status (p<0.001), C11:0 (p=0.022), C14:0 (p=0.017), C18:0 (p<0.001), C18:1 ω9 (p=0.027) and C18:3 ω6 (p=0.001) were found to be statistically significant, while the C18:3 ω3 (p=0.069) was nearly significant on total milk fat. It was revealed that the variables given in Table 2 had an effect of 24% on total milk fat and the established model was statistically significant (R^2=0.238) (p<0.001). The model created as a result of the regression analysis was shown in the equation below.

Total Milk Fat = 7.638 + (0.944 x Breed) + (-1.164 x Pregnancy Status) + (0.933 x C11:0) + (-0.138 x C14:0) + (0.375 x C18:0) + (-0.100 x C18:1 ω9) + (-1.431 x C18:3 ω3) + (-3.257 x C18:3 ω6)

According to this model, Holstein cows increased total milk fat by 0.944 units more than Simmental cows. Pregnant cows reduced total milk fat by 1.164 units compared to non-pregnant cows. One unit increase in C11:0 and C18:0 fatty acids increased the total milk fat by 0.933 and 0.375 units, respectively. Finally, one unit increase in C14:0, C18:1 ω9, C18:3 ω3, and C18:3 ω6 fatty acids decreased the total milk fat by 0.138, 0.100, 1.431 and 3.257 units, respectively.

4. Discussion and Conclusion

Milk quality, composition and fatty acid profile vary depending on genetic, environmental, and phenotypic factors. In addition, milk fat, which is one of the unique components of milk and its importance for health, is always considered as a current quality parameter (12). In this study, milk samples collected on the same control day were evaluated. Thus, the factors of breed and pregnancy status and fatty acid profile that have an effect on total milk fat were evaluated by limited and similar environmental conditions.

Table 3: The variables removed from regression model

Tablo 3: *Regresyon modelinden çıkarılan değişkenler*

Variables	**β**	**t**	**p**
C13:0	0.083	1.349	0.178
C14:1 ω5	-0.039	-0.670	0.504
C15:0	0.028	0.542	0.588
C15:1 ω5	-0.018	-0.351	0.726
C16:0	-0.078	-1.047	0.296
C16:1 ω7	0.039	0.636	0.525
C17:0	0.063	0.985	0.326
C17:1 ω8	0.019	0.315	0.753
C18:2 ω6	-0.086	-0.960	0.338
C20:0	0.019	0.339	0.735
C20:1 ω7	-0.001	-0.027	0.979
C20:2 ω6	-0.010	-0.157	0.875
C20:3 ω6	-0.043	-0.774	0.440
C20:4 ω6	0.079	1.338	0.182
C20:3 ω3	0.038	0.680	0.497
C20:5 ω3	-0.010	-0.172	0.864
C21:0	-0.060	-0.937	0.350
C22:0	-0.047	-0.841	0.401
C22:1 ω9	-0.054	-0.940	0.348
C22:2 ω6	-0.060	-0.984	0.326
C22:6 ω3	-0.046	-0.816	0.415
C23:0	-0.024	-0.389	0.697
C24:0	-0.029	-0.548	0.584
C24:1 ω9	0.051	0.861	0.390

β: regression coefficient, t: t statistic

Multiple linear regression analysis is a statistical method that aims to mathematically reveal the relations between a dependent variable and the independent variables that are thought to affect the dependent variable (9). There are different studies such as estimation of blood parameters using the composition of milk and fatty acids, determination of milk fatty acids content using different methods, evaluation of adulteration in milk fat, and the effects of milk composition parameters on fatty acids (12-15). The determination of the effects of breed, pregnancy status and fatty acids on total milk fat by multiple linear regression analysis showed that it is unique in the literature.

Milk fat can be affected by various factors such as breed, nutrition, lactation, metabolic and health status (16). Similar to our study, studies were reported that Holstein cows' milk is higher than Simmental cows in terms of milk fat (17,18). In addition, studies were shown that pregnancy is effective on milk composition parameters in cows (19,20). In another study, it was reported that pregnancy affects milk composition and varies according to pregnancy stages

(21). On the other hand, the fact that the pregnancy stages of the cows were not determined in the study was considered as a limitation.

The data set in the study showed that there are generally significant relationships between milk fat and fatty acids. In consequence of the multiple linear regression analysis, it was found that there was a positive relationship between milk fat and C11:0 and C18:0 fatty acids, and a negative relationship between C14:0, C18:1 ω9, C18:3 ω3, and C18:3 ω6 fatty acids. In a study, similar to our study, it was emphasized that the relationships between milk fat and C14:0, C18:0 and C18:1 ω9 fatty acids were at a significant level, and that the C18:3 ω3 level should be evaluated (22). While medium-chain fatty acids are synthesized de novo in the mammary gland, fatty acids longer than C16:0 pass into milk through the circulatory system (23,24). The statistical significance of medium-chain fatty acids in our study suggested that it may be related to the activity in mammary epithelial cells. In addition, it was thought that C11:0 fatty acid might have a positive relationship with milk fat, since it plays a role in fat metabolism (25). Fatty acids longer than C16:0 usually originate from the ration (24). Although the cows in the study were fed with similar rations, it was thought that the relationship might have resulted from the sample size and genotypic differences in breeds (26,27).

The multiple explanatory coefficient (R^2) shows to what extent the independent variables explain the change in the dependent variable (9). In our study, the R^2 value was found to be 0.238. Although the R^2 value was not very high, it was thought that the explanation rate by these variables is sufficient.

As a result, it was determined that breed, pregnancy status and C11:0, C14:0, C18:0, C18:1 ω9, C18:3 ω6 fatty acids had a statistically significant effect on milk fat under the same environmental conditions. Considering the importance of milk fat in terms of economy and health, it is thought that milk fat can be increased by applying appropriate breeding protocols. In addition, examining different factors that may affect milk fat in future studies will further improve the explanatory coefficient of the model to be established.

Acknowledgement

We would like to thank Saray Agriculture and Livestock Inc. for their kindly help.

Conflict of Interest

The authors declared that there is no conflict of interest.

Funding

This work was supported by the Scientific Research Project Fund of Hatay Mustafa Kemal Üniversitesi under the project number 21.GAP.004.

Authors' Contributions

Motivation / Concept: Ufuk KAYA, Hüseyin ÖZKAN, Murat Onur YAZLIK
Design: Ufuk KAYA, Hüseyin ÖZKAN, Aytaç AKÇAY, Akın YAKAN
Control / Supervision: Aytaç AKÇAY, Akın YAKAN
Data Collection and / or Processing: Ufuk KAYA, Hüseyin ÖZKAN, Murat Onur YAZLIK, Güven GÜNGÖR, Baran ÇAMDEVİREN, İrem KARAASLAN, Sevda DALKIRAN, Hasan Hüseyin KEÇELİ, Akın YAKAN
Analysis and / or Interpretation: Ufuk KAYA, Güven GÜNGÖR, Aytaç AKÇAY
Literature Review: Ufuk KAYA, Hüseyin ÖZKAN
Writing the Article: Ufuk KAYA, Hüseyin ÖZKAN
Critical Review: Murat Onur YAZLIK, Aytaç AKÇAY, Akın YAKAN

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules, and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules.

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