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Examination of Milk Samples, obtained from the Different Cattle breeds in the First Lactation, by Means of Discriminant Analysis

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ABSTRACT

By means of discriminant analysis, it can be identified which class the individuals (data) desired to be classified. In this study, it was aimed to identify that the milk samples belong to which breed, applying discriminant analysis to the milk samples taken from the cattle breeds (19 heads of Jersey, 53 heads of Holstein and 27 heads of Charolaise) in the first lactation. As a conclusion of the study, proper classification actualized in the rate, which can be considered quite high like 90.6%, according to the milk components (fat, protein, lactose, density, pH and conductivity) of Holstein Cattles. This rate actualized as 63.2% in Jersey and 25.9% in Charolaise. It was identified that the rate of general classification of non-linear analysis, used in discriminating Holstein, Jersey and Charolaise breeds from their milk components, was 67.7%.

1. Introduction

Milk is a porcelain white liquid, which has a specific and which is secreted in certain times in mammary glands of female mammals for them to be able to feed their young their kids, which contains all nutrients the kid has to receive until it reaches the position of being able to feed itself in the necessary rates (Çetiner, 2017). Cow milk is food that is rich in terms of mineral substances (especially calcium and phosphor). Calcium and phosphor needs of adults can be completely met by one liter of milk. Since calcium in milk is in an appropriate form, the milk is valued in the best way as a food (Demirci, 1981). In the group of milk and dairy products, the foods made of milk such as yoghurt, cheese and milk powder take place.

These foods are important resource of nutrient elements such as calcium, phosphor, B2 and B12 vitamins. Especially adult women, children and young people, those in all age groups have to consume the products in this group (Ünal and Besler, 2008). Milk does not have a constant composition and can differ according to the factors such as the species, breed age lactation period of animal, enterprise, region, calving season, feeding, animal health and the daily number and duration of milking. Milk yield mostly has importance for breeder and milk composition for milk industry. For example, the quality, efficiency and standard production of dairy products such as drinking milk, butter, yoghurt, cheese and powdered depend on the richness of composition of the raw milk coming to the processing plants of and the low variability of the composition (Yaylak et al., 2007). Fat content of milk differs according to the breeds. For example, Holstein contains fat of 3-3.5%; Brown Swiss, 3.8%; Jersey, 6.0%; Ayrshire, 4.5; Guernsey, 5.0% and Simmental 4.2 % (ESK, 2022).

That a total amount of dry substance in milk is more shows that milk is more suitable for the products such as cheese, milk powder, coagulated milk. The protein and fat content of the milk is extremely important to production of cheese. Knowing the factors changing composition of milk considerably helps to milk processing plants in planning their processes and forming marketing processes according to the coming raw milk (Yaylak et al., 2007). In no. 2019/64 official statement associated with classification of raw cow milk, published in Official Journal, numbered 31019, on the date of January 25, 2020, it was stated that the values of protein and fat would be considered in classification of raw cow milk (like in European Union Countries).

Discriminant analysis, taking into consideration, a number of features of individuals (independent variables), is a multi-variable statistical method, which is used in dividing the individuals into groups they belong to at optimal level with minimum fault, deciding which features (independent variables) are effective and stating that the individual is drawn from which group (Çiftçi, 2019). As a result of the analysis of the milk samples of

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[1] This study is a summary of the first author's master's thesis.

the cows of other breeds that are widely grown, a general correct classification rate can be determined and it will be advantageous to use the milk of unknown origin to determine which breed it belongs to.

There are many studies, in which discriminant analysis is used in the different areas of breeding. Kocabaş et al. (2003), in the studies they carried out and applied discriminant analysis by using physical properties of milk, stated that classification of unclear origin fleeces could be correctly made as Akkaraman or Anatolian Merino. In another study, again carried out on fleece, it was stated that Akkaraman or Anatolian Merino breeds could be properly classified in the rate of 81.9% (İlhan et al., 2009). Mahmood and Naeem (2011) made discriminant analysis for identifying the physical and chemical characteristics of water buffalo milk and demonstrated that discriminant analysis could be utilized in interpreting complex dataset. In related to this subject, Gençer (1996) examined the structural and behavioral features of ecotypes of honeybees in Central Anatolia and their various crossbreeds by means of discriminant analysis and, as result, showed that the right decisions could be made.

Although discriminant analysis is used in the different breeding areas, identifying the breeds from the milk compositions of Jersey, Charolaise and Holstein breeds could not be earlier met in the literature. Thanks to this, the current original study, adding the new information to the literature, is expected to fill a gap in this area.

In this study, utilizing discriminant analysis, it was aimed to classify the compositions (fat, protein, lactose, density, pH, conductivity) of milk samples of Jersey, Charolaise and Holstein breeds.

2. Material and Method

2.1. Materials

The material of this study consists of the milk samples taken from 19 heads of Jersey, 53 heads of Holstein and 27 heads of Charolaise cows in the first lactation, bred in the private enterprises in the different provinces of Konya. The values of fat (%), lactose (%), density (kg/m^3), pH, conductivity (µS/cm) of the milk samples, taken by centrifugal tubes of 50 ml, from 99 heads of cow in the first lactation were identified by measuring once for each sample by Lactoscan MMC-30 milk analysis device.

2.1. Methods

Discriminant analysis is examined under two main groups as linear and quadratic discriminant analysis. Linear discriminant analysis can be applied in case that sample data matrices, drawn from multi-variable populations exhibiting normal distribution, equal to intergroup variance covariance matrices ($S_1 = S_2 = \cdots = S_k$). If inter group variance covariance matrices are not equal, quadratic discriminant analysis is made. Whether or not intergroup variance covariance matrices are equal is controlled by means of *Box* M Test, developed by Box in 1949, is in the form of $Box\ M = MxC$ and shows Chi Square (X^2) distribution with $((k-1)/2)(p(p-1))$ freedom degree (Sangün, 2007).

$$BoxM = MxC \approx x^2_{\left[a,\left((k-1)/2\right)\left(p(p-1)\right)\right]}$$

Where, it is calculated by means of

$M = \sum_{i=1}^{k}(n_i - 1)\ln|S| - \sum_{i=1}^{k}(n_i - 1)\ln|S_i|$. S: is common variance covariance matrix calculated as follows:

$$S = S_{ortak} = \frac{\sum_{i=1}^{k}(n_i - 1)S_i}{\sum_{i=1}^{k} n_i - k}$$

n_i, denotes the number of observation in i^{th}feature

k, the number of group;

$|S|$, determinant of common variance covariance matrix;S_i , covariance matrix belonging to i^{th} group; and

$|S_i|$, determinant of variance covariance matrix belonging to i^{th} group.

C is the number of observation in the groups and is obtained by means of the following formulas:

If, $n_1 \neq n_2 \neq \cdots \neq n_k$,

$$C = 1 - \frac{2p^2 + 3p - 1}{6(p+1)(k-1)}\left(\sum_{i=1}^{k} - \frac{1}{\sum_{i=1}^{k}(n_i - 1)}\right)$$

If, $n_1 = n_2 = \cdots = n_k = n$

$$C = 1 - \frac{(2p^2 + 3p - 1)(k+1)}{+(p+1)\big(k(n-1)\big)}$$

In the formula, p denotes the number of features. In discriminant analysis, observation matrix X is obtained by combining observation matrices X_1 and X_2 containing observations regarding the totals of π_1 and π_2. From these data matrices, sample average vectors and covariance matrices are calculated as follows (Öztürk, 2006).

$$\bar{X}_1 = \frac{1}{n_1}\sum_{j=1}^{n_1} X_{1j}\,;\ S_1 = \frac{1}{n_1 - 1}\sum_{j=1}^{n_1}(X_{1j} - \bar{X}_1)(X_{1j} - \bar{X}_1)'$$

$$\bar{X}_2 = \frac{1}{n_2}\sum_{j=1}^{n_2} X_{2j}\,;\ S_2 = \frac{1}{n_2 - 1}\sum_{j=1}^{n_2}\big(X_{2j} - \bar{X}_2\big)(X_{2j} - \bar{X}_2)'$$

Accepting that the populations examined have the same covariance matrix (Σ), sample covariation matrices, union of S_1 an S_2 S_p (pooled variance covariance matrix) is calculated as follows (Özdamar, 2004).

$$S_{pooled} = \frac{(n_1 - 1)S_1 + (n_2 - 1)S_2}{n_1 + n_2 - 2}$$

Common covariance matrix can also be calculated in the form of $\hat{\Sigma} = E(X - \bar{x}_i)(X - \bar{x}_i)$. By means of a separation function to maximize intergroup difference, it will be possible to separate the groups from each other. Therefore, a common separation function is formed. Classification function associated with each group can be written in the form of:

$$Y_i = b_{0i} + b_{1i}X_1 + b_{2i}X_2 + \cdots + b_{pi}X_p$$

i=1, 2 in the equation represents the number of group; b_{i0} constant value b_{ij}(j = 1,2 ... p) canonic components and p is the number of variable. $\bar{X}_i$ as group average vector, constant value b_{0i} and coefficients vector b_{ij} can be calculated in the form of

$$b_{i0} = -\left(\frac{1}{2}\right)\bar{x}_i' S^{-1}\,\bar{x}_i$$

$b_{ij} = S^{-1}(\bar{X}_i)$ i = 1, 2…g, j=1, 2…p. Separation function for two groups is calculated as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + \cdots + b_pX_p$$

Canonic components b_j (j=1, 2…p) are found by average difference factor $(X_1 - X_2)$ with the following equation.

$$b_i = S^{-1}(\bar{X}_i - \bar{X}_j)$$

If covariance matrices of the groups are not equal ($S_1 \neq S_2$), two groups of quadratic analysis are made and, instead of common variance (S), taking the differences of covariance (S_1-S_2) matrices of the groups, coefficients vector bj is calculated by the following equation for quadratic separation function (Kılıç et al., 2013).

$$b_j = (S_1^{-1} - S_2^{-2})(\bar{X}_1 - \bar{X}_2)$$

3. Results and Discussion

In Jersey, Charolaise and Holstein cattle, means and standard deviations belonging to milk components (fat, density, lactose, protein, pH and conductivity) are given in Table 1.

Table 1
The means and standard deviations of milk components

Milk Components	Breeds		
	Jersey $\bar{X} \pm S_{\bar{x}}$	Charolaise $\bar{X} \pm S_{\bar{x}}$	Holstein $\bar{X} \pm S_{\bar{x}}$
Fat	4.78±0.31^{A}	5.21±0.43^{A}	4.14±0.33^{A}
Density	42.60±1.13^{A}	36.12±1.12^{B}	35.43±0.54^{B}
Lactose	6.74±0.19^{A}	5.84±0.16^{B}	5.62±0.10^{B}
Protein	4.45±0.13^{A}	3.90±0.11^{B}	3.76±0.07^{B}
pH	7.12±0.02^{A}	6.98±0.04^{B}	6.94±0.02^{B}
Conductivity	4.59±0.11^{A}	4.39±0.09^{A}	4.50±0.04^{A}

A, B: p<0.01

In Table 1, in the samples belonging to Jersey breed, it is seen that the values belonging to the other milk components, other than fat, are higher than Charolaise and Holstein cows. According to milk components, unidirectional analysis was applied to cattle breeds and the features that turned out different were subjected to Duncan test, one of multiple comparison tests. As also seen in Table 1, the differences between cattle breeds in terms of density, lactose, protein and pH features were found statistically significant (p<0.01) and in terms of fat and conductivity, insignificant (p>0.05). In Table 2, covariance matrices belonging to milk components of cattle in Jersey, Charolaise and Holstein breeds are given. Correlation coefficient between milk components of cattle from Jersey, Charolaise and Holstein breeds are given in Table 3.

Table 2
Covariance Matrices Belonging to Milk Components

Breeds	Milk Components	Fat	Density	Lactose	Protein	pH	Conductivity
Jersey	Fat	1.818	3.953	0.802	0.538	0.066	0.153
	Density	3.953	24.424	4.016	2.695	0.183	-0.220
	Lactose	0.802	4.016	0.680	0.456	0.035	-0.012
	Protein	0.538	2.695	0.456	0.306	0.023	-0.008
	pH	0.066	0.183	0.035	0.023	0.008	0.027
	Conductivity	0.153	-0.220	-0.012	-0.008	0.027	0.216
Charolaise	Fat	4.998	-2.701	0.271	0.189	-0.128	-0.430
	Density	-2.701	33.727	4.493	2.992	-0.063	0.173
	Lactose	0.271	4.493	0.691	0.461	-0.027	-0.020
	Protein	0.189	2.992	0.461	0.308	-0.018	-0.014
	pH	-0.128	-0.063	-0.027	-0.018	0.043	0.009
	Conductivity	-0.430	0.173	-0.020	-0.014	0.009	0.219
Holstein	Fat	5.811	3.003	1.178	0.794	-0.108	-0.344
	Density	3.003	15.688	2.644	1.765	-0.013	-0.409
	Lactose	1.178	2.644	0.532	0.356	-0.016	-0.103
	Protein	0.794	1.765	0.356	0.238	-0.011	-0.069
	pH	-0.108	-0.013	-0.016	-0.011	0.014	0.012
	Conductivity	-0.344	-0.409	-0.103	-0.069	0.012	0.096

Table 3
Correlations between Milk Components

Breeds		Fat	Density	Lactose	Protein	pH
	Density	0.593**				
	Lactose	0.721**	0.985**			
Jersey	Protein	0.721**	0.985**	1.000**		
	pH	0.538*	0.404	0.460*	0.461*	
	Conductivity	0.244	-0.096	-0.032	-0.032	0.628**
	Density	-0.208				
	Lactose	0.146	0.931**			
Charolaise	Protein	0.153	0.928**	1.000**		
	pH	-0.278	-0.053	-0.158	-0.159	
	Conductivity	-0.411*	0.064	-0.052	-0.054	0.089
	Density	0.315*				
	Lactose	0.670**	0.915**			
Holstein	Protein	0.675**	0.913**	1.000**		
	pH	-0.377**	-0.027	-0.181	-0.185	
	Conductivity	-0.461**	-0.333*	-0.457**	-0.457**	0.319*
	Density	0.169				
	Lactose	0.478**	0.945**			
Overall	Protein	0.482**	0.943**	1.000**		
	pH	-0.182	0.230*	0.143	0.141	
	Conductivity	-0.325**	-0.039	-0.132	-0.133	0.260**

*: p<0.05; **: p<0.01

As can be seen from Table 3, for overall milk samples, considerably high and statistically significant correlations (p<0.01) were identified between density and lactose (0.945), between density and protein (0.943) and between lactose and protein (1.000).

For checking whether or not the assumption that variance covariance matrices are homogeneous is provided, Box's M test is used (Sangün, 2007). As a result of the analysis made, it was identified that the value of Box's M was 486.551. According to this result, variance covariance matrices are not equal. As a result of this assumption, deciding to make quadratic analysis on the data, analysis was continued.

For identifying how much important the separation functions formed are, the values of canonic correlation, eigenvalue and Wilks Lambda statistics were referred to. In the analysis, Jersey, Charolaise and Holstein breeds were coded as dependent variables and milk components as independent variables, and quadratic discriminant analysis was made. The values showing importance control of separation functions are given in Table 4 and Table 5.

Table 4
The values of Eigenvalue Statistics

Function	Eige value	Variance (%)	Cumulative (%)	Canonical Correlation Value and Square
1	0.596	87.4	87.4	0.611 / 0.373
2	0.086	12.6	100	0.281 / 0.079

Table 5
The values of Wilks Lambda Statistics

Function	Wilks Lambda	Chi-Square	DF	Sigma
1	0.577	51.696	10	0.000
2	0.921	7.734	4	0.102

Depending on the magnitude of the statistical value of eigenvalue a large part of variance is accounted for by that function. In general, that eigenvalue is bigger than 0.40 is accepted as good (Cangül, 2006). According to this, a large part of the variance in dependent variable is accounted for by the first function, where eigenvalue is 0.596.

Canonic correlation measures the relationship between separation scores and groups and shows total variance explained (Altay and Yiğit, 2021). The square of this value expresses total variance, which separation function accounts for on the dependent variable. According to this, when the values in Table 4 are examined, it is seen that 1st function accounts for total variance on the dependent variable in the rate of 37.3% and, 2nd function, in the rate of 7.9%.

Wilks lambda statistics takes values between 0 and 1. Big values of Wilks Lambda expresses that group means are not different. The smaller the value of Wilks Lambda is, the more discriminating power of model increases (Cangül, 2006). The value of Wilks lambda expresses the rate of variance that cannot be explained between the groups. According to Table 5, depending on the value of Wilks lambda, the variance that cannot be explained for the 1st and 2nd function turned out 57.7% and 92.1%, respectively; and this expression reveals that the 1st function is more effective. The importance of Wilks Lambda expresses whether or not the functions

formed are not discriminated from each other. According to this, two separation functions formed according to the importance of Wilks Lambda taking place in Table 5 can significantly separate the groups from each other. In other words, the groups of 1st and 2nd separation functions formed can be significantly separated from each other ($p<0.01$).

The basis of discriminant analysis is to find a function to provide identification of main mass of the individual (Cangül, 2006). Separation functions used in discriminant analysis are formed by means the following formulas according to the coefficient values of canonic separation functions taking place in Table 6.

$$Y = b_0 + b_1X_1 + b_2X_2 + \cdots + b_pX_p$$

Where, Y denotes separation score; h_0, constant; b's, separation coefficients; X's, independent variables. As a result of discriminant analysis made, two pieces of separation functions were obtained. According to the coefficients of canonic separation function, the 1st and 2nd separation functions are as follows:

$$Y_1 = -38.320 - 0.006\ Fat + 0.049\ Density + 0.749\ Lactose + 4.371\ pH + 0.349\ Conductivity$$

$$Y_2 = -12.098 + 1.690\ Fat + 1.453\ Density - 10.564\ Lactose + 2.349\ pH - 0.771\ Conductivity$$

The values of canonic separation functions stated above are given in Table 6.

Table 6
The coefficients of canonic separation functions

Function	Independent Variables	Coefficient of Function	Constant
1	Fat	-0.006	-38.320
	Density	0.049	
	Lactose	0.749	
	pH	4.371	
	Conductivity	0.349	
2	Fat	1.690	-12.098
	Density	1.453	
	Lactose	-10.564	
	pH	2.349	
	Conductivity	-0.771	

In discriminant analysis, the achievement of analysis is the real percentage of classification. With this expression, depending on the magnitude of real classification percentage, it is proved that analysis is extremely successful. By means of analysis, the falsely or correctly classified observation data according to the breeds and achievement percentage are given in Table 7.

Table 7
The results and achievement percentages according to discriminant analysis

Predicted Group	Breeds	Number			Correct Classification Rate
		Jersey	Charolaise	Holstein	
	Jersey	12	1	1	63.2
	Charolaise	1	7	4	25.9
	Holstein	6	19	48	90.6
Total		19	27	53	

According to Table 7, in terms of milk features, the breeds are divided into the correct classes in the rate of 67.7% ((12 + 7+ 48)/ 99 = 67.7%). While 12 from 19 Jersey breed cows took place in the correct class, 7 cows (1 Charolaise, 6 Holstein) took place in the wrong group. While 7 from 27 Charolaise breed cows were correctly classified, 20 of them were wrongly classified (1 Jersey 19 Holstein). While 48 from 53 Holstein cows were correctly classified, only 5 (1 Jersey, 4 Charolaise) of them were wrongly classified. In addition, analysis results were expressed as percentage, Jersey breed cows were correctly classified in the rate of 63.2% and wrongly, in the rate of 36.8%. While Charolaise breed cows were properly classified in the rate of 25.4% and wrongly in the rate of 74.1%, Holstein breed cows were correctly classified in the rate of 90.6% and wrongly in the rate of 9.4%. According to discriminant analysis, the distribution of group (breed) data are given in Figure 1.

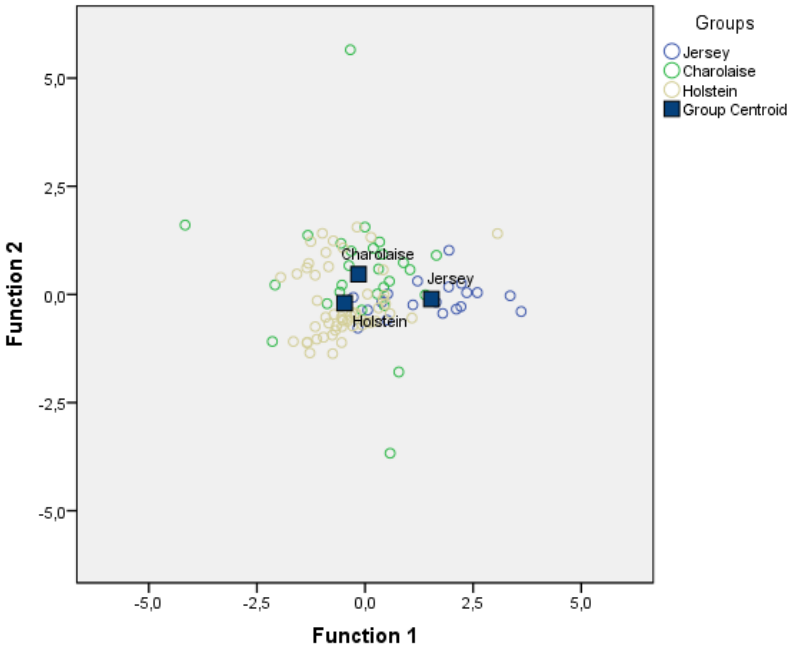

Figure 1
According to discriminant analysis, graphical view of the distributions of group data

As also seen from Figure 1, Holsteins formed a group and also Jerseys, a distinct group. Although Charolaise breed cows are divided as a distinct group, it is seen that some part of the cows (19 heads of Charolaise cow) takes place in Holstein, while one of them takes place in Jersey.

4. Conclusion and Suggestions

Discriminant analysis is a statistical technique, which enables researchers to study on the difference between 2 and more sample groups, in general, it utilizes some mathematical equations in grouping units. These equations, called separation functions are used to identify the common features of the groups in such way that it will enable to identify the most similar groups. Discriminant analysis is made to find separation functions enabling to discriminate the groups from each other, and, by means of these functions, to reveal separation variable that affects the separation the most and, by means of separation functions calculated, to identify that the newly observed unit will be included in which group in such a way that separation error will be minimum (Bayram, 2002).

As a result of the discriminant analysis made in this study, according to milk features (fat, protein, lactose, density, pH and conductivity) of Holstein cows, correct classification actualized in a rate that can be accepted as considerably high like 90.6%. This rate was 63.2% for Jerseys and 25.9% for Charolaise. It was identified that the rate of correct classification of nondirectional discriminant analysis, used in discriminating the milk features of Holstein, Jersey and Charolaise breeds, was 67.7%. Namely, in the milks, unknown to which breed, using the features such as fat, density, conductivity, that they belong to which breed can be correctly identified in the rate of 67.7%. However, it may be more accurate to determine a general correct classification rate as a result of taking milk samples from cows belonging to other breeds that are widely raised and examining them with separation analysis, and using milk of unknown origin to determine which breed it belongs.

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