

# Classification of Holstein Dairy Cattles in Terms of Parameters Some Milk Component Belongs by Using The Fuzzy Cluster Analysis

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## Abstract

This study was carried out on classification of Holstein Friesian breed dairy cattles in terms of some milk component parameters and on investigating the relevant parameters in the resulting cluster structures. Within the scope of this study, thirteen different criteria were used including somatic cell count (SCC), milk fat (%), milk protein (%), milk lactose (%), casein (%), urea (%), dry matter (%), non-fat dry matter (%), density (g/cm<sup>3</sup>), acidity (°SH), free fatty acids (mmol/10L), citric acid (%) and freezing point (°C). As a result of the analysis using Fanny algorithm based on the principle of fuzzy equality, the fuzziness level was found to be minimum when a total of 282 cattles were divided into 2 clusters with the accuracy rate of 97.5%. Accordingly, the cattles were classified in terms of the investigated characteristics in 2 different clusters in which 25 cattles were in Cluster 1 and the rest of the cattles were in Cluster 2. When the resulting cluster structures were studied, it was found that Cluster 2 has a more stable clustering than Cluster 1. When evaluating the change in milk components according to the clusters, it was concluded that somatic cell count, dry matter (%), milk fat (%) and density (g/cm<sup>3</sup>) have significant differences between clusters (P<0.05), while the other parameters were found statistically non-significant (P>0.05).

**Keywords:** Fuzzy clustering, Milk composition, Fanny algorithm, Holstein

## Bulanık Kümeleme Analizi İle Siyah Alaca Süt Sığırlarının Bazı Süt Bileşenlerine Ait Parametreler Bakımından Sınıflandırılması

### Özet

Bu çalışma, Siyah Alaca ırkı süt sığırlarının bazı süt bileşenleri bakımından bulanık kümeleme analizi ile sınıflandırılması ve oluşan küme yapılarında ilgili parametrelerin incelenmesi üzerine yürütülmüştür. Araştırma kapsamında somatik hücre sayısı (SHS), süt yağı (%), süt proteini (%), süt laktöz (%), kazein (%), üre (%), kuru madde (%), yağsız kuru madde (%), yoğunluk (g/cm<sup>3</sup>), asitlik (°SH), serbest yağ asidi (mmol/10L), sitrik asidi (%) ve donma noktası (°C) olmak üzere on üç farklı ölçüt kullanılmıştır. Bulanık kümeleme analizinde bulanık eşitlik ilkesine dayalı Fanny algoritması kullanılarak yapılan analiz sonucunda ise toplam 282 adet inek %97.5 doğru sınıflandırma oranı ile 2 ayrı kümeye ayrıldığında bulanıklık düzeyinin minimum olduğu görülmüştür. Buna göre inekler incelenen özellikler bakımından 25 tanesi küme 1'de, 257 tanesi de küme 2'de yer alacak şekilde 2 farklı kümede sınıflandırılmıştır. Oluşan küme yapıları incelendiğinde ise küme 2'nin küme 1'e göre daha kararlı bir küme oluşturduğu tespit edilmiştir. Kümelere göre süt bileşenlerinin değişimi değerlendirildiğinde ise SHS, süt yağı, kuru madde (%), süt yağı (%) ve süt yoğunluğunun (g/cm<sup>3</sup>) kümeler arası önemli bir (P<0.05) farklılık gösterdiği, diğer parametrelerin ise istatistiksel açıdan önemli bir farklılık göstermediği (P>0.05) sonucuna varıldı.

**Anahtar sözcükler:** Bulanık kümeleme, Süt kompozisyonu, Fanny algoritması, Siyah Alaca

## INTRODUCTION

In animal breeding, knowing the differences or similarities of the individuals in a population based on the investigated characteristics is important for both for breeding studies and for revealing the genetic profiles of the individuals in the herd [1]. One of the common methods used in animal breeding for this purpose is the clustering analysis. This method has been applied successfully in many

subjects, such as distinguishing breeds or populations, determining the genotype similarities of the individuals and the classification of morphological characteristics [1-5].

Cluster analysis is a method which is used to classify according to similarities or dissimilarities of the ungrouped and scattered data or independent variables [6-9]. The purpose of this method, which is based on unsupervised learning, is to provide a grouping of the units showing similar characteristics in a way that homogeneous within



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themselves by bringing them together <sup>[10-12]</sup>. In this way, both the process time is shortened and the scattered data is provided in a more general form (group), and also useful and summary information can be presented to the researchers <sup>[9]</sup>. In the cluster analysis, each unit is assigned to a cluster with a final decision, and according to the classic cluster concept, a unit is either a member of the relevant cluster (Membership 1) or remain outside the cluster without being a member (Membership 0) <sup>[13]</sup>. Therefore, researchers may encounter some unstable situations.

As reported in the literature by several researchers, different clustering methods, depending on the distance criteria used may give different results. In addition, it can be seen that some units which are taking place in different clusters in clustering algorithms in which approximately the same results are obtained, may be in the condition of being instabile (uncertain) in their cluster membership. Similarly, not knowing how to group the data before the analysis in the cluster analysis can reveal the uncertainty in making a final decision while classifying according to the similarity of some units. So, in one way, the identification of the outliers which are difficult to assign to a cluster will help making more reliable interpretations <sup>[9,14-16]</sup>. Fuzzy clustering analysis is recommended as a more suitable method in detection of cases involving such uncertainties. In this method, there is a situation that each unit in the cluster belongs to a cluster with the membership degrees ranging between [0,1]. Thus, a unit can belong to multiple clusters with different membership degrees. In this context, it can also said that the fuzzy clustering analysis, unlike the classical cluster analysis, provide flexibility to cluster limits and contains more detailed information <sup>[13,17-19]</sup>. Therefore, in the study, fuzzy clustering analysis was used to classify the dairy cattles in terms of analyzed characteristics, and it was aimed to study how these characteristics changed in the resulting cluster structures.

There are limited number of studies in the literature related to the fuzzy clustering analysis which is only recently used in animal breeding. For example, some of these studies in which the fuzzy clustering was used are; in determining phylogenetic relationships in sheep by Geng et al.<sup>[20]</sup> in the classification of body measurements of sheep by Kılıç and Özbeyaz <sup>[15]</sup> and Karakaya and Bafra; the examination of animal behavior by Cohen et al.<sup>[21]</sup>, Görgülü <sup>[22]</sup> in the classification of cattle in terms of some milk yield characteristics.

The purpose of this study was to classify the dairy cattles according to thirteen different milk component [somatic cell count (SCC), milk fat (%), protein (%), lactose (%), casein (%), urea (%), dry matter (%), non-fat dry matter (%), density (g/cm<sup>3</sup>), acidity (°SH), free fatty acid (mmol/10L), citric acid (%) and the freezing point (°C)] traits and to determine the criteria effective in clustering by analyzing the change in the resulting cluster structures.

## MATERIAL and METHODS

### Material

The study was conducted in experimental farm of East Mediterranean Agricultural Research Institute in Adana, TURKEY. The animals included in the study consisted of 282 Holstein cows. The Holstein cows were 5 to 6 years of age and weighed between 500 and 550 kg. The feed ration contained silage, wheat straw, alfaalfa dry hay, with an addition of feed concentrate. The experiment was continued for 4 months.

The SCC in milk samples obtained from cows in the morning was analyzed by DCC which is a De Laval brand measuring instrument. Firstly, the SCC was determined separately for each breast lobe. After necessary cleaning, the udder to be measured the first milk was discharged by milking a few times, then milk sample was taken to plastic sample tubes. This taken sample was taken to the measuring tapes, after performing its homogenization by turning upside-down a few times by closing the mouth of the tube.

The SCC was evaluated in the barn in a very short time (about 45-60 seconds) by taking De Laval measuring tape from the plastic cups by which the samples do to not interfuse in the mammary lobes of milk samples. Determining the number of cells was done by measuring according to the principle of counting somatic cells stained with a DNA-specific fluorescent probe propidium iodide, approximately 60 µl milk samples were taped, installed tape is placed in the measuring window of the De Laval cell counter, and the SCC was determined by evaluating 1 µl <sup>[23]</sup>.

The milk analyzes were performed with the FOSS MilkoScan™ 120 instrument, and the values of the milk fat was determined by the Röse Gottlieb; the milk protein by the Kjeldahl method; the non-fat dry matter by the heating oven method; the lactose by the Boehringer Mannheim Enzymatic kit; the density by the Anton Paardan DMA 38 density measuring instrument; the acidity by titration with 0.25 M NaOH; free fatty acid, fat by titration using a pH electrode and the citric acid was determined by the Boehringer Mannheim Enzymatic Kit <sup>[24]</sup>.

### Methods

Fuzzy clustering analysis is emerging as a suitable method when the clusters does not separated from each other clearly or the some units are creating unstable conditions for cluster membership <sup>[25-27]</sup>. There are two main methods of fuzzy clustering analysis. The first one is Fuzzy c-means (FCM) which is based on c-partition and the second one is the hierarchical clustering method based on the fuzzy equality principle <sup>[28-30]</sup>. In this study, the Fanny algorithm which is based on the fuzzy equality principle was used. The fuzzy clustering technique used

in this algorithm aims to minimize the objective function as shown in Equation 3 below, and has some limitations. These were given in Equation 1 and 2, respectively. The limitations are,

$$1. U_{iv} \geq i=1,2,\dots,n \text{ and } v=1,2,\dots,k \quad (1)$$

$$2. \sum_{v=1}^k u_{iv} = \%100 \quad i=1,2,\dots,n \quad (2)$$

The objective function is expressed as follows:

$$C = \sum_{v=1}^k \frac{\sum_{i,j=1}^n u_{iv}^2 u_{jv}^2 d_{(ij)}}{2 \sum_{j=1}^n u_{jv}^2} \quad (3)$$

Where in,  $d_{(ij)}$ : the distance between the  $i^{\text{th}}$  and  $j^{\text{th}}$  units (similarity),  $u_{iv}$ : the unknown membership of the  $i^{\text{th}}$  unit to the  $v^{\text{th}}$  cluster,  $u_{jv}$ : unit the unknown membership of the  $j^{\text{th}}$  unit to the  $v^{\text{th}}$  cluster,  $k$ : number of clusters, and  $n$ : the total number of units<sup>[31,32]</sup>.

In the study, the degree of fuzziness in the cluster was determined by using Dunn's partition coefficient ( $F_k$ ). Dunn's partition coefficient ( $F_k$ ) also known as a coefficient that is used to understand how far is the fuzzy clustering away from the exact clustering<sup>[13]</sup>. The general mathematical expression of the Dunn partition coefficient is indicated as in Equation 4. The value of this coefficient can be  $1/k$  at minimum and can be 1 at maximum. Accordingly, the value range of the Dunn partition coefficient is defined as  $[1/k, 1]$ <sup>[33]</sup>.

$$F_k = \sum_{i=1}^n \sum_{v=1}^k \frac{u_{iv}^2}{n} \quad (4)$$

The Normalized Dunn Coefficient  $F'_k(u)$  is obtained when this coefficient is normalized regardless of the number of clusters, and is calculated by the equation given in Equation 5. This coefficient, also known as the non-fuzziness index, is in the range of  $[0,1]$ <sup>[14]</sup>.

$$F'_k(u) = \frac{F_k(u) - \left(\frac{1}{k}\right)}{1 - \left(\frac{1}{k}\right)} = \frac{kF_k(u) - 1}{k - 1} \quad (5)$$

In determining the number of clusters in the study, Kaufman partition coefficient  $D(U)$  and the Normalized Kaufman coefficient  $D_c(U)$  was used as well as the Normalized Dunn Coefficient  $F'_k(u)$ . These coefficients are calculated by using the equations given in Equation 6 and Equation 7, respectively<sup>[34]</sup>. In determining the appropriate number of clusters, the Normalized Dunn Coefficient  $F'_k(u)$  was considered to be high and the value of Normalized

Kaufman coefficient was considered to be low as the criteria in the study<sup>[34]</sup>.

$$D(U) = \frac{1}{n} \sum_{v=1}^k \sum_{i=1}^n (h_{iv} - u_{iv})^2 \quad (6)$$

$$D_c(U) = \frac{D(U)}{1 - \left(\frac{1}{k}\right)} \quad (7)$$

The other coefficient which was considered in the study in order to determine the number of clusters was the Silhouette Coefficient ( $SC_i$ ), and this coefficient is also a widely used index to determine the stability of the cluster structures<sup>[7]</sup>. It was determined according to the Mean Silhouette index ( $SC$ ) obtained by calculating the mean of these values that how well all of the units clustered such as in  $k$  number of cluster. It is considered to be appropriate clustering when this value is above 0.50, and the number of clusters corresponding to the Maximum ( $SC$ ) value is taken as the optimal number of clusters<sup>[16]</sup>. All analyzes in this study was performed using the NCSS 2001 software package<sup>[35]</sup>.

## RESULTS

In the research, the numbers of clusters between  $k=2$  and 10 were increased one by one in order to define appropriate cluster number in fuzzy clustering analysis. For this purpose, Mean Silhouette coefficient values ( $SC$ ) for each cluster number were obtained as in Table 1 containing Silhouette coefficient values ( $SC_i$ ) and average of all. When the average Silhouette coefficient values ( $SC$ ) at Table 1 were analyzed, it can be said that if the number of cluster were  $k=2$ , cluster 2 ( $SC_i=0.8773$ ) had more stable structure than cluster 1 ( $SC_i=0.2033$ ).

The maximum ( $SC$ ) coefficient value was obtained with the two fuzzy clusters. Accordingly, it can be said that the appropriate number of clusters according to the ( $SC$ ) coefficient is  $k=2$ . In addition, the ( $SC$ ) coefficient value was generally found higher than 0.5 for the number of other clusters, and this also showed that the appropriate number of clusters for clustering structure has been reached.

When the Dunn partition coefficient ( $F_k$ ) and Normalized Dunn Coefficient  $F'_k(U)$  values in Table 2 were analyzed in order to determine the degree of the fuzziness in the cluster, it was found that when the cluster number is  $k=2$ ,  $F_k$  value was found as 0.94; and the  $F'_k(u)$  value was found as 0.89. According to these values, it can be said that the cluster is closer to fuzzy clustering when the cluster number is  $k=2$ . To determine the number of clusters, when Normalized Kaufman coefficient  $D_c(U)$  and Normalized Dunn Coefficient  $F'_k(u)$  coefficient values were analyzed; the  $F'_k(u)$  value was at its highest and the

**Table 1.** The ( $\overline{SC}$ ) and ( $\overline{SC}$ ) values according to the number of clusters**Tablo 1.** Küme sayılarına göre ( $\overline{SC}$ ) ve ( $\overline{SC}$ ) değerleri

No of Clusters	Average Silhouette Coefficients $SC_i$											
	1	2	3	4	5	6	7	8	9	10	( $\overline{SC}$ )	
2	0.2033	0.8773										0.82
3	0.3463	0.3398	0.7066									0.60
4	0.4123	0.5920	0.5610	0.2047								0.50
5	0.8522	0.3265	0.6104	0.6645	0.5546							0.55
6	0.5360	0.8522	0.5455	0.5540	0.5831	0.5782						0.57
7	0.8522	0.5742	0.4621	0.5451	0.5110	0.5360	0.6201					0.54
8	0.8457	0.5123	0.4728	0.6317	0.4604	0.4460	0.5652	0.5878				0.53
9	0.7978	0.6177	0.8352	0.5054	0.4604	0.6431	0.6599	0.2709	0.5878			0.56
10	0.7978	0.5294	0.6599	0.6227	0.6070	0.3653	0.8352	0.6177	0.2709	0.5550		0.54

**Table 2.** The partition coefficients and the accurate classification rates according to the number of clusters**Tablo 2.** Küme sayılarına göre ayırıştırma katsayıları ile doğru sınıflandırma oranları

Number of Clusters (k)	$F_k$	$F'_k(u)$	$D(U)$	$D_c(U)$	ACR(%)
2	0.94	0.89	0.01	0.03	97.5
3	0.85	0.79	0.04	0.07	95.7
4	0.80	0.74	0.06	0.08	95.8
5	0.82	0.78	0.04	0.06	92.7
6	0.81	0.78	0.06	0.08	95.8
7	0.80	0.76	0.06	0.08	95.7
8	0.79	0.76	0.06	0.08	94.9
9	0.80	0.78	0.06	0.07	95.7
10	0.79	0.77	0.06	0.07	95.7

$F_k$ : Dunn Coefficient,  $F'_k(u)$ : Normalized Dunn Coefficient,  $D(U)$ : Kaufman Coefficient,  $D_c(U)$ : Normalized Kaufman Coefficient, ACR (%): Accuracy classification rate

$D_c(U)$  was at its lowest for  $k=2$ . It can be said that the appropriate number of cluster is 2. In addition, as seen in Table 2, the accurate classification rate was determined as 97.5% as a result of discriminant analysis conducted by using cluster membership values for the number of clusters  $k=2$ . The accurate classification rate being high also showed that the number of clusters is 2 can be also considered as an indication for this number to be appropriate.

The optimal cluster number was determined to be  $k=2$  according to the results of the evaluations. As a result of the fuzzy clustering analysis, a total of 282 cattles were divided into two clusters, 25 cattles were in Cluster 1 and 257 cattles were in Cluster 2. The mean values of milk component parameters which are analyzed within the study for both clusters were given in Table 3.

When the cluster structures in Table 3 were examined, the values of investigated parameters for Cluster 1 and Cluster 2 were found, SCC: 420.32 -77.71, Dry matter (%): 12.86-12.36; Non-fat dry matter (%): 8.60-8.69; Fat (%): 4.34-3.71; Protein (%): 3.23-3.24, Lactose (%): 4.53-4.62; Casein (%): 2.55-2.58; Urea (%): 0.02-0.03; Density ( $g/cm^3$ ): 1.02-1.03; Free

**Table 3.** The mean and standard deviation values of milk components in the cluster parameters**Tablo 3.** Kümelerdeki süt bileşen parametrelerinin ortalama ve standart sapma değerleri

Criteria	Cluster 1 ( $n_1=25$ )	Cluster 2 ( $n_2=257$ )
The somatic cell count (SCC)	420.32±207.92	77.71±36.37**
Dry matter, %	12.86±1.26	12.36±1.22*
Non-fat dry matter, %	8.60±0.24	8.69±0.45 <sup>ns</sup>
Fat, %	4.34±1.18	3.71±1.11**
Protein, %	3.23±0.25	3.24±0.37 <sup>ns</sup>
Laktose, %	4.53±0.15	4.62±0.23 <sup>ns</sup>
Casein, %	2.55±0.18	2.58±0.29 <sup>ns</sup>
Urea, %	0.02±0.004	0.03±0.005 <sup>ns</sup>
Density, $g/cm^3$	1.02±0.001	1.03±0.001*
Free fatty acid, mmol/10L	3.62±1.33	3.05±1.52 <sup>ns</sup>
Citric acid, %	0.13±0.03	0.13±0.03 <sup>ns</sup>
Freezing point, °C	0.53±0.02	0.53±0.03 <sup>ns</sup>

\*  $P<0.05$ ; \*\*  $P<0.01$ ; <sup>ns</sup>:  $P>0.05$

fatty acid (mmol/10L): 3.62-3.05; citric acid (%): 0.13-0.13 and the freezing point (°C): 0.53-0.53, respectively.

The differences between clusters for each of these parameters were determined by independent samples t-test, and the homogeneity of variance was determined by Levene's test.

As a result of this study, the differences of SCC, dry matter (%), density (g/cm<sup>3</sup>) and milk fat (%) which are one of the investigated the parameters between clusters were found statistically significant (P<0.05) and the differences of the clusters between the other parameters were found non-significant (P>0.05).

## DISCUSSION

In this study which was carried out on Holstein dairy cattle breeds, cattles are classified in terms of some milk component parameters by using fuzzy clustering analysis. As a result of fuzzy clustering analysis, cattles were divided into 2 cluster with 97.5% correct classification rate to be minimum fuzzy level. Accordingly, cattles were grouped as 25 of them were in cluster 1, 257 of them were in cluster 2. When the changes in the cluster structure of the milk parameters studied in this research were examined, it is determined that somatic cell count (SCC) showed a significant (P<0.01) difference. When the cluster structures obtained by fuzzy clustering analysis, the Somatic cell count in Cluster 2 was found as SCC <100.000 cell/ml, and was found SCC <500.000 cell/ml in Cluster 1 as seen in *Table 3*.

Somatic cell count (SCC) is one of the important criteria that can be used in determining milk quality and in revealing whether the cattles in the herd have the mastitis case [36-39]. The SCC value in a regular milk is generally required to be SCC <200.000 cell/ml, and it is it has been considered to be abnormal when it is above this value [38,40]. Within the framework of this information, as a result of fuzzy clustering analysis, that the SCC value was found above 200.000 cell/ml in Cluster 1.

The milk fat rate was determined as 4.34% in Cluster 1, and as 3.71% in Cluster 2, and a significant difference (P<0.01) was determined between clusters (*Table 3*). The study results for the milk fat which has importance in terms of the pricing of the milk [41] was found to be higher than the value of 3.5% which is declared in Turkish Food Codex [42]. Milk dry matter was another milk component which showed a significant (P<0.05) decrease between the clusters in the study.

The value of milk dry matter in Cluster 1 was determined as 12.86% and as 12.36% in Cluster 2, and was found lower in Cluster 2 than Cluster 1 (P<0.05). The values within both clusters of milk dry matter which is important for the nutritional value of milk [43] were found lower than the value

of 13.62% reported by Sahin and Kasıkcı [44], and found in compliance with the value of 12% which is declared in Turkish Food Codex [42]. The milk density which gives information whether any cheating was done on milk [37] was found 1.02 g/cm<sup>3</sup> in Cluster 1, and 1.03 g/cm<sup>3</sup> in Cluster 2, and a statistically significant (P<0.05) difference was found between clusters (*Table 3*). When the milk density values for both clusters were analyzed, it was found in compliance (TS1018) with the values of 1.028 - 1.039 g/cm<sup>3</sup> reported for raw milk standard [45]. Differences between clusters in terms of dry matter, milk fat and density may affected by SCC. There are many research results on this manner [37,46,47]. Although no statistically significant difference was found between clusters, the milk protein value (%) was found higher than the values reported by many researchers on this subject as being 3.23%-3.24%, respectively [37,48].

The milk casein was found as 2.55%-2.58% in the clusters, and was observed in the amount of less casein in Cluster 1. The value of the non-fat dry milk matter was found as 8.60 - 8.69% in the clusters and was found compatible with the rate of 8.5% declared in Turkish Food Codex [42]. It was found that the freezing point and the citric acid (%) values had the same value in both clusters, and were determined as 0.53°C and 0.13%, respectively (*Table 3*). The amount of milk urea (%) in the custers was found between the values of 0.03%-0.02%. The free fatty acid was found as 3.62 mmol/10L%-3.05 mmol/10L% in the clusters, the amount of milk lactose was determined as 4.53%-4.62%.

Consequently, as a result of classifying the dairy cattles by fuzzy clustering analysis according to some milk components, it has been understood that the main diversity criteria for the clusters was the SCC. The other characteristics [dry matter (%), density (g/cm<sup>3</sup>) and milk fat (%)] were determined to have minor effects on the clustering. In this respect, in the classification studies conducted in the field of animal breeding, it has been thought that using the fuzzy clustering analysis would enable the researchers to make a more realistic classification, especially in situations involving uncertainty.

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