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RESEARCH ARTICLE

Estimation of Milk Yield Losses from Subclinical Mastitis in Dairy Cows

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ABSTRACT

In order to estimate the prevalence of subclinical mastitis, somatic cell content in cow's milk is the main indicator. It was aimed at revealing the relationship between breed, age, number of lactations, lactation periods, average milk yield of cows, and somatic cell count (SCC) used in mastitis diagnosis. The material of the study consisted of milk from 300 mammary lobes belonging to 75 cows from different breeds (Holstein and Holstein Crossbred) aged between 3 and 8 years in dairy cattle enterprises in Efeler district of Aydın province between December 2020 and February 2021. To determine the association between SCC and milk yield, multiple regression analysis was used. There was no significant difference in SCC between crossbred Holstein and Holstein cows. It was determined that SCC increased with increasing age (≤4 and 5≥). In the study, it was determined that the daily milk yield of the cows was 21.1 kg, and it was found that the milk yield loss showed significant differences according to the SCC. In the analysis, there is a negative relationship between SCC and milk yield; an increase of 1 unit in SCC was estimated to result in a daily loss of 0.71 kg of milk output per cow.

Key words: Cow, milk yield loss, somatic cell count, subclinic mastitis

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Süt İneklerinde Subklinik Mastitisten Kaynaklanan Süt Verim Kayıplarının Tahmini

ÖZ

İnek sütündeki somatik hücre konsantrasyonu, subklinik mastitis prevalansının tahmin edilmesinin ana göstergedir. Bu çalışmada ineklerin ırk, yaş, laktasyon sayısı, laktasyon dönemleri ve ortalama süt verimi ile mastitis tanısında kullanılan somatik hücre sayısı (SHS) arasındaki ilişkiyi ortaya çıkarmak amaçlanmıştır. Araştırmanın materyalini, Aydın ili Efeler ilçesindeki süt sığırcılığı işletmelerinde, Aralık 2020 ile Şubat 2021 tarihleri arasında 3 ila 8 yaşları arasındaki farklı ırklardan (Holstein ve Holstein Melez) 75 ineğe ait her meme lobundan (toplam 300) alınan süt örnekleri oluşturmuştur. SHS ve süt verimi arasındaki ilişkiyi belirlemek için çoklu regresyon analizi kullanılmıştır. Melez Holstein ve Holstein inekleri arasında SHS bakımından anlamlı bir fark bulunmamıştır. SHS'nin yaş arttıkça (≤4 ve ≥5 yaş) arttığı belirlenmiştir. Çalışmada, ineklerin günlük süt veriminin 21.1 kg olduğu ve SHS'ye göre süt verim kaybının anlamlı farklılıklar gösterdiği bulunmuştur. Analizde, SHS ve süt verimi arasında negatif bir ilişki olduğu; SHS'deki 1 birimlik artışın, inek başına günlük 0.71 kg süt verimi kaybına neden olduğu tahmin edilmiştir.

Anahtar Kelimeler: İnek, somatik hücre sayısı, subklinik mastitis, süt verimi kaybı

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INTRODUCTION

For the majority of people in the world, milk and dairy products represent some of their most basic dietary sources. The average milk yield per cow is rising as a result of the rising demand for dairy products worldwide (Lucy 2001). The increase in milk yield has resulted from genetic selection as well as improved cow nutrition and herd management. One of the major problems affecting high milk yield is poor udder health, especially due to mastitis (De Vliegher et al. 2003). The onset of clinical mastitis and the development of intramammary infection (IMI) result in large financial losses for dairy farmers (El-Awady and Oudah, 2011).

Mastitis is an inflammatory disorder of the mammary gland that can be brought on by microorganisms, disease-related tissue damage, and chemical, physical, or traumatic incidents (Bae et al. 2017). As a result of mastitis disease, the disease has economic importance as significant losses occur due to decreased quantity and quality of milk produced, antibiotic treatment, and increased veterinary care costs (Jilo et al. 2017).

There are two basic types of mastitis: subclinical and clinical. It is commonly acknowledged that subclinical mastitis accounts for the majority of the economic costs associated with mastitis. From an economic point of view, for many cattle farms, subclinical mastitis is considered to be the most economically important type of mastitis due to its long-term impact on total milk yield (Halasa et al. 2007).

The disease-related decrease in product and the forfeiture of production benefits can be characterized as the economic losses resulting from mastitis. The first of these is represented by the milk that must be thrown out following antibiotic treatment, and the second is the benefit of the milk that this disease will prevent from ever being produced (Kossaibati and Esslemont 1997).

Direct and indirect expenses are the two categories of costs associated with mastitis. Veterinary services, diagnosis, treatment, extra labor costs, and discarded milk (during treatment) are all considered direct expenses. Known as hidden costs, indirect expenses are described as costs that are not always evident to the milk producer. Indirect losses due to subclinical mastitis (SCM) can be listed as decreased milk yield, early slaughter losses due to the disease, and poor milk quality (Nielsen 2009).

The detection of subclinical mastitis is a very difficult task for producers, but its detection is very important to save both producers and animals from many problems (Kabir et al. 2017).

Although subclinical mastitis cases cannot be diagnosed clinically because clinical symptoms are not observed, since the disease manifests itself through an increase in the number of somatic cells and bacteria in milk, it can be detected indirectly by looking at the level of somatic cell count (SCC) in milk. At the same time, milk yield losses occurring at different levels of subclinical mastitis can be determined by quantitative methods (Yalcin, et al. 1999a; Sumon*,* et al. 2020).

It has been observed that somatic cell concentration in cow's milk is the main indicator for estimating the prevalence of subclinical mastitis. Cows with subclinical mastitis have no visible signs but have a high somatic cell count (SCC, defined as the number of somatic cells per milliliter of milk). High SCC in milk indicates the presence of pathogens in the udder and is an indicator of intramammary infection (IMI) and also a measure of response to infection (Pyörälä 2003; Heringstad et al. 2006).

To estimate the possible milk yield losses caused by subclinical mastitis, it is important to define healthy or uninfected. The threshold for a healthy udder has been considered to be SCC ≤50 000 (Seegers et al. 2003) or about 70 000 (Djabri et al. 2002; Schukken et al. 2003). Some authors have defined a healthy animal as having a slightly higher SCC, i.e., ≤100 000 (Hand et al. 2012). SCC less than 100,000 is considered to be uninfected, and there is no significant milk yield loss due to subclinical mastitis. The new definition of subclinical mastitis assumes a new case if SCC reaches >100 000 after a test day with SCC <50 000 (Halasa et al. 2009). Therefore, the choice of an appropriate threshold to identify an uninfected mammary depends on the purpose. At a lower threshold, more cases of CBE (increased sensitivity and fewer false negatives) are identified, whereas using a higher threshold (increased specificity) may result in fewer false positive results (Pantoja et al. 2009).

The problem of subclinical mastitis is extremely complicated. So, the dairy industry is very interested in developing a simple, economical, and effective way to forecast the correlations between high SCC, subclinical mastitis, and possible loss of milk yield in dairy cows (Jeretina et al. 2017).

Furthermore, because harmful organisms like Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli, Mycobacterium bovis, and Mycoplasma spp. can be found in the milk collected from the afflicted cow, subclinical mastitis has zoonotic significance. For this reason, it is critical that customers have early detection and treatment of subclinical mastitis (Dhakal et al. 2007).

In this study, the estimation results of milk yield losses due to subclinical mastitis on dairy cattle farms are evaluated. In addition, it was aimed at revealing the relationship between breed, age, number of lactations, lactation periods, average milk yield of cows, and somatic cell count used in mastitis diagnosis.

MATERIALS and METHODS

Experimental Design

The data and samples required for the study were obtained from dairy cattle farms operating in the Efeler District of Aydın Province between December 2020 and February 2021. In the study, 10-15 ml milk samples were taken from 300 udder lobes of 75 Holstein and Holstein Crossbred cows, aged between 3 and 8 years, which did not show clinical symptoms, and used in sterile plastic tubes. Information on daily milk yield, number of lactations, and lactation period was obtained from the farms visited for sample collection.

Taking Milk Samples

Somatic cell counts were determined in milk samples taken from each udder lobe of 75 animals. For this purpose, milk samples were taken from four udder lobes (300 milk samples in total) of each cow during evening milking and analyzed on the same day. Before taking the samples, the sampler disinfected his or her hands and the udders of the cows. After discarding the first 5 ml of milk to remove saprophytic bacteria from each teat, 10-15 ml milk samples were taken into sterile plastic tubes (Zajac et al. 2018; Kabir et al. 2019).

Preparation of Milk Films

Milk films were prepared within 1 hour after the milk samples were collected. For this purpose, 10-15 µl of milk were taken from each milk sample using a micropipette and spread on 1 cm² square areas on a clean microscope slide. After the milk films were prepared, they were dried at room temperature (Zajac et al. 2018; Kabir et al. 2019).

Dyeing of Milk Films

The prepared milk films were stained according to the Broadhurst-Paley staining method. For this purpose, the slides were immersed in xylene for 2 minutes and 95% ethyl alcohol for 2-5 minutes and filtered. Then the slides were immersed in Broadhurst-Paley stain for 30 seconds. Finally, the slides were rinsed by immersion in three separate distilled waters and dried at room temperature (Broadhurst and Paley 1939; Moraes et al. 2018).

Microscopic Somatic Cell Count

The stained milk films were examined under an immersion objective using a light microscope (Leica DMLB Meyer Instruments, Inc., Houston, TX). The diameter of the image area under the immersion objective was measured at 195 µm with a micrometer, and the working factor (WF) was calculated at 13400. For somatic cell counting, 25 fields were counted in each milk film. For reliability, somatic cells were counted on two milk films prepared from each milk sample and averaged. The average cell count was then multiplied by WF to calculate the number of cells in 1 ml of milk (Zajac et al. 2018).

Statistical Analyses

Regression analysis was performed using the SPSS Statistics 18 package program for statistical calculations of the data obtained from the study. In the study, the number of lactations, average daily milk yield, age, breed, and lactation periods of the cows were determined. According to the data obtained, the cows;

Age = (years); 1st (≤ 4 years; 2nd (≥ 5 years).

Breed = 1. (Holstein), 2. (Holstein hybrid)

Average daily milk yield (kg) = 1st (1-19,4 kg), 2nd (19,5-23,1 kg), 3rd (23,2 kg ≥).

Number of lactations: 1st (first 3 lactations), 2nd (4th lactation and above)

Lactation period (months): 1st (1-3; early period), 2nd (4-6; middle period), 3rd (7-10; late period)

The somatic cell count was subdivided into 0 (below 200 000), 1 (200 001-500 000), 2 (500 001-1 million), 3 (over 1 million) and then subjected to the necessary analyses.

The quantitative relationship between milk yield and somatic cell count was estimated by multiple regression analysis. The model used was:

SVi = SCCi + LACNUMBERi + LACPERIODi + AGEi + RACEi

Equation:

SVi: Milk yield on the first visit day (kg/day/cow), SCCi: The number of somatic cells in each milliliter of milk on the day of the first visit, LACNUMBERi: Number of lactation of the cow on the first visit day, LACPERIODi: lactation period on the day of the first visit, AGEi: age of the cow on the day of the first visit, RACEi: breed of cow on the day of the first visit.

RESULTS

Model Estimation Results

The regression estimation results are presented in Table 1. There was a negative correlation between SCC and lactation number and a positive correlation between breed, age, and lactation period. The relationship between milk yield, SCC, and number of lactations was found to be statistically significant ($P<0.01$). The significant F statistic ($p<0.001$) indicated that the model was significant as a whole, and the adjusted R2 value of 0.464 indicated that the independent variables included in the model explained 46.4% of the variation in milk yield.

Durbin-Watson test results were analyzed for an autocorrelation problem, and it was concluded that there was no such problem (DW= 1.787). The multicollinearity problem (multicollinearity) was

investigated by analyzing the correlation matrix between the independent variables, and it was determined that there was no high correlation between any variables.
In the estimated model, the most important variable affecting the variation in milk yield was the increase in somatic cell count. According to the SCC result, the milk yield loss caused by the increase of l units was estimated to be 0.71 kg/cow/day.
According to 2019 data from the Turkish Statistical Institute (TurkStat), milk is obtained from 6.580.753 milking cows in Turkey. The loss of milk yield in our country due to SCC was estimated at 1.425.062 metric tons. As a result of the calculation made with the 2023 second-period raw milk current prices, the monetary equivalent of the yield loss was calculated as $ 602,286,428. When calculating the monetary equivalent of the loss of yield, the loss due to the decrease in milk quality caused by the disease and the loss in case of recurrence of the disease were not taken into account.

Table 1: Predicted milk yield regression estimation results

	b	SE	P
Fixed	2.471	0.640	0.000
SCC	-0.708	0,237	0.004
Race	0.089	0.192	0.644
Age	0.251	0.237	0.293
LacPeriod	0.114	0.120	0,345
LacNumber	-0.009	0.224	0.002

R=0.681 R2 =0.464F Value: 20.31 (P<0.001) Durbin-Watson= 1.787

DISCUSSION

One of the most important problems encountered on dairy cattle farms is mastitis. This disease causes millions of dollars of economic losses every year due to reasons such as the decrease in milk yield and quality, disposal of mastitic milk, medicine, and veterinary costs, removal of animals from the herd, decrease in the market value of animals, and protection and control practices in mastitis (Yalçın 2000).
Mastitis generally occurs in two forms: clinical and subclinical. Clinical mastitis has external signs that can be easily observed in the udder of the cow. However, subclinical mastitis is not recognized because the udder does not show a clinical picture and continues for a long time. Approximately 70-80% of milk yield losses due to mastitis are caused by subclinical mastitis (De Graves and Fetrow 1993; Yalçın 2000). Traumatic, bacterial, viral, parasitic, and chemical factors play a role in the occurrence of mastitis. Factors such as breed, age, milk yield level, lactation period and number, anatomical reasons, milking method, seasonal and climatic conditions, nutrition, barn and shelter conditions, metabolism, and hormonal balance of the animal play a role as predisposing factors (Contreras and Rodríguez 2011). Since the data obtained in this study were taken during the same period, and factors such as barn conditions, udder hygiene, and milking method were similar; other factors other than breed, age, milk yield, lactation period, and number were not taken into consideration in the study.
In previous scientific studies on mastitis in dairy cattle farms, the number of losses due to the disease varies between countries depending on factors such as calculation methods, loss items (milk, treatment, labor, reformation, etc.), disease form (clinical/subclinical) and severity (mild, severe), incidence rate, and prices/wages (veterinarian, medicine, milk, and labor) (Sarıözkan 2019). For example, losses of 22-31 € per cow per year in the USA (Kaneene and Hurd, 1990; Miller et al. 1993), 19-32 € in France (Fourichon et al. 2001), 3 € in Germany (Reinsch and Dempfle 1998), 102-279 € per case in the UK (Kossaibati and Esslemont 1997; McInerney et al. 1992), €240 in Germany (Clair et al. 2019), €440 in Canada (Aghamohammadi et al. 2018), and $80.09 in Iran (Sadeghi-Sefidmazgi et al. 2011). In a study conducted in 21 enterprises in Tunisia, it was estimated that there was an annual milk yield loss of 524 kg per cow (Mtaallah et al. 2002). Mungube et al. (2002) stated that the annual economic loss in milk yield per cow was between 29.1 and 66.6 USD. In Turkey, while an average loss of 315 TL per infected animal (equivalent to 271-1277 L milk) and 113 TL

per cow was reported in 2006 (Yalçın et al. 2010), an average loss of 244 TL per infected animal (equivalent to 158-1204 L milk) and 110 TL per cow was reported in 2014 (Yıldız and Yalçın 2014).

In this study, with the current prices of 2023, the amount of loss per infected animal in mastitis cases ($91.52) is equivalent to 216.55 L of milk. It is thought that the fact that the majority of the animals used in the study were cattle on family farms and that the traditional breeding model was applied to the farms was effective in the high rates of subclinical mastitis. To better determine the effect of the SCC increase, it may be useful to study more samples in large herds with standardized breeds, ages, number of lactations, and lactation periods raised in the same environmental conditions. In conclusion, in this study, the difference between the milk yield of cows and the SCC increase was found to be statistically significant, and it can be said that mastitis has a significant effect on decreasing milk yield. The average daily milk yield of the cows was determined to be 21.1 kg, and it was determined that milk yield loss varied significantly according to the SCC of the cows. Demir and Ekşi (2019) estimated that the milk yield loss caused by an increase of l units in CMT was 1.92 kg/cow/day. Dohoo et al. (1984) calculated 1.21-2.09 liters of yield loss per cow per day due to mastitis. Yalcin et al. (1999a) estimated the loss at 0.7 kg per cow. In another study, Yalcin et al. (1999b) calculated milk yield loss as 1.01 kg.

CONCLUSION and RECOMMENDATIONS

In conclusion, our statistical analyses identified a linear inverse relationship between somatic cell count (SCC) and milk yield. Milk production losses were estimated at 0.71 kg/cow/day per unit increase in SCC. As a result of the intensive polyculture production in the livestock sector in Turkey, producers cannot allocate enough time to dairy cattle breeding and cannot specialize in production, leading to yield loss. Moreover, their low level of technical and formal education prevents them from keeping up with the latest knowledge and advancements in their industry, which decreases their success. This situation results in a breakdown of enterprise controls and follow-up, a rise in illnesses, and ultimately a decrease in production. Ultimately, all these factors prevent the producers from working profitably and efficiently. It will be useful to inform the producers about the diseases and the extent of the losses they cause at the enterprise level, and some of these losses can be avoided by closing the technical knowledge deficits of the producers with the training programs to be carried out.

Since the clinical signs of subclinical mastitis are not visible in the field, it is important to determine the economic dimension of this disease, which is neglected by many producers in the sector today, and to take the necessary measures. To determine the economic weight of the disease, it would be more appropriate to extend the record-keeping system in enterprises and to carry out field research in different regions.

Conflicts of Interest: No conflicts of interest are declared by the authors.

Author Contributions: MT, Investigation, data collection and analysis, writing original draft preparation, writing review; ÖG, Laboratory analysis, manuscript proofreading and editing. All authors have read and approved the finalized manuscript.

Ethical Approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained with in the frame work of academic and ethical rules.

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