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Effects of Somatic Cell Count and Various Environmental Factors on Milk Yield and Foremilk Constituents of Red-Holstein Cows

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ABSTRACT

In this study, the effects of somatic cell count (SCC) and various environmental factors on milk yield and foremilk constituents in Red-Holstein (RH) cows raised under Mediterranean climatic conditions in Aydın Province in Turkey were determined. A total of 891 foremilk samples collected from 129 cows during morning and evening milkings in winter and summer seasons between 2009 and 2012 were analysed for protein content (PC), lactose content (LC), non-fat dry matter content (NFDMC), and SCC. SCC data were split into 6 groups (<100,000 cells mL⁻¹; 100,000-199,999 cells mL⁻¹; 200,000-299,999 cells mL⁻¹; 300,000-399,999 cells mL⁻¹; 400,000-750,000 cells mL⁻¹; and >750,000 cells mL⁻¹; before statistical analysis. LS MEANS of sampling time milk yield (STMY), PC, LC, NFDMC, and $Log_{10}SCC$ for winter and summer seasons were 11.27±0.253 kg and 10.59±0.226 kg (P<0.05), 3.24±0.024% and 3.04±0.021% (P<0.01), 4.44±0.028% and 4.48±0.025% (P>0.05), 8.68±0.045% and 8.50±0.040% (P<0.01), and 5.051±0.0508 (112,461 cells mL⁻¹) and 4.914±0.0428 (82,035 cells mL⁻¹) (P<0.05), respectively. Compared to the first SCC group, STMY, LC, and NFDMC decreased to 1.1 kg (9.4%), 6.4%, and 3.8% in the fifth group and 1.49 kg (12.7%), 11.3%, and 6.3% in the sixth group, respectively (P<0.05). In conclusion, LC could be used as a mastitis marker due to a distinct decrease in LC as SCC increases. Additionally, due to significant reductions in the STMY, PC, and NFDMC in the summer season, some precautions are required to prevent possible heat stress in this herd and the herds managed in the regions with hot summer months.

Keywords: Somatic cell count; Protein content; Lactose content; Milk yield loss

Kırmızı-Alaca Sığırların Süt Verimi ve Ön Süt Bileşenleri Üzerine Somatik Hücre Sayısı ve Bazı Çevresel Faktörlerin Etkileri

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ÖZET

Bu çalışmada, Akdeniz iklim koşullarının egemen olduğu Aydın'da yetiştirilen Kırmızı-Alaca sığırların süt verimi ve ön süt bileşenleri üzerine somatik hücre sayısı (SHS) ve bazı çevresel faktörlerin etkileri araştırılmıştır. 2009-2012

yılları arasında yılda iki kez (kışın ve yazın) 129 baş inekten sabah ve akşam sağımlarında alınan toplam 891 ön süt örneğinde protein oranı (PO), laktoz oranı (LO) ve yağsız kuru madde oranı (YKMO) ile SHS belirlenmiştir. İstatistik analiz öncesinde SHS verileri 6 gruba (<100,000 hücre mL⁻¹; 100,000-199,999 hücre mL⁻¹; 200,000-299,999 hücre mL⁻¹; 300,000-399,999 hücre mL⁻¹; 400,000-750,000 hücre mL⁻¹; ve >750,000 hücre mL⁻¹) ayrılmıştır. Kış ve yaz mevsimleri için denetim zamanı süt verimi (DZSV), PO, LO, YKMO ve Log₁₀SHS en küçük kareler ortalamaları sırasıyla 11.27±0.253 kg ve 10.59±0.226 kg (P<0.05), % 3.24±0.024 ve % 3.04±0.021 (P<0.01), % 4.44±0.028 ve % 4.48±0.025 (P>0.05), % 8.68±0.045 ve % 8.50±0.040 (P<0.01) ve 5.051±0.0508 (112,461 hücre mL⁻¹) ve 4.914±0.0428 (82,035 hücre mL⁻¹)'dir (P<0.05). Birinci SHS grubu ile karşılaştırıldığında, DZSV, LO ve YKMO, beşinci SHS grubunda sırasıyla 1.1 kg (% 9.4), % 6.4 ve % 3.8, altıncı SHS grubunda ise sırasıyla 1.49 kg (% 12.7), % 11.3 ve % 6.3 azalmıştır (P<0.05). Sonuç olarak, SHS yükseldikçe LO'nın belirgin bir şekilde düşmesi LO'nın bir mastitis göstergesi olarak kullanılabileceğini göstermektedir. Ayrıca, DZSV, PO ve YKMO'nda yaz mevsiminde görülen önemli düşüşler bu sürüde ve yöredeki diğer sürülerde sıcak yaz aylarında görülebilecek sıcaklık stresine karşı çeşitli önlemler almayı gerektirmektedir.

Anahtar Kelimeler: Somatik hücre sayısı; Protein oranı; Laktoz oranı; Süt verim kaybı

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1. Introduction

Mastitis is one of the most costly diseases in dairy herds all over the world. Due to a high genetic correlation between somatic cell count (SCC) and mastitis (Boichard & Brochard 2012; Koeck et al 2012), SCC is used as a good genetic predictor of mastitis in breeding programs (Berry et al 2011; Gernand et al 2012).

SCC is mainly affected by mammary gland infection, cow level factors such as the genotype of the cow and morphology of the udder, parity, lactation stage, season, stress, herd size, milking interval, milking system, and milking management (Haas 2003; Skrzypek et al 2004; Ahn et al 2005).

A genetic antagonism also exists between milk production and mastitis (Rupp & Boichard 2003; Berry et al 2011). Hagnestam-Nielsen et al (2009) reported that due to subclinical mastitis in Swedish Holstein and Red-Holstein (RH) cows, the 305-d milk yield losses were 155 kg in primiparus and 445 kg inmultiparus cows. Erdem et al (2010) estimated the losses caused by SCC in daily milk yield and 305d milk yield in Jersey cows as 14.96% and 13.95%, respectively. In Lithuanian Black and White herds, milk yield loss was determined as 14.4%, when SCC increased from 200,000 cells mL⁻¹ to 800,000 cells mL⁻¹ (Jouzaitiene et al 2006). Atasever & Erdem (2009) reported that the increase in SCC resulted in 17.44% loss in daily milk yield and 15.38% loss in 305-d milk yield in Holstein-Friesian (HF) cows. According to Atasever & Erdem (2009) the financial loss estimated per cow was \$217.8 in HF in Turkey.

SCC also affects the chemical composition of milk. As SCC increases, the crude protein level in milk decreases slightly (Litwinczuk et al 2011), and a distinct reduction is observed in lactose level (Barlowska et al 2009). As a result, lactose content in milk has been suggested as a mastitis marker (Forsbäck 2010).

The purpose of this study was to determine the factors affecting sampling time milk yield (STMY), foremilk constituents (protein content (PC), lactose content (LC) and non-fat dry matter content (NFDMC)) and SCC and to estimate the effects of SCC levels on STMY and foremilk constituents in RH cows raised in Aydın province of Turkey.

2. Material and Methods

The data was collected from a RH herd raised in Aydın province, Turkey. Aydın has a Mediterranean climate. The geographic coordinates of the farm are 37° 45' 48.15" N and 27° 17' 34.45" E. The long term monthly highest average temperature, and "Temperature Humidity Index" estimated from the long term monthly average temperature and relative humidity for the region are 33.5 °C and 72.97 in June, 36.1 °C and 76.15 in July, and 35.4 °C and 75.49 in August. The average calving interval, lactation length, lactation milk yield, and 305-d milk yield of the RH herd were 443±4.9 d, 349±4.0 d, 8,509±120.1 kg, and 7,679±87.5 kg, respectively (Koç 2012).

The farm was visited in winter and summer seasons in each year from 2009 to 2012 to collect milk samples from the cows during morning and evening milkings. The milking interval in summer season was 12 hours, whereas the interval became 11-13 hours in winter season depending on daylight. A total of 891 foremilk samples from 129 cows were taken. One milk sample was taken from each cow for each milking. After cleaning the teats (i.e., prestimulation) and fore stripping, 2-3 strips from each teat were collected and mixed into sterile sampling cups. Milk samples were stored in a refrigerator and carried to the laboratory in an icebox on the next day. Then, the samples were analyzed for PC, LC, NFDMC, and SCC by using Bentley 150 Infrared Milk Analyzer and Bentley Soma count 150.

SCC data were split into 6 groups. The first group represented the SCC less than 100,000 cells mL⁻¹, the second group between 100,000 and 199,999 cells mL⁻¹, the third group between 200,000 and 299,999 cells mL⁻¹, the fourth group between 300,000 and 399,999 cells mL⁻¹, the fifth group between 400,000 and 750,000 cells mL⁻¹, and the sixth group higher than 750,000 cells mL⁻¹. Before the statistical analysis, SCC data were transformed to a base-10 logarithm scale (Log₁₀SCC). The statistical model used for STMY, PC, LC, NFDMC, and Log₁₀SCC is as follows:

$$y_{ijklmno} = \mu + a_i + b_j + c_k + d_l + f_m + g_n + (bd)_{jl} + (bf)_{jm} + (cd)_{kl} + e_{ijklmno}$$
(1)

Where; $y_{ijklmno}$ is the observation of STMY, PC, LC, NFDMC, or $Log_{10}SCC$; μ is the overall mean; a_i is the year effect (i= 2009, 2010, 2011 and 2012); b_j is the season effect (j= winter and summer); c_k is the parity effect (k= 1, 2, 3, 4, 5+); d_1 is the lactation month effect (l= 1, 2,....11, 12+); f_m is the milking time effect (m= morning and evening); g_n is the SCC group effect (n= 1, 2, 3, 4, 5, 6; except for $Log_{10}SCC$ data); (bd)_{jl} is the season x lactation month interaction effect; (bf)_{jm} is the parity x lactation month interaction effect; and $e_{ijklmno}$ is the normally distributed random error with mean zero and unknown variance σ^2 .

The data were analysed using the GLM procedure of SAS, and the differences between the least-squares means of fixed factor levels were considered to be statistically significant at P<0.05 (2-tailed), based on Tukey's adjustment type I error rate.

3. Results and Discussion

STMY, PC, LC, NFDMC, and $\text{Log}_{10}\text{SCC}$ LS MEANS are given in Table 1. The means of STMY, PC, NFDMC and $\text{Log}_{10}\text{SCC}$ in winter season were higher than those in summer season (P<0.05). STMY mean (11.63±0.217 kg) of the morning milking was 1.4 kg higher than that of the evening milking (P<0.01), whereas PC mean (3.17±0.020%) of the evening milking was higher (P<0.01) than that of the morning milking (3.11±0.021%).

With an increase of 2.08 kg, the mean of STMY reached to 15.25 ± 0.438 kg in the second month of lactation and then decreased gradually during the rest of the lactation period (Table 1). In contrast to STMY, PC decreased in the second month of lactation and then increased gradually during the rest of the lactation months. On the other hand, a decrease in LC started from the fourth month of lactation and this decrease continued to the end of lactation (Table 1, Figure 2).

It was observed in this study that $Log_{10}SCC$ increased as parity advanced. SCC of the fifth parity (148,936 cells mL⁻¹) was significantly higher than those of the first (94,234 cells mL⁻¹) and the second (64,408 cells mL⁻¹) parities (P<0.05). This finding is in agreement with the findings of Koç (2008; 2009), Hagnestam-Nielsen et al (2009) and Gernand et al (2012).

The mean of Log_{10} SCC (144,212 cells mL⁻¹) in the first lactation month gradually decreased until the fourth month of lactation (P<0.05) and then a fluctuated increase was detected until the end of lactation. The third and fourth lactation months were also found to be significantly different from the months 1, 8, 9, 11, and 12 (P<0.05). The SCC pattern observed during lactation is in agreement with Haas

Table 1- LS MEANS and standard errors of sampling time milk yield (STMY), protein content (PC), lactose content (LC), non-fat dry matter content (NFDMC) and Log₁₀ somatic cell count (Log₁₀SCC) in Red Holstein cows

Çizelge 1- Kırmızı-Alaca ineklerin denetim zamanı süt verimi (DZSV), protein oranı (PC), laktoz oranı (LC), yağsız kuru madde oranı (NFDMC) ve Log_{10} somatik hücre sayısı (Log_{10} SHS) en küçük kareler ortalamaları ve standart hataları

		STMY, kg	PC, %	LC, %	NFDMC, %	Log ₁₀ SCC
Year	п	**	**	**	NS	**
2009	199	11.61±0.280ª	3.18±0.027 ^a	4.48±0.031ab	8.63±0.050	5.038±0.0565ª
2010	102	10.53±0.383 ^{ab}	2.99±0.036b	4.57±0.042ª	8.64±0.068	5.005±0.0794ª
2011	335	10.12±0.246 ^b	3.22±0.023ª	4.41±0.027 ^b	8.58±0.044	4.780±0.0478 ^b
2012	255	11.47±0.264 ª	3.16±0.025ª	4.39±0.029b	8.52±0.047	5.107±0.0521ª
Season		*	**	NS	**	*
Winter	435	11.27±0.253ª	3.24±0.024ª	4.44±0.028	8.68±0.045ª	5.051±0.0508ª
Summer	456	10.59±0.226 ^b	3.04±0.021b	4.48±0.025	8.50 ± 0.040^{b}	4.914±0.0428b
Parity		NS	NS	**	**	**
1	355	11.20±0.248	3.09±0.024	4.68±0.027 ^a	8.78±0.044ª	4.738±0.0459ª
2	210	11.10±0.303	3.17±0.029	4.49±0.034b	$8.66 {\pm} 0.054^{ab}$	4.927±0.0605 ^{ab}
3	127	11.32±0.370	3.16±0.035	4.37±0.041bc	8.51±0.066bc	4.978±0.0764 ^{bc}
4	61	10.34 ± 0.480	3.16±0.046	4.42±0.053bc	8.58±0.085 ^{abc}	5.097 ± 0.1008^{bc}
5+	138	10.70±0.301	3.12±0.029	4.35±0.033°	8.43±0.053°	5.173±0.0617°
Lactation month		**	**	**	NS	**
1	65	13.17 ± 0.509^{ab}	$3.04{\pm}0.048^{\text{cdf}}$	4.66±0.056 ^{ab}	8.72±0.090	5.159±0.1073ª
2	66	15.25±0.438ª	2.75±0.042e	4.69 ± 0.048^{a}	8.42 ± 0.077	$4.858{\pm}0.0894^{ab}$
3	54	13.59 ± 0.503^{ab}	$2.85{\pm}0.048^{\text{ef}}$	4.65±0.056 ^{ab}	8.48 ± 0.089	4.670±0.1025b
4	61	12.48 ± 0.629^{bd}	$2.91{\pm}0.060^{\text{def}}$	4.67±0.070 ^{ab}	8.57±0.111	4.571±0.1306b
5	76	12.97 ± 0.626^{ab}	$3.00{\pm}0.059^{\text{cdf}}$	4.57±0.069abc	8.55±0.111	4.797±0.1300 ^{ab}
6	63	11.48 ± 0.497^{bc}	3.11 ± 0.047^{bcd}	4.52±0.055abc	8.62 ± 0.088	5.068±0.1030 ^{ab}
7	45	9.29 ± 0.722^{cef}	$3.16{\pm}0.068^{bcd}$	4.44 ± 0.080^{abcd}	8.53±0.128	4.963±0.1517 ^{ab}
8	46	9.79±0.614 ^{cdef}	$3.20{\pm}0.058^{bc}$	4.38±0.068 ^{bcd}	8.56±0.109	5.173±0.1298ª
9	81	9.53±0.405 ^{ce}	$3.29{\pm}0.038^{ab}$	4.35±0.045 ^{cd}	8.64±0.072	5.094±0.0845ª
10	66	8.13 ± 0.577^{ef}	3.46±0.055ª	4.18±0.064 ^d	8.66±0.102	5.076±0.1217 ^{ab}
11	50	7.85±0.613 ^{ef}	3.48±0.058ª	4.21±0.068 ^d	8.69±0.108	5.260±0.1292ª
12+	218	7.66 ± 0.335^{f}	3.43±0.032ª	4.23±0.037 ^d	8.67±0.059	5.103±0.0689ª
Milking time		**	**	NS	NS	NS
Morning	449	11.63±0.217ª	3.11±0.021ª	4.47±0.024	8.58±0.038	4.963±0.0412
Evening	442	10.23±0.212b	3.17±0.020b	4.45±0.023	8.60±0.038	5.002±0.0409
SCC group (cells/mL)		**	NS	**	**	-
1 (<100,000)	498	11.73±0.198ª	3.13±0.019	4.70±0.022ª	8.85±0.035ª	-
2 (100,000-199,999)	141	11.23±0.292 ^{ab}	3.16±0.028	4.59±0.032b	8.75±0.052 ^{ab}	-
3 (200,000-299,999)	81	10.73±0.379 ^{ab}	3.14±0.036	4.51±0.042bc	8.64 ± 0.067^{b}	-
4 (300,000-399,999)	46	$11.05{\pm}0.487^{ab}$	3.12±0.046	4.39±0.054°	8.51 ± 0.086^{bc}	-
5 (400,000-750,000)	63	10.63 ± 0.421^{ab}	3.13±0.040	4.40±0.047°	8.51 ± 0.075^{bc}	-
6 (>750,000)	62	10.24 ± 0.432^{b}	3.16±0.041	4.17 ± 0.048^{d}	8.29±0.076°	-
Season* Milking time	891	**	**	NS	NS	NS
Parity* Lac. month	891	**	**	**	**	*
Season* Lac.month	891	**	**	**	**	NS

NS, non significant; *, P<0.05; **, P<0.01. Different letters (a,b,c,d,e,f) indicate significant differences (P < 0.05)

(2003), Hagnestam-Nielsen et al (2009) and Gernand et al (2012). A similar pattern for Brown-Swiss and HF cows was also reported by Koç (2008; 2009).

The effects of the SCC on STMY, LC and NFDMC were found to be statistically significant (P<0.01). The highest STMY mean was found in the first SCC group (11.73 \pm 0.198 kg), which was 1.49 kg (12.7%) higher than that of the sixth group (P<0.05).

Compared to STMY, the effect of SCC on LC was more distinct. LC mean decreased gradually from the first SCC group (4.70 \pm 0.022%) to the sixth SCC group (4.17 \pm 0.048%). The mean of the first SCC group for LC differed significantly from that of the last SCC group and means of these two groups were also different from those of other SCC group was found to be similar to the third SCC group (P<0.05), but different (P<0.05) from those of other groups.

A pattern determined in SCC groups for LC was also detected for NFDMC. The mean NFDMC ($8.85\pm0.035\%$) in the first SCC group decreased to $8.29\pm0.076\%$ in the sixth SCC group (P<0.05) and the mean of the first SCC group was similar to that of the second group, but different from those of other groups. The mean of the sixth SCC group was also found to be significantly different from those of the second and third SCC groups (P<0.05).

Compared to the first SCC group in which the cows were accepted as healthy, STMY, LC and NFDMC of RH cows decreased about 1.1 kg (9.4%), 6.4% and 3.8% in the fifth SCC group, and 1.49 kg (12.7%), 11.3% and 6.3% in the sixth SCC group, respectively. These results support the idea that milk yield and constituents are significantly affected by the increase of SCC.

Season x milking time interaction was found to be statistically significant for STMY (P<0.01) and PC (P<0.01). Parity x lactation month interaction was statistically significant (P<0.05) for all traits and season x lactation month interaction was statistically significant (P<0.05) for all traits except for $Log_{10}SCC$.

STMY in winter-morning milking was found to be different from both morning and evening milkings in summer and also from winter-evening milking (P<0.05; Figure 1). PC in both milkings in winter were also found to be different from those of summer milkings (P<0.05).



Figure 1- Fluctuations in STMY (kg) and PC (%) depending on milking time (M, Morning; E, Evening) and milking season (W, Winter; S, Summer)

Şekil 1- Sağım zamanı (M, Sabah; E, Akşam) ve sağım mevsimine (W, Kış; S, Yaz) göre DZSV (kg) ve PO (%)'nın değişimi

A gradual increase in PC and a fluctuated increase in NFDMC occurred in all parities, after the decrease both in PC and NFDMC were observed in the second month of lactation (Figure 2). On the other hand, the decrease in $Log_{10}SCC$ lasted up to the third month of lactation and then a fluctuated increase in $Log_{10}SCC$ was observed. The increase in the SCC is more obvious for the fourth and the fifth parities.

STMY means of the first half of the lactation in winter milkings were higher than those of the first half of the lactation in summer milkings (Figure 3). The winter milking means of PC in all lactation months except for the third lactation month were higher than those of summer milkings. A pattern similar to PC was also observed in NFDMC. These findings are consistent with the results obtained from HF and Montbeliarde breeds by Koç (2011). On the other hand, a gradual decrease in LC was observed at the second half of the lactation in both seasons as seen in Figure 3.



Figure 2- Fluctuations in STMY (kg), PC (%), LC (%), NFDMC (%) and Log₁₀SCC parity means during lactation period (P1, the first farity; P2, the secod parity; P3, the third parity; P4, the fourth parity and P5, the fifth parity)

Şekil 2- DZSV (kg), PO (%), LO (%), YKMO (%) ve Log₁₀SHS'nin laktasyon sırası ortalamalarının laktasyon dönemine göre değişimi(P1, birinci laktasyon sırası; P2, ikinci laktasyon sırası; P3, üçüncü laktasyon sırası; P4, dördüncü laktasyon sırası ve P5, beşinci laktasyon sırası)

SCC decreased gradually up to the fourth month of lactation for both seasons (Figure 3). Inconsistent increases, however, were also observed until the end of lactation. Except for the first and second months of lactation in winter, SCC means of other lactation months were higher in winter season than in summer season (Figure 3). The lower SCC level in summer detected in this study is not consistent with the findings of Erdem et al (2007) and Koç (2011). Significant milking time and season effects on STMY could be explained by the different milking interval depending on the season. Additionally, significant decreases in PC, NFDMC, and STMY, especially in the first half of the lactation, and an increase in SCC in the first two lactation months in summer season could be resulted from a possible heat stress in lactating cows of this herd. Due to the higher temperature and relative humidity, Temperature Humidity Index was over 72 from June to August in the region (Koç 2012). As a



Figure 3- Fluctuations in STMY (kg), PC (%), LC (%), NFDMC (%) and Log₁₀SCC season means during lactation period

Şekil 3- DZSV (kg), PO (%), LO (%), YKMO (%) ve Log₁₀SHS mevsim ortalamalarının laktasyon dönemine göre değişimi

result, the milk production and milk constituents were negatively affected. On the other hand, a higher SCC level observed in the middle and at the end of lactation in winter milkings could be explained by higher rainfall in the region in winter months.

As mentioned for ruminants by Silanikove (1992) and Marai & Habeeb (2010), the decrease in milk yield, PC and NFDMC in summer season in this study could be resulted from the reduction in feed intake and secretion of the milk constituents and the lack of secretory function of the udder which were negatively affected by hot weather.

The finding of the significant effects of SCC on STMY, LC and NFDMC is in agreement with the findings of Barlowska et al (2009), Hagnestam-Nielsen et al (2009), Atasever & Erdem (2009), Erdem et al (2010). Litwinczuk et al (2011) found a progressive decline in daily milk yield as SCC increased in Polish HF cows and concluded that HF cows were more sensitive to mammary gland infections than Simmental and Jersey cows.

The loss in the daily milk yield due to the increase in SCC in present study is similar to the findings of Hagnestam-Nielsen et al (2009) for Swedish HF and RH cows. The daily milk yield loss in RH in this study was lower than in Jersey cows (Erdem et al 2010) and in HF cows (Atasever & Erdem 2009; Juozaitiene et al 2006).

Unlike the results of Litwinczuk et al (2011), the effects of SCC on PC were not found to be significant. However, Forsbäck (2010) stated that the PC did not change significantly despite high SCC, because of the decline in casein content and an increase in whey protein in milk during inflammation.

The results of this study demonstrated that a significant decrease occurred in LC due to the increase in SCC as reported by Barlowska et al (2009) and Forsbäck (2010). The reduction found in NFDMC as SCC increased was mainly depending on the decrease in LC. Forsbäck (2010) indicated that even a moderate increase in SCC resulted in a reduction in LC and also added that this reduction in LC was due to decreased synthesis and loss of circulation.

4. Conclusion

In conclusion, some important and gradual reductions were detected in the STMY and NFDMC as SCC increased. These reductions were severe at greater SCC. Also, a distinct decrease in lactose level occurred as SCC increased. The finding of this study supported the idea that LC of milk could be used as a marker for mastitis. A significant decrease in STMY, especially in the first half of lactation, a decrease in PC through the whole lactation period, and an increase in SCC in the first two lactation months indicated a possible heat stress in RH cows in this herd. Taking some precautions to decrease SCC levels especially in cows at the beginning of lactation in hot summer months would prevent important economic losses in this herd and the herds managed in the regions with hot summer months.

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