

## The Use of Teat-End Hyperkeratosis to Predict Somatic Cell Count and Milk Quality of Holstein Cows Raised in Egypt

S. Abdelghany, N. H. Fahim, F. Samir, & M. A. Radwan\*

Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt

\*Corresponding author: [m.radwan883@agr.cu.edu.eg](mailto:m.radwan883@agr.cu.edu.eg)

(Received 27-08-2020; Revised 12-10-2020; Accepted 04-11-2020)

### ABSTRACT

The study aimed to investigate the association between teat-end hyperkeratosis (THK) and somatic cell count (SCC) and to assess the effect of SCC on milk yield, fat, and protein percentages of Holstein cows. A total of 740 udder quarters of 185 Holstein cows kept in three commercial herds in Egypt were enrolled in the study from May to September 2019. Foremilk samples were collected from udder quarters for assessing milk SCC, fat, and protein percentages. On the same cows, a digital picture of each teat end orifice was taken with a digital camera. Cows' teats were assessed for THK and classified into two different groups of hyperkeratosis; mild and severe THK. SCC was converted to five classes of somatic cell score (SCS). Simple linear regression was performed to determine the loss of milk yield, fat, and protein percentages. SCC was significantly affected by THK ( $p < 0.0001$ ). About 57% of teats were classified as mild THK and 43% as severe THK. The teats with severe THK recorded higher SCC in milk. It was observed that SCS had a significant effect on fat and protein percentages ( $p < 0.0001$ ). A similar trend was shown for the effect of SCS on milk yield ( $p < 0.05$ ), where the highest values of milk traits were obtained for the lowest SCS. The loss of milk was 220 g/day. Also, fat and protein percentages decreased by 0.11% and 0.08%, respectively, for each unit increase in SCS, from zero to four. In conclusion, severe THK can lead to high SCC, which negatively affects milk traits.

*Keywords: hyperkeratosis; milk quality; Holstein; Egypt; somatic cell count*

### INTRODUCTION

Mastitis is considered as the main cause of economic losses in dairy farms in terms of treatment costs, milk quantity, and quality reduction (Le Maréchal *et al.*, 2011; Ruegg & Pantoja, 2013). The incidence of mastitis can be linked with the cow (internal factors), including breed, stage of lactation, parity, milk yield, reproductive problems, and teat and udder conformation. Also, there are factors related to farm management aspects (external factors) such as animal housing, nutrition, udder hygiene, and milking practices (De Vliegher *et al.*, 2012; Miles *et al.*, 2019).

Pinzón-Sánchez & Ruegg (2011) reported that the clinical mastitis (CM) symptoms were graded as mild (abnormal milk, such as clotted milk), moderate (abnormal appearance of milk accompanied by the mammary gland swelling or redness), and severe (changes in milk secretion, mammary gland, and health disorders such as fever). While Vissio *et al.* (2014) reported that sub-clinical mastitis (SCM) is represented up to 80% of the total losses of mastitis disease and is usually demonstrated as an elevation of the SCC. SCM diagnostic approaches are usually based on SCC measurements, where somatic cells are the reflection of resistance and sensitivity of cows to mastitis, particularly SCM (Sharma *et al.*, 2011). Also, SCC is a useful diagnostic tool to detect intra-

mammary infection and as milk assessment in terms of quality, hygiene, and mastitis control (Alhussien & Dang, 2018a). Somatic cells consist of white blood cells (leukocytes) and epithelial cells, which represent about 75% and 25%, respectively. Whereas the function of white blood cells is engulfing and killing bacteria (Sharma *et al.*, 2011). Milk from uninfected cows typically has  $SCC < 200,000$  cells/mL, while cows with  $SCC \geq 200,000$  per mL of milk without clinical symptoms are considered SCM cases (Smith *et al.*, 2001), while the Egyptian standards considered udder is affected by SCM when SCC exceeds 500,000 cells/mL (Egyptian standards of raw milk, 2010).

Somatic cells represent the second line of defense against intra-mammary infection like mastitis, while the primary line of defense is the teat apex and teat canal, so keeping teat skin and teat-end integral is an essential part of successful mastitis prevention (Alhussien & Dang, 2018a). The factors influencing the teat-end changes can be divided into two groups of factors: the first group associated with a cow, which includes teat-end form, the length of the teat, milk yield, days in milk, and parity (Neijenhuis *et al.*, 2000). The second group is linked with machine factors such as vacuum level, slow or overmilking, and pulsation rate, in addition to harsh weather conditions (Mein *et al.*, 2001).

Mein *et al.* (2001) classified changes in the teat-end condition into four categories: (1) Short-term effects include the change in teat color, teat swelling, hardness, and teat orifice; (2) Medium-term effects include change in teat skin condition and vascular injury; (3) Longer-term effects include change in THK; and (4) Very long-term effects include the change in teat size and tissue fibrosis. Moreover, the researchers defined THK as a thickening of the skin that lines the teat canal and surrounds the external teat orifice, and it was classified into four categories: no ring, slightly rough ring, rough, and very rough.

Holstein is the most recognized and common dairy breed in the world. It is considered the most predominant exotic dairy breed in the commercial herds in Egypt. Most studies on Holstein in Egypt focused on productive and reproductive performance. Detecting mastitis depended on measuring milk SCC or California Mastitis Test (CMT), with no available reports depicting a relationship between THK and SCC under the Egyptian conditions. Therefore, the objectives of this study were to investigate the relationship between THK score and milk SCC and to assess the effect of SCC on milk yield, fat%, and protein% of Holstein cows raised in three commercial herds in Egypt.

## MATERIALS AND METHODS

### Study Location

This study was conducted on three commercial dairy farms in Egypt during the period from May to September 2019. The first farm is located in Alexandria Governorate (220 km northwest of Cairo, at a latitude 31°12'56.3"N and a longitude 29°57'18.97"E); the second farm is located in Sharqia Governorate (115 km northeast of Cairo, at a latitude 30° 36' 58.25"N and a longitude 31° 44' 6.50"E) and the third farm is located in Fayoum Governorate (120 km southwest of Cairo, at a latitude 29°18'35.82"N and a longitude 30°50'30.48"E).

### Animals

A total number of 185 Holstein cows were enrolled in this study. Cows in the three farms were in the first 100 days of lactation and from the first to the fourth parity. Cows participating in the study didn't show any visible signs of clinical mastitis such as swelling, heat, and hardness or abnormalities in milk such as watery appearance, flakes, or clots. Regarding animal ethical code of ethics, all participated authors followed the international, national, and institutional guidelines (Institutional Animal Care and Use Committee (CU-IACUC), Cairo University with approval number: (CU II F 31 20) for humane animal treatment and complied with relevant legislation.

### Herd Management

Management practices in the three farms were almost similar, where the cows were housed in free-stall barns with a sandy floor and shaded with corrugated

metal sheets. Animals were fed total mixed ration (TMR) throughout the year, according to National Research Council requirements. The TMR consisted of corn, soybean, wheat bran, corn silage, alfalfa hay, and vitamins and minerals supplementation. Rations were given three times a day, and freshwater was available all day. Cows were machine milked three times daily in all farms at intervals of 8 hours, beginning at 05:00 am. The milking routine that was applied in Sharqia and Fayoum Farms was foremilk stripping, pre-dipping the teats, drying the teats with a clean, dry cloth or towel, attaching the milking unit, and post-dipping. Alexandria farm was conducted the same milking routine with washing udder as additional practice and then wiped the teats with a towel per cow before stripping step to ensuring no water remained on the udder. The milking vacuum level in the three farms was set at 42 kPa. The daily milk yield of the enrolled cows in three farms was recorded using a herd management software system (Dairy Comp 305).

### Milk Sampling and Analysis

Milk sampling was using sterile tubes. 50 mL of foremilk samples were collected during the second milking (at 01:00 pm) from 740 udder quarters. Milk samples were kept in the icebox until transport to the laboratory at the Faculty of Agriculture, Cairo University. Samples were stored at 4°C and analyzed within 6-12 hours after collection. Fat%, protein%, and SCC were analyzed using Lactoscan MCC Combo, Ultrasonic milk analyzer, and somatic cell counter in one device (Stara Zagora, Bulgaria). Cows participating in this study were inspected just once.

### Somatic Cell Score (SCS)

To achieve the normality of distribution, SCC had been transformed to SCS according to the equation of Ali & Shook (1980) as follows:  $SCS = \log_2 (SCC/100,000) + 3$ . Values of SCS were: (0) <25,000 SCC/mL; (1) =25,000:49,999 SCC/mL; (2) =50,000:99,999 SCC/mL; (3) =100,000:199,999 SCC/mL; (4) ≥200,000 SCC/mL. The same authors add that the advantage of this scale (SCS) was associated linearly to losses due to mastitis infection in milk yield.

### Teat-End Hyperkeratosis (THK)

THK is a term used to describe a thickened smooth keratin ring or extending fronds of keratin around the teat orifice. To identify changes in teat end for roughness and callosity, THK was assessed and classified. THK was evaluated immediately after milking and before post-dipping of teats. All observations were carried out by the same person. THK was classified into four categories (Figure 1) according to Mein *et al.* (2001) as follows: class 1 (no ring), which means smooth teat end with a small even orifice; class 2 (smooth or slightly rough ring), which means a raised ring around the orifice with no roughness or slightly rough and no fronds of old keratin; class 3 (rough ring), which means a raised roughened ring with isolated fronds of old keratin ex-

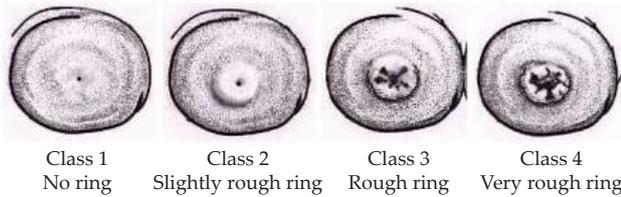


Figure 1. Scoring system for teat-end hyperkeratosis (Mein et al., 2001).

tending 1-3mm from the orifice; and class 4 (very rough ring), which means the rim of the ring roughened and cracked with old keratin extending more than 4mm from the orifice.

Emre & Alaçam (2015) studied Holstein cows and evaluated THK into six classes: (0) Normal teat-end orifice, (1) Smooth ring, (2) Slightly rough ring of keratin, (3) Moderate raised, smooth ring, (4) Rough ring, and (5) Very rough ring. The authors merged the six classes of THK into three categories: (1) Normal teats, (2) Mild hyperkeratosis, and (3) Severe hyperkeratosis.

In the present study, it was difficult to differentiate between classes 1 and 2 when assessing the most teats. So, we merged them into one category. The same was done for classes 3 and 4. Thus, the four classes were combined with being two categories, category 1 (no ring and slightly rough ring teats) versus category 2 (rough ring and very rough ring teats). THK scoring was detected via the photography method with a digital camera Kaiser Baas X4 Action Camera (12-megapixel resolution 4000x3000 4:3; Melbourne, Australia). The photos of THK were uploaded to a computer and scored in the laboratory instead of the direct optical assessment in the milking parlor to avoid interference with milking routines. Subsequently, results of individual SCC were matched with a score of THK. The distribution of udder quarters, according to the parameters under study, is presented in Table 1.

**Studied Traits**

1- Quarter daily milk yield (QDMY): This trait was calculated according to Bogucki (2018) as follows: right front quarter produced 21.3%, left front quarter 21.3%, right rear quarter 28.6%, and left rear quarter 28.8% of the cow’s total daily milk yield. 2- Somatic Cell Count (SCC). 3- Milk Fat percentage. 4- Milk Protein percentage.

**Statistical Analysis**

Data were analyzed using XLSTAT version 2019.1.2. according to the two subsequent models:

Model 1: was used for analyzing SCC.

$$Y_{ijklm} = \mu + T_i + F_j + P_k + Q_l + e_{ijklm}$$

where,  $Y_{ijklm}$  was SCC in each quarter per each cow;  $\mu$ = overall mean,  $T_i$  was the effect of the  $i$ th THK ( $i=1, 2$ ); where 1 was no ring or slightly rough ring teat, and 2 was rough or very rough ring teat,  $F_j$  was the effect of the  $j$ th farm ( $j=1, 2, 3$ ); where 1 was Alexandria, 2 was

Sharqia, and 3 was Fayoum;  $P_k$  was the effect of the  $k$ th parity ( $k=1, 2, 3, 4$ );  $Q_l$  was the effect of the  $l$ th quarter position ( $l = 1, 2, 3,4$ ); where 1 was right front, 2 was left front, 3 was right rear, and 4 was left rear); and  $e_{ijklm}$  was a random residual effect.

Model 2: was used for analyzing QDMY and fat and protein percentages.  $Y_{ijkl} = \mu + S_i + F_j + P_k + e_{ijkl}$  where,  $Y_{ijkl}$  was dependent variables;  $\mu$  was overall mean;  $S_i$  was the effect of the  $i$ th SCS ( $i= 0, 1, 2, 3, 4$ ); where  $0<25,000$  SCC/mL,  $1=25,000:49,999$  SCC/mL,  $2=50,000:99,999$  SCC/mL,  $3=100,000:199,999$  SCC/mL, and  $4\geq 200,000$  SCC/ mL;  $F_j$  was the effect of the  $j$ th farm ( $j= 1, 2, 3$ ); where 1 was Alexandria, 2 was Sharqia, and 3 was Fayoum;  $P_k$  was the effect of the  $k$ th parity ( $k= 1, 2, 3, 4$ ); and  $e_{ijkl}$  was residual error.

All possible interactions were tested and found not significant; therefore, they were excluded from the models. Simple linear regression of QDMY and fat and protein percentages on SCS classes were studied to assess the effect of SCS on milk yield, fat%, and protein%.

**RESULTS**

**Somatic Cell Count (SCC)**

The least-squares means and standard errors of SCC as affected by THK, farm, parity, and udder quar-

Table 1. Percentage distribution of the udder quarters according to parameters studied

Variables	No.	Percentage
THK		
1	425	57.40
2	315	42.60
SCS		
0	238	32.16
1	80	10.81
2	89	12.03
3	105	14.19
4	228	30.81
Farm		
Alexandria	280	37.84
Sharqia	340	45.94
Fayoum	120	16.22
Parity		
1 <sup>st</sup>	196	26.49
2 <sup>nd</sup>	288	38.92
3 <sup>rd</sup>	108	14.59
4 <sup>th</sup>	148	20.00
Quarter position		
Right front	185	25.00
Left front	185	25.00
Right rear	185	25.00
Left rear	185	25.00

Note: THK= Teat-end hyperkeratosis (1= no ring or slightly rough ring teat, 2= rough or very rough ring teat); SCS= Somatic cell score (0<25,000 Somatic cell count, 1=25,000: 49,999 SCC, 2=50,000:99,999 SCC, 3=100,000:199,999 SCC, 4≥200,000 SCC); No.= number of udder quarters.

ter position were presented in Table 2. The overall mean of SCC was about 195,000 cells/mL. THK had a significant effect ( $p < 0.0001$ ) on SCC. The highest mean of SCC was observed with the score 2 (442,657 cells /mL), representing 42.6% of the total examined teats. Furthermore, SCC was significantly influenced by the farm ( $p < 0.003$ ). The lowest mean of SCC was about 175,000 cells/mL in Alexandria farm.

Table 2 shows that there was no significant effect between parity and udder quarter position on SCC. Where, the lowest milk SCC was recorded during the second parity (213,750 cells/mL), while the highest SCC value was observed in the fourth one (302,024 cells/mL). Regarding udder quarter position, the values of SCC in the four quarters ranged from 220,000 cells/mL (in right front) to 305,000 cells/mL (in right rear).

### Quarter Daily Milk Yield (QDMY)

The least-squares means and standard errors of QDMY as affected by SCS, farm, and parity were presented in Table 3. The overall mean of QDMY was 6.14 kg, which means that the average daily milk yield is about 25 kg for each cow in the three farms. The SCS significantly affected QDMY ( $p < 0.05$ ). The values of QDMY ranged from 6.14 kg for score zero ( $SCC < 25,000$ /mL) to 5.22 kg for score 4 ( $SCC \geq 200,000$ /mL) (Table 3).

The farm had a significant effect ( $p < 0.0001$ ) on QDMY, Alexandria farm had a higher value for QDMY (6.94 kg) than the other two farms, this may be attributed to the lowest milk SCC of Alexandria farm (175,000

cells/mL). In contrast, the Fayoum farm had the lowest value of QDMY (3.86 kg), which could be associated with the highest SCC value, i.e., 332,935 cells/mL (see Table 2).

Parity also had a significant effect ( $p < 0.001$ ) on QDMY, which increased gradually by increasing the parity number. The lowest QDMY was found with the first parity (5.28 kg), while the highest QDMY was observed in the fourth (6.28 kg).

### Fat and Protein Percentages

The least-squares means and standard errors of fat and protein percentages as affected by SCS, farm, and parity are presented in Table 4. The overall means of fat and protein percentages in foremilk were 2.80% and 3.51%, respectively, of studied Holstein cows. SCS had a significant effect ( $p < 0.0001$ ) on fat and protein percentages, where the highest values of fat and protein percentages were obtained with SCS class zero ( $SCC < 25,000$  cells/mL), and the lowest values of fat and protein percentages were observed with SCS class 4 ( $SCC \geq 200,000$  cells/mL).

The farm had a significant effect on the fat percentage ( $p < 0.001$ ), but there was no significant effect on protein percentage. The highest fat percentage was found at Fayoum farm (2.93%), and the lowest value was recognized in Alexandria farm (2.68%). However, parity had no significant effect on the percentages of fat and protein. The first parity showed the highest fat percentage value after that, and fat content tended to decrease in the next parities (Table 4).

Table 2. Least-squares means (LSM) and standard error (SE) of somatic cell count (SCC) as affected by teat-end hyperkeratosis, farm, parity, and udder quarter position

Classification	No.	SCC /mL	
		LSM±SE	P-value
Overall mean	740	194,891±16,939	
THK			<0.0001
1	425	66,861±24,000 <sup>a</sup>	
2	315	442,657±25,924 <sup>b</sup>	
Farm			0.003
Alexandria	280	175,191±31,711 <sup>a</sup>	
Sharqia	340	256,151±26,915 <sup>ab</sup>	
Fayoum	120	332,935±35,254 <sup>b</sup>	
Parity			0.270
1 <sup>st</sup>	196	246,816±34,108	
2 <sup>nd</sup>	288	213,750±27,981	
3 <sup>rd</sup>	108	256,445±44,946	
4 <sup>th</sup>	148	302,024±36,754	
Quarter position			0.210
Right front	185	219,364±35,214	
Left front	185	225,992±33,727	
Right rear	185	305,658±32,864	
Left rear	185	268,022±32,593	

Note: THK= Teat-end hyperkeratosis (1= no ring or slightly rough ring teat, 2= rough or very rough ring teat); No.= number of udder quarters. Means in the same column with different superscripts differ significantly ( $p < 0.05$ ).

Table 3. Least-squares means (LSM) and standard error (SE) of quarter daily milk yield (QDMY) as affected by somatic cell score, farm, and parity

Classification	No.	SCC /mL	
		LSM±SE	P-value
Overall mean	740	6.14±0.13	
SCS			<0.05
0	238	6.14±0.21 <sup>a</sup>	
1	80	5.91±0.31 <sup>ab</sup>	
2	89	5.70±0.28 <sup>ab</sup>	
3	105	5.53±0.33 <sup>ab</sup>	
4	228	5.22±0.19 <sup>b</sup>	
Farm			<0.0001
Alexandria	280	6.94±0.19 <sup>a</sup>	
Sharqia	340	6.31±0.15 <sup>b</sup>	
Fayoum	120	3.86±0.21 <sup>c</sup>	
Parity			<0.001
1 <sup>st</sup>	196	5.28±0.20 <sup>a</sup>	
2 <sup>nd</sup>	288	5.37±0.22 <sup>a</sup>	
3 <sup>rd</sup>	108	5.88±0.25 <sup>ab</sup>	
4 <sup>th</sup>	148	6.28±0.17 <sup>b</sup>	

Note: SCS= Somatic cell score (0<25,000 Somatic cell count, 1=25,000:49,999 SCC, 2=50,000:99,999 SCC, 3=100,000:199,999 SCC, 4≥200,000 SCC); No= number of udder quarters. Means in the same column with different superscripts differ significantly ( $p < 0.05$ ).

**Regression Analysis**

Based on a simple linear regression analysis of QDMY and fat and protein percentages on SCS, the following equations have resulted from the regression analysis:

**The first equation.**  $y = -0.220x + 6.367$ , ( $R^2=0.991$ )  
 Where y was dependent variable (QDMY), (-0.220) was the regression coefficient of QDMY on SCS, which determines the amount of decrease in QDMY due to the increasing SCS, by one unit from zero to four to be 220g (Figure 2), x was independent variable (SCS), and (6.367) was the intercept.

**The second equation.**  $y = -0.116x + 2.992$ , ( $R^2=0.995$ )

Where y was dependent variable (Fat%), (-0.116) was the regression coefficient of fat% on SCS, which determines the amount of decrease in fat percentage at the quarter level due to increasing SCS, by one unit from zero to four equals to 0.11% (Figure 3), x was independent variable (SCS), and (2.992) was the intercept.

**The third equation.**  $y = -0.078x + 3.845$ , ( $R^2=0.961$ )  
 Where y was dependent variable (Protein%), (-0.078) was the regression coefficient of protein% on SCS, which determines the amount of decrease in protein percentage at the quarter level due to increasing SCS, by one unit from zero to four equals to 0.078% (Figure 3), x was independent variable (SCS), and (3.845) was the intercept.

Table 4. Least-squares means (LSM) and standard error (SE) of fat and protein percentages as affected by somatic cell score, farm, and parity

Classification	No.	Fat %		Protein %	
		LSM±SE	P-value	LSM±SE	P-value
Overall mean	740	2.80±0.03		3.51±0.02	
SCS <sup>3</sup>			<0.0001		<0.0001
0	238	2.90±0.05 <sup>a</sup>		3.74±0.04 <sup>a</sup>	
1	80	2.83±0.07 <sup>ab</sup>		3.60±0.05 <sup>b</sup>	
2	89	2.80±0.07 <sup>ab</sup>		3.51±0.05 <sup>b</sup>	
3	105	2.67±0.06 <sup>cb</sup>		3.37±0.05 <sup>c</sup>	
4	228	2.58±0.05 <sup>c</sup>		3.27±0.04 <sup>c</sup>	
Farm			0.001		0.741
Alexandria	280	2.68±0.05 <sup>b</sup>		3.50±0.03	
Sharqia	340	2.66±0.04 <sup>b</sup>		3.49±0.03	
Fayoum	120	2.93±0.05 <sup>a</sup>		3.47±0.04	
Parity			0.071		0.654
1 <sup>st</sup>	196	2.84±0.05		3.50±0.04	
2 <sup>nd</sup>	288	2.76±0.04		3.47±0.03	
3 <sup>rd</sup>	108	2.74±0.06		3.51±0.04	
4 <sup>th</sup>	148	2.68±0.05		3.52±0.04	

Note: SCS= Somatic cell score (0<25,000 Somatic cell count, 1=25,000:49,999 SCC, 2=50,000:99,999 SCC, 3=100,000:199,999 SCC, 4≥200,000 SCC); No= number of udder quarters. Means in the same column with different superscripts differ significantly (p<0.05).

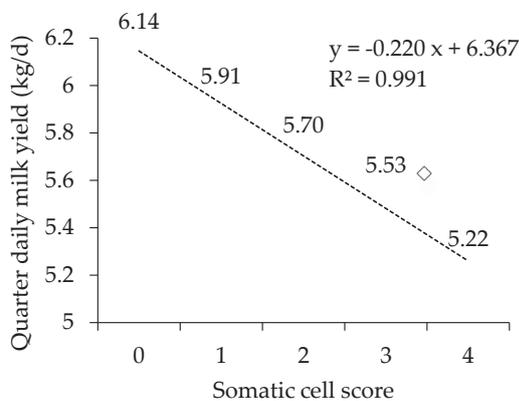


Figure 2. Effect of somatic cell score on quarter daily milk yield (-◇-)

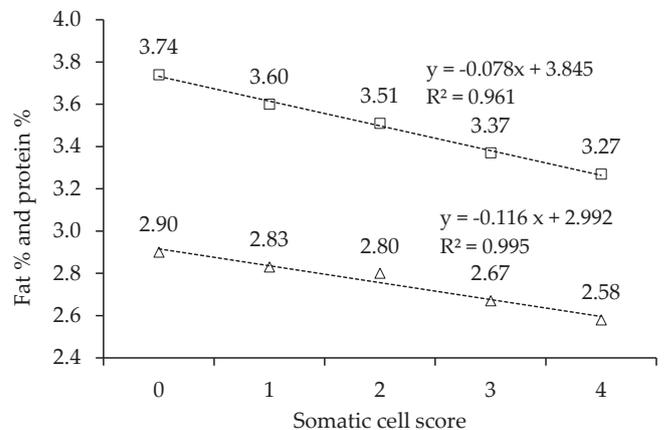


Figure 3. Effect of somatic cell score on fat % (-△-) and protein % (-□-).

## DISCUSSION

### Somatic Cell Count (SCC)

The classification of THK into scores can be useful as an important tool to identify the role of THK as a risk factor for the incidence of mastitis (Manzi *et al.*, 2012). In this study, udder quarters with severe THK have a higher SCC. This result is similar to that found by Emre & Alaçam (2015) on Holstein cows, who reported that a severe THK was associated with SCC  $\geq 200,000$  cells/mL. Mitev *et al.* (2012); Cardozo *et al.* (2015); Juozaitiene *et al.* (2019) showed an extreme degree of hyperkeratosis associated with an increase of milk SCC as a reaction to intramammary infection such as subclinical mastitis. Mein (2012) interpreted the increase of SCC with a severe THK because teats with thickness or roughness have less tightly teat canals after milking, so they can be penetrated more easily by microorganisms. In contrast, the study conducted by Zoche-Golob *et al.* (2015) on Holstein cows in Germany concluded that there was no effect of various THK scores on the risk of new intramammary infections or mastitis.

All farms performed almost the same milking routine, but in Alexandria farm, there was better control during milking, and bedding was cleaner than that of the other two farms. This result explains the importance of applying a good milking routine as well as good environmental hygiene in dairy farms. The significant effect of the farm on SCC were also by Sadeghi-sefidmazgi & Rayatdoost-baghal (2014), Sandrucci *et al.* (2014), Cardozo *et al.* (2015) in Holstein dairy farms.

Although SCC increased with the increasing number of parities, the differences did not reach the level of significance. A similar result was mentioned by Alhussien & Dang (2018b) on Karan Fries (Holstein-Friesian  $\times$  Tharparkar) cows in India. They found no differences in milk SCC up to the fourth parity, but for cows with more than four parities, SCC increased significantly. Mitev *et al.* (2012) observed a higher milk SCC with an increasing parity number, which could be due to the longer effect of the milking machine and occurring teat end changes, promoting the invasion of bacteria and thus increasing SCC. The significant effect of parity on SCC was also reported by (Asadpour *et al.*, 2015; Sebastino *et al.*, 2020).

The effect of the udder quarter position on SCC was not significant, although the rear quarters had higher SCC than the front quarters. The same conclusion was found by Schwarz *et al.* (2010); Badiuzzaman *et al.* (2015); Singh *et al.* (2017), studied on Holstein Friesian cows, crossbred dairy cows (Sahiwal  $\times$  Deshi), and Frieswal dairy cows, in Germany, Bangladesh, and India, respectively. The authors attributed the association between increasing SCC and rear quarters may be due to rear quarter teats are often closer to the floor, particularly in older cows, and would, therefore, be more readily contaminated or injured with greater exposure to environmental effects. Guarín *et al.* (2017) in Wisconsin, USA, on Holstein cows, demonstrated that teat length and teat-end shape were not associated with SCC, while there was a tendency for the reduced SCC

with the increased teat barrel diameter regardless of quarter position. Manzi *et al.* (2012) also stated that there was no association between susceptibility to mastitis and teat-end shape. On the contrary, Miles *et al.* (2019) studied Holstein cows in New York, observed that teats with a flat teat-end shape were associated with elevated SCC and more susceptible to mastitis. Also, Sharma *et al.* (2016) concluded that inverted and flat teat-end shape and large teat length had a significant incidence of SCM, in Tharparkar cows in India.

### Quarter Daily Milk Yield (QDMY)

The results of our study indicate that the QDMY decreased by increasing SCS. The percentage of samples of score 4 equals about 30%. Silva *et al.* (2018); Franzoi *et al.* (2019) in Brazil and Italy, respectively, reported similar findings to those observed in this study for decreasing milk yield with the increase of SCS in Holstein cows. A decrease in milk yield with an increase of SCS is attributed to two main groups of reasons. The first group includes physical damage of epithelial cells in the udder, the mammary gland's synthetic and secretory ability would be substantially decreased (Le Maréchal *et al.*, 2011). The second group involves change most energy orientation from milk production towards the immune system, and also decreased feed intake due to pain and less activity (Alhussien & Dang, 2018b). Furthermore, cows do not return to their performance before mastitis. Even after antibiotic treatment, the milk yield level does not improve, where clinical mastitis was associated with a loss of about 400 kg of milk at the next lactation (Bar *et al.*, 2007).

Management practices have been consistently related to SCC levels in milk, resulting in a positive or negative effect on milk production, so farmers need to develop their management practices to monitor subclinical mastitis. The current finding on the farm's effect on milk yield was confirmed by Sadek *et al.* (2014) on Holstein cows in Egypt.

There was a positive association between parity and QDMY. This result agrees with the findings of Yang *et al.* (2013), who stated that on Holstein cows in the north of China, daily milk yield in the first parity was lower than other parities ( $p < 0.05$ ), where the daily milk yield was 25.94, 28.45, 28.81, 29.05 kg/day in the first, second, third, and fourth parities, respectively. In another study carried out in Mexico, Ríos-Utrera *et al.* (2013) reported that milk yield per lactation and milk yield per day increased with increasing lactation number of Holstein Friesian and Brown Swiss cows.

### Fat and Protein Percentages

The obtained overall means for fat and protein percentages estimated from the foremilk (2.80%, 3.51%) were close to those reported by Khatun *et al.* (2019), who found that the means of fat and protein percentages were 2.83% and 3.41%, respectively, using foremilk samples from Holstein-Friesian cows in Australia. Most of the studies on raw milk composition of Holstein cows in Egypt reported that milk fat

percentage ranged from 2.97% to 3.74% (Zeinhom *et al.*, 2016; Nasr & El-Tarabany, 2017). In a study of Holstein cows in Denmark, to investigate the effects of sampling time during milking on the concentration of milk components, including fat percentage, Nielsen *et al.* (2005) found that milk fat increases significantly as the milking process progresses. The findings in this study have shown a clear association between the increase of milk SCS and the decrease of fat and protein percentages. These results are in agreement with the findings of Franzoi *et al.* (2019), who found that the greatest values of fat and protein percentages were observed with the lowest SCS class for Holstein Friesian, Brown Swiss, and Simmental cows. Also, Kul *et al.* (2019) reported that high milk SCC was associated with a reduction in the content of milk fat and protein of Holstein cows. Milk fat globule membranes are susceptible to the action of lipase enzymes, produced by leukocytes that invade the mammary gland in response to infection. This leads to a breakdown of triglycerides, oxidation of fatty acids, and hydrolysis of lipoproteins, this is a reason that explains why fat percentage decreased by the increase of SCC (Le Maréchal *et al.*, 2011). Also, the same authors reported that when milk SCC increases, protein profile changes, whey protein levels increase, and  $\alpha$ - and  $\beta$ -casein decrease, and these changes may be due to the hydrolysis of milk protein, besides, synthesis of casein is reduced during mastitis.

Fayoum farm had the lowest daily milk yield (3.86 kg), and Alexandria farm had the highest daily milk yield (6.94 kg). Thus, the Fayoum farm had the highest milk fat percentage. In other words, the fat percentage tended to increase with a decrease in milk production. Feltes *et al.* (2016); Wongpom *et al.* (2017) showed a significant negative correlation between milk yield and fat percentage on Holstein cows in Brazil and Thailand, respectively.

There was no significant effect of parity on fat and protein percentages. This result agrees with the findings of Gurmessia & Melaku (2012), who reported the not significant differences among primiparous and multiparous cows in fat and protein percentages of milk, using crossbred Holstein Friesian cows in Ethiopia. Heinrichs *et al.* (2016) found that milk fat and protein percentages decreased by 0.2% and 0.03%, respectively, each year from the first to the fifth lactation. This decline in fat and protein percentages could be attributed to increasing milk yield with advancing parity. In contrast, Kul *et al.* (2019) recorded a higher milk fat content in the second parity and subsequently decreased ( $p < 0.05$ ).

### Regression Analysis

In the current study, there was a reduction in milk production by 220 g/day. Furthermore, fat and protein percentages decreased by 0.11% and 0.08%, respectively, at the quarter level, for each SCS class higher than zero. Halasa *et al.* (2009) reported that the expected loss of milk production, fat, and protein for cows with SCC (200,000 cells /mL, corresponding to SCS =4) were 0.31 and 0.58 kg/d for milk production, 9 and 13 g/d for fat, and 6 and 10 g/d for protein, for primiparous and mul-

tiparous cows, respectively. With each SCS unit, more than one, milk production, fat, and protein decreased by 0.43, 0.01, and 0.01 kg /d, respectively (Franzoi *et al.*, 2019). The same authors reported that very low SCC <6,250 cells/mL had negative effects on milk yield and quality. Therefore, SCC among 12,500:25,000 cells/mL (SCS from zero to one) was considered the best SCS for milk yield and quality. Moreover, optimal SCS estimated ( $R^2$ ) for milk yield, fat, and protein percentages, based on a third-order polynomial regression, which resulted in  $R^2 \geq 0.96$  ( $p < 0.001$ ). Further studies are needed, with consideration of collecting monthly milk samples throughout the lactation period, which would likely provide a lot of information about THK or teat-end condition as a diagnostic tool to monitoring udder health and milk quality.

### CONCLUSION

From the results obtained in the present study, it could be concluded that there is a relationship between THK and SCC. The severity of THK was associated with an increase in SCC in milk and a greater risk of subclinical mastitis at the quarter level. As a result, milk production and its fat and protein percentages were decreased. Using THK assessment to detect milk SCC could be used as a simple and inexpensive tool to assess milk quality at the farm level. Also, to evaluate milking practices that cause the severity of THK, particularly in developing countries.

### CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with any financial, personal, or other relationships with the other people or organizations related to the material discussed in the manuscript.

### ACKNOWLEDGEMENT

The authors would like to express their gratitude to Dr. Mohamed Abd El-Aziz Ibrahim, Professor of Animal Husbandry, Faculty of Agriculture, Cairo University, for his great help in the statistical analysis of data. Also, the authors would like to appreciate Dr. Rabie Ragab Sadek Professor of Animal Husbandry, Faculty of Agriculture, Cairo University, for his valuable advice through writing the manuscript. Funding of this study provided by Academy of Scientific Research and Technology (ASRT) through Agricultural Research in the Mediterranean (ARIMNET), project CDCMCT ARIMNet2 2017 for young researchers, grant agreement N° 618127.

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