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Impact of Subclinical Mastitis on Milk Quality in Different Seasons

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ABSTRACT

This study was carried out to evaluate the influence of subclinical mastitis on chemical constituents of cow's milk samples in different seasons; which collected from 444 apparently healthy Holstein Frisian dairy animals during the period of December 2016 to November 2017 in a private dairy farm located in Fayoum district, Egypt. The results of chemical analysis of the milk samples along the four seasons of the year which done by the Lactoscan SLC milk analyzer device revealed that; the values of mean for (Fat%, Protein%, SNF%, Lactose%, Salt% and SCC/ml) in winter were (2.22±0.06, 2.90±0.08, 7.79±0.22, 4.14±0.12, 0.72±0.02 and $6.9\times10^5\pm1.9\times10^4$ respectively).While the mean values of the same parameters in spring were (2.72±0.23, 2.58± 0.22, 6.93±0.58, 3.67±0.31, 0.64±0.05 and $9.2\times10^5\pm7.7\times10^4$ respectively). In the summer, the mean values of (Fat%, Protein%, SNF%, Lactose%, Salt% and SCC/ml) were (2.35± 0.24, 2.80 ±0.28, 7.55±0.76, 4.00±0.40, 0.70±0.07 and $7.7\times10^5\pm7.7\times10^4$ respectively), while the mean values in the autumn were (2.89±0.09, 2.69±0.08, 7.25±0.21, 3.84±0.11, 0.67±0.02 and $11.0\times10^5\pm3.2\times10^4$ respectively). The analysis of findings specified that; there is a significant difference ($P<0.05$) in fat % and SCC between winter and autumn, however there is a significant difference ($P<0.05$) between (winter and autumn) and (winter and spring) in the parameters of protein, SNF and lactose content. The results reveal that there is a strong negative correlation between SCC and fat %; however there is a strong positive correlation between SCC and salt %.

Key words: Chemical constituents, Subclinical mastitis, Seasons

INTRODUCTION

Milk is considered as one of the most important foods for human and animals, and acts as a complete diet due to its crucial components such as carbohydrate, proteins, fats, vitamins and minerals (Mia *et al.*, 2016). Milk composition is highly dependent, that can be greatly affected by many factors as animal's health status, especially of the mammary gland health, photoperiod effect of different seasons**,** animal's diet (as higher concentrate intake during dry season), genetic factors and the milk storage temperature (Nóbrega and Langoni, 2011).

Also the inflammation of the mammary gland leads to changes in the chemical composition of milk either due to local effects on udder tissue or due to the movement of some normal milk components out of the alveolar lumen into the perivascular space or entering of the serum components to the milk (Paixao *et al.*, 2017). The Poly-Morph Nuclear cells (PMN) normally flow freely or roll through capillaries with only minimal adherence to vessel walls. Adhesion molecules are expressed and PMN adhere to the endothelium of smaller blood vessels and pass between cells lining the vessel during inflammation. The speed of the influx of PMN is believed to be a key factor in the resolution of an infection and the severity of the disease (Sharma *et al.*, 2017).

The phagocytic capability of PMN of cow's milk can consume parts of butter fat globules and casein, so milk composition can be affected by a wide array of factors as the inflammatory reaction of the mammary gland to the infection and the season. So the elevated Somatic Cell Count (SCC) has a multitude effects on the quantity, quality of the milk and may cause some changes in the spectra of milk because of alterations in milk proteins as well as in the concentrations of some minerals which altered during SCM (Lima *et al.*, 2018). So, early identification of udder health problems is essential for dairy farmers and veterinarians to ensure not only the animal well-being but also the milk quality (Youssif *et al.*, 2019).

Climate changes are likely to be one of the main challenges that affect mastitis incidence, also has a great effect on the variation of biochemical composition and technological properties of milk and dairy products. During the summer, daytime was always high which is considered the upper critical cause for lactating dairy

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animals. Moreover, during the night-time also cows couldn't be completely recovered from the heat stress imposed by the daytime. In contrast, the spring and autumn periods was characterized by a period of thermal comfort (Bernabucci *et al.,* 2015). Reduction of some milk chemical parameters percentage has already been reported by several authors in heat-stressed dairy cows**.** Therefore, this research was carried out for description the changes in milk composition which occur during subclinical mastitis at different seasons.

MATERIALS AND METHODS

Collection of samples

Cow's milk samples (n=444) were collected according to Radostits *et al.* (2010) from apparently healthy Holstein Frisian dairy animals during the period from December 2016 to November 2017 in a private dairy farm located in Fayoum district, Egypt, which suspected to harbor SCM based on: California Mastitis Test (CMT) according to Schalm *et al.* (1971), Electrical Conductivity (EC) according to Linzell and Peaker (1971) and Somatic Cell Count (SCC). The investigation period was divided into the four seasons of the year.

Determination the major constituents of collected milk samples

The cow's milk samples that expressed positive for SCM were examined by Lactoscan SLC milk analyzer device for measuring of (Fat %, Lactose %, Protein %, Salt % and SNF %).

Determination of somatic cell count (SCC/ml) according to Zecconi *et al.* (2002)

The Somatic Cell Count was estimated for 1145 quarter milk samples which suspected to harbor SCM using Bentley soma count 150 (115 Volts/ 60Hz/ 2Amps) (Chaska, MN 55318, United States), The device measured the total number of milk SCC per ml of milk (MSCC/ml). According to the National Mastitis Council (2015); Cow's SCC number above 200,000 were used as the threshold for subclinical infection.

Statistical analysis according to SPSS, version 25

The analysis of variance (ANOVA) test was conducted to test the possible significance ($P \leq 0.05$) among mean values of parameters using Fishers Least Significance Difference (LSD).

RESULTS

The statistical analysis of constituents of the examined SCM milk samples along the four seasons

The results of chemical analysis of the SCM cow's milk samples that collected from 444 apparently healthy Holstein Frisian dairy animals during the period of December 2016 to November 2017 (Table 1) revealed that; the mean values of (Fat%, Protein%, SNF%, Lactose%, Salt% and SCC/ml) in winter were (2.22± 0.06, 2.90±0.08, 7.79±0.22, 4.14±0.12, 0.72±0.02 and $6.9\times10^5\pm1.9\times10^4$ respectively). While the mean values of the same parameters in spring were (2.72±0.23, 2.58± 0.22, 6.93±0.58, 3.67±0.31, 0.64±0.05 and $9.2\times10^5\pm 7.7\times10^4$ respectively). In the summer, the mean values of (Fat%, Protein%, SNF%, Lactose%, Salt% and SCC/ml) were (2.35±0.24, 2.80±0.28, 7.55±0.76, 4.00±0.40, 0.70± 0.07 and $7.7\times10^5\pm7.7\times10^4$ respectively), while the mean values in the autumn were (2.89±0.09, 2.69±0.08, 7.25± 0.21, 3.84±0.11, 0.67±0.02 and $11.0\times10^5\pm3.2\times10^4$ respectively).

Significant difference between seasons in different chemical parameters of SCM milk samples

The analysis of findings statistically specified that; there is a significant difference ($P<0.05$) in fat % and SCC between winter and autumn, however there is a significant difference ($P<0.05$) between (winter and autumn) and (winter and spring) in the parameters of protein, SNF and lactose % (Table 2).

Correlation between somatic cell count and other parameters

The results in (Table 3) reveal that there is a strong negative correlation between fat % and the somatic cell count (r=-0.65), However there is a strong positive correlation (r=0.64) between salt % and SCC.

DISCUSSION

Milk is the stable, complete diet and one of the most important foods to humans and animals, because it contains a broad scope of dietary constituents with vital significance like butter fat, protein, lactose, minerals and vitamins (Javaid *et al.,* 2009)**.** Reduced quality is manifested through a reduction in butter fat, protein, lactose % and an increase in (MSCC) and salts percentage. Sub-clinical mastitis is the inflammatory reaction of mammary gland parenchyma regardless of the cause, described by bacterial, physical, chemical changes in milk and pathological changes in the glandular tissues (Radostits *et al.*, 2010).

Generally, fat % is the base of milk sale and the animals that have Intra-mammary Infection (IMI) express lower butter fat % resulting in a loss for the farmer. One of the most apparent reflection of the inflammatory response to the SCM is the increased influx of immune reactive cells from blood into milk especially the SCC, these cells including lymphocytes, macrophages, PMNs and some epithelial cells that considered the main part of the natural defense mechanism (Sharma *et al.,* 2017).

Table 1: Mean ± SE values of different parameters of the examined SCM milk samples along the four-year seasons.

Season	No.	Fat %	Protein %	SNF%	Lactose %	Salt %	No*.	SCC/ml
Winter	143	2.22 ± 0.06	2.90 ± 0.08	7.79 ± 0.22	4.14 ± 0.12	0.72 ± 0.02	369	$6.9\times10^5 \pm 1.9\times10^4$
Spring	65	2.72 ± 0.23	2.58 ± 0.22	6.93 ± 0.58	3.67 ± 0.31	0.64 ± 0.05	168	$9.2\times10^5 \pm 7.7\times10^4$
Summer	139	2.35 ± 0.24	2.80 ± 0.28	7.55 ± 0.76	4.00 ± 0.40	0.70 ± 0.07	358	$7.7\times10^5 \pm 7.7\times10^4$
Autumn	97	2.89 ± 0.09	2.69 ± 0.08	7.25 ± 0.21	3.84 ± 0.11	0.67 ± 0.02	250	$11.0\times10^5 \pm 3.2\times10^4$

No. = Number of examined SCM milk samples; No.* = Number of examined SCM quarter milk samples.

Table 2: Significant difference between seasons in different parameters of SCM milk samples.

Dependent variable	Season		Sig. difference
Fat %		Spring	0.176
	Winter	Summer	0.731
		Autumn	0.014*
		Spring	0.009*
Protein %	Winter	Summer	0.474
		Autumn	0.023*
		Spring	0.008*
SNF %	Winter	Summer	0.473
		Autumn	0.021*
Lactose %		Spring	0.008*
	Winter	Summer	0.463
		Autumn	0.019*
		Spring	0.014*
Salt %	Winter	Summer	0.508
		Autumn	0.041*
		Spring	0.204
SCC/ml	Winter	Summer	0.696
		Autumn	0.003*

***** Significant difference, P-value <0.05.

Table 3: Correlation between Somatic Cell Count (SCC) and milk constituents.

Milk constituents	Correlation coefficient (r)
Fat %	-0.65
Protein %	-0.14
SNF %	-0.15
Lactose %	-0.15
Salt %	0.64

The leukocytes para-cellular diapedesis was enhanced by the damaged milk-synthesizing cells causes decreased tight junction integrity and hence exchange of constituents between blood and milk. The function of PMN in milk is to engulf and digest the invading bacteria, when PMN enter milk they also engulf other particles such as butter fat globules and casein (Darbaz *et al.,* 2018).

Vlkova *et al., (*2017) stated that SCC is the most accurate indicator of SCM and the milk that has SCC exceeding (200,000) cells/ ml generally indicative to Intra-mammary Infection.

The presented data revealed that the highest mean value of SCC/ml of SCM quarter milk samples was ($11.0\times10^5\pm3.2\times10^4$ cells/ml) and this value is nearly similar to results that obtained by Jagadeesh *et al.* (2016). The highest mean value of SCC was recorded in the autumn, followed by spring, summer and the winter respectively.

The results recorded in this study showed decreased the mean values of fat%, Solids Not Fat (SNF%), lactose% and protein% of the subclinical mastitic cow's milk samples in the four seasons of the year than legal level according to (Egyptian standards 1-154/2005) and these results are in accordance with previously reported results (Swami *et al.,* 2017).

The present data revealed that the minimum finding of milk protein, lactose and SNF contents were observed in spring followed by autumn, summer and winter respectively, and this result is in accordance with other data (Zaman *et al.,* 2016). The reduction in milk constituents in spring may be related to the photoperiod effect which involves a series of hormonal changes that affect milk production, Dry Matter Intake (DMI), feeding behavior, reproduction, and growth with a consequent dilution effect on protein (Bertocchi *et al.,* 2014).

However the lowest mean value of milk fat% which observed in winter season, followed by summer, spring and autumn respectively, and these results are in agreement with the data of (bouchelaghem, 2015)**.** In this study, the reduction of fat % may be due to elevation of MSCC caused by SCM and the increase rate of breakdown and lipolysis of butter fat during IMI (Hamid *et al.,* 2012).

The ability of the mammary epithelium to synthetize and secrete the major milk constituents is reduced during udder inflammation. Also, lactose incompletely leaks into blood through the damaged Blood Milk Barrier, while the secretion of lactoferrin is concurrently up-regulated (Isae and Kurtu, 2018)**.** These changes were linked with many factors such as season, environmental conditions, breed, feeding and age**.** However lactose considered as the main osmotically active constituents in milk of healthy quarters, its concentration can only be depressed if the decrease of synthesis is recompensed by the elevated influx of blood-borne electrolytes. Therefore, the lesser lactose concentration is based on the severity of tight junctions' destruction. In this study; the lactose concentrations were not obviously affected and this is may be related to the intact mammary epithelium that prevent extensive electrolyte influx (Alemu *et al.,* 2013)**.**

Lactose and protein were major components of SNF, as the legal value of the SNF % is not less than 8.25 in cow's milk according to (Egyptian standards 1-154/2005), the drop in SNF % in this study was mainly due to decreased of the major components of SCM milk samples**.** In the present study, the decrease in SNF % may be attributed to breakdown of protein due to increase in the activity of a proteolytic enzymes resulting from SCM (Swami *et al.,* 2017).

When inflammation or any destruction to the epithelial tissue takes place, it lead to increase in permeability of the "paracellular" junctions which allows other mammary disorders, plasma constituents may exudate and cause changes in percentage of electrolytes contained in cow's milk so more Na^+ diffuse into the mammary gland, resulting in rise in Na and Cl content that lead to increase the milk Electrical Conductivity. This composition is considered relatively constant, but the concentration of minerals varies with the season and other factors (Gaucheron, 2005).

According to data illustrated in this study; the highest salt content was found in winter season with a mean value of 0.72±0.02 followed by summer, autumn and spring respectively and these results are in accordance with the data reported by Sant'Anna and de Costa (2011)**.** The salt % of the SCM milk samples varied within a small range from one season to another and the difference in its % is may be also related to changes in animal feeding behavior and dilution factor which is related to different pasture composition (more succulent, less fibrous material available for the animal cause drop in the milk mineral output, which cannot be compensated by the provided concentrate) (Hamed *et al.,* 2017).

The statistical analytical results presented in this study specified that; there is a significant difference (P<0.05) in fat % and SCC for SCM milk samples between winter and autumn, moreover there is a significant difference (P<0.05) between (winter and autumn) and (winter and spring) in other parameters (protein, SNF, lactose and salt content) and these results are in line with other data (Nakov and Trajcev, 2013). The climatic issue may be considered one of the main factors that affects the milk constitutes, the main causes of these variations among different seasons can be included the characteristics of the feed (such as cows kept inside and fed silage during winter, kept on pasture, fed fresh grass during spring-summer) and feeding techniques (Hamed *et al.*, 2017).

Milk somatic cell count is the most commonly applied evaluation indicator of the health state of the mammary gland of a cow. Many studies have demonstrated that increased SCC in cow's milk results in decreased milk production and affects milk composition, which causes a reduction in the major milk constituents in cows suffered from SCM (Nesma *et al.*, 2020).

Somatic cells are epithelial, leukocytes and erythrocytes cells which increase in response to bacterial infection, tissue injuries & stress and contain many Lysosomal Enzymes, many of which pass into the milk, where they cause increased proteolysis and lipolysis causes changes in physicochemical properties (Ştefça *et al.*, 2014). According to the obtained results; there is a strong negative correlation between fat % and the SCC (r= - 0.65), however there is a strong positive correlation (r=0.64) between salt % and the SCC and these results are in accordance with other data reported by Swami *et al.* *(*2017), whose recorded that the somatic cell count had a significant effect on milk fat % (P<0.05), as milk fat % decreased with elevations in SCC. In addition, milk salt% gradually increases significantly with increased SCC.

Conclusions

Based on the findings of the present study; it can be concluded that the SCM is characterized by biochemical changes in composition of milk depending on the severity of mammary infection. So, milk composition especially contents of fat, protein and lactose were found to be lower with a significant increase in Salt % and SCC of SCM milk samples. This study showed that measurements of SCC can be an effective screening test for abnormalities in milk constituents. It is also concluded that; there is a strong negative correlation between SCC and fat % and a strong positive correlation between SCC and salt %. Regarding the seasons of the year; the data showed that the spring has the highest negative effect on protein, SNF and lactose, while autumn on SCC and winter on fat and salt %.

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