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Effect of Spirulina on Somatic Cell Count and Milk Quality

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Abstract

Subclinical mastitis is a major problem threating the cows' industry in Egypt. This study aimed to investigate the impact of SCC on the milk composition and evaluate the effect of spirulina supplementation on SCC and milk quality. Total of 270 milk samples were examined using CMT, BacSomatic and MilkoscanTMFT1 system. For evaluation of spirulina effect, ten cows were supplemented with spirulina powder (20 g per head for one month). The prevalence of SCM according to CMT and SCC was 32.2% and 54.4%, respectively. SCC negatively correlated with Fat, protein, lactose, and casein% which had Means of 2.94±0.75, 3.48±0.37, 4.82±0.23, and 2.58±0.39, respectively at SCC (<200×10^{3}cell/ml) while, at SCC above 400×10^{3}cell/ml were 2.43±0.95, 3.24±0.33, 4.61±0.22, and 2.4±0.38, respectively. There was a significant decrease in the average of SCC from 6638.9±4675.9×10^3 to 361.1±321.4×10^{3}cell/ml after 3 weeks of spirulina supplementation. The Mean of Fat, protein, lactose, and casein% were increased from 2.84±0.29, 3.02±0.4, 4.49±0.4 and 2.34±0.28, respectively to reach 3.62±0.16, 3.65±0.43, 4.86±0.41 and 2.59±0.3, respectively and Milk yield increased from average of 21.7±3.23 kg/day to 24.2±2.39kg after 21 days of spirulina treatment. Therefore, milk quality and quantity can be improved by using Spirulina supplementation which reduces SCC.

KEYWORDS
Spirulina, Somatic cell count, Subclinical mastitis, Milk quality.

INTRODUCTION

Milk and its derivatives as cheese, yoghurt, cream, and pasteurized milk are integral food for all age groups of people. Moreover, it is considered one of the most nutritive foods as contain essential nutrients such as fat, protein, carbohydrates, minerals, and vitamins that benefit health. So, it is a cruel necessity to be safely consumed and free from any contaminants as microbiological, physical, and chemical substances (Trombete *et al*., 2014; Shah, 2021).

Somatic cell count is a valuable used indicator of mastitis. Inflammation of mammary gland during mastitis contributes to increase somatic cell count (SCC). SCC is the number of somatic cells in one millilitre of milk with a normal physiological level of less than 200×10^3 cells/mL in healthy milk (Kaskous, 2021). Milk of healthy udder has SCC of a wide range of cells including macrophages (58%), lymphocytes (28%), polymorph nuclear cells (PMN, 12%), and epithelial cells (2%) (Winter, 2010; Alhussien and Dang, 2018). Conversely, the SCC of infected udder has a significant variation in cell proportions with a special increase of PMN cells of up to 90% that protect the udder from invading microbes (Paape *et al*., 2002; Alhussien and Dang, 2018).

Elevation of SCC has a direct reflection on milk quality and quantity which was reported to has a negative correlation with fat, protein, total solids, solids-non-fat contents, and milk yield, in contrast, a positive correlation with lactose content (Zecconi *et al*., 2020; Lianou *et al*., 2021). Interestingly, increasing SCC poses a risk to dairy animal productivity represented by a reduction in milk yield as a result of the damage of milk-producing tissue caused by mastitis pathogens and their toxins (Guimaraes *et al*., 2017; Talukder and Ahmed, 2017).

As a generalized concept, the production of good quality dairy products relies on using high-quality milk with low SCC to avoid additional costs from lower yield, manufacturing difficulties, and final product losses (Sousa *et al*., 2007). Moreover, high SCC milk is a booby trap with a high bacterial load that intimidates human health (Abdi *et al*., 2021).

Over the last number of years, feed additives such as Spirulina supplementations were identified to have many positive benefits making it an optimal choice to improve milk quality by increasing the protein and lactose content of milk and prolonging the lactation period (Christaki *et al*., 2017). The high nutritive value of Spirulina is owing to its high protein content (60-70% by dry matter) with most essential amino acids and fatty acids and a wide range of vitamins and minerals which provides milk with the most nutritionally favorable constituents without the appearance of any side effect on the animal performance on the short term (Jamil *et al*., 2015; Manzocchi *et al*., 2020; El-Deeb *et al*., 2022).

The current research aimed to investigate the prevalence of subclinical mastitis, study the impact of somatic cell count on

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the milk compositional parameters such as fat, protein, lactose, casein, total solid and solid non-fat contents, and evaluate the effect of spirulina supplementation on SCC, milk quality and production.

MATERIALS AND METHODS

Prevalence of subclinical mastitis according to California mastitis test (CMT) and SCC

A total of 270 lactating Holstein cows which belonged to three farms (farm A: n.= 90; farm B: n.= 60 and farm C: n.=120 cows) in Gamsa area, at Dakahlia Governorate, Egypt were examined for subclinical mastitis (SCM) using the California mastitis test according to Schalm *et al*. (1971). Moreover, all milk samples (270 samples) were collected according to Radostits *et al*. (2007) and transferred directly in an ice box to the laboratory of the Animal Health Research Institute for detection of SCC using BacSomatic (AOAC, ISO 13366-2:2006, ISO 16140-2:2016, ICAR guidelines) according to Zecconi *et al*. (2003) and milk parameters (fat, protein, total solid, solid non-fat, lactose, and casein) were assessed using MilkoscanTMFT1 system (Electromagnetic Company Compatibility (EMC) Directive 2014/30/EU, Low Voltage Directive (LVD) 94/62/EU, Packing and Packing waste directive 94/62/EU, WEEE Directive 2012/19/EU, REACH Directive 1907/2006/EC.

Effect of spirulina supplementation on SCC and milk quality and quantity

Experimental design

The experiment was carried out at farm B in Gamsa area. Ten Holstein cows apparently healthy and suffering from subclinical mastitis according to CMT and confirmed by BacSomatic were separately bounded as an experimental group under the same management factors of the farm. The cows were balanced for lactation period, parity, body weight, and age, and were supplemented with flash start- spirulina powder (Amoun Vet Company, Egypt, package of 5 kg containing 250 gm Spirulina) with a dose rate 20 g per head for one month (Christaki *et al*., 2012).

Milk sampling and analysis

Milk samples were collected before and after spirulina supplementation with one-week intervals during the period of the study at 0, 7, 14, and 21 days. Samples were subjected to compositional analysis for the detection of milk parameters (fat, protein, lactose, casein, and urea) by MilkoscanTMFT1 system, and SCC using BacSomatic.

Milk productivity of supplemented cows (milk yield/kg) was calculated at the farm during the period of the study with one-week intervals at 0, 7, 14, and 21 days.

Statistical Analysis

The obtained results were recorded as numbers and percentages, and the average and standard deviation were calculated by SPSS software version 16 (Statistical Package for Social Science).

RESULTS

Prevalence of SCM according to CMT and SCC

In the present study, CMT was used to investigate the prevalence of subclinical mastitis as a field test, the results revealed that 32.2% (87/270) of cows had subclinical mastitis and 67.8% as healthy cases (Table 1). SCC was detected using BacSomatic and the overall prevalence of subclinical mastitis was 54.4% (147/270) which was distributed as 22.2% (60/270) of samples with SCC (200- 400×10^3) and 32.2% (87/270) with SCC (>400×10^3) while the percentage of healthy cases was 45.6% (123/270) of samples with SCC <200×10^3cell/ml (Table 1). Prevalence of SCM in farm A, farm B, and farm C was 64.4%, 10%, and 19.2%, respectively according to CMT, however, it was 74.4%, 28.3%, and 52.5%, respectively on the base of SCC detection.

Table 1. Prevalence of subclinical mastitis according to CMT and SCC of all examined milk samples

Farms	Total	CMT				According to SCC (×10^3)						Total SCM	
		Healthy Cases (Negative)		SCM cases (>400×10^3) positive		Healthy cases		Subclinical cases					
						(<200)		(200-400)		(>400)			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Farm A	90	32	35.6	58	64.4	23	25.6	9	10	58	64.4	67	74.4
Farm B	60	54	90	6	10	43	71.7	11	18.3	6	10	17	28.3
Farm C	120	97	80.8	23	19.2	57	47.5	40	33.3	23	19.2	63	52. 5
Total	270	183	67.8	87	32.2	123	45.6	60	22.2	87	32.2	147	54.4

Table 2. Correlation between milk parameters level and somatic cell count of all examined samples.

Milk Parameters (Mean±SD)	SCC (×10^3)		
	> 200	200 - 400	> 400
Fat%	2.94±0.75	2.7±0.74	2.43±0.95
Protein%	3.48±0.37	3.37±0.29	3.24±0.33
Lactose%	4.82±0.23	4.69±0.39	4.61±0.22
Casein%	2.58±0.39	2.58±0.39	2.4±0.38
SNF %	9.06±0.63	8.9±0.54	8.46±0.81
TS %	12.2±1.69	11.75±1.12	11.21±1.79

Milk composition and SCC

Milk composition was detected using MilkoscanTMFT1 system and the levels of milk parameters were expressed as Mean. Fat, protein, lactose, casein, SNF and TS% were found to have Mean of 2.94±0.75, 3.48±0.37, 4.82±0.23, 2.58±0.39, 9.06±0.63 and 12.2±1.69, respectively at SCC (> 200×10^{3}cell/ml) while, at SCC (200-400×10^{3}cell/ml) were 2.7±0.74, 3.37±0.29, 4.69±0.39, 2.58±0.39, 8.9±0.54, and 11.75±1.12 respectively and in samples with SCC above 400×10^{3}cell/ml were 2.43±0.95, 3.24±0.33, 4.61±0.22, 2.4±0.38, 8.46±0.81 and 11.21±1.79, respectively, (Table 2).

Effect of spirulina supplementation on SCC

SCC levels of spirulina supplemented cows were detected by BacSomatic and showed a significant decrease for all cows with an average of 6638.9±4675.9×10^{3}cell/ml before supplementation of spirulina and reduced to reach an average of 361.1±321.4×10^{3}cell/ml after 3 weeks of spirulina treatment (Table 3).

Effect of spirulina supplementation on milk quality and quantity

MilkoscanTMFT1 system was used for detecting milk composition and the results were expressed as Mean. Fat%, protein%, lactose%, and casein% were 2.84±0.29, 3.02±0.4, 4.49±0.4 and 2.34±0.28, respectively before spirulina addition with an average SCC of 361.1±321.4×10^3 cell/ml and showed a significant increase to reach 3.62±0.16, 3.65±0.43, 4.86±0.41 and 2.59±0.3, respectively after 21 days of spirulina supplementation. The average of urea content (mg/l) decreased during the period of

Table 3. Effect of Spirulina supplementation in the feed of dairy cows onsomatic cell count SCC of milk.

Animal number	SCC (×10^3) Before spirulina	SCC (×10^3) After 7 days	SCC (×10^3) After 14 days	SCC (×10^3) After 21 days
1	5020	5404	749	58
2	3339	968	321	460
3	1616	855	628	588
4	8837	8227	874	855
5	5043	888	855	848
6	16122	15276	8739	129
7	1226	363	131	115
8	12215	1292	644	78
9	6100	5800	750	430
10	6871	5100	270	50
Average	6638.±4675.9	4417.3±4702.5	1396.1±2592.7	361.1±321.4

Table 4. Correlation between levels of the measured milk parameters (Mean) and Spirulina supplementation in the feed of dairy cows.

Milk parameters (mean±SD)	Spirulina supplementation			
	0 day	7 days	14 days	21 days
SCC (×10^3)	6638.9±4675.9	4417.3±4702.5	1396.1±2592.7	361.1±321.4
Fat%	2.84±0.29	3.19±0.17	3.49±0.15	3.62±0.16
Protein%	3.02±0.4	3.30±0.5	3.51±0.67	3.65±0.43
Lactose%	4.49±0.4	4.69±0.3	4.70±0.35	4.86±0.41
Urea mg/l	31.01±6.3	30.27±6.2	30.49±6.6	25.3±5.7
Casein%	2.34±0.28	2.39±0.43	2.56±0.49	2.59±0.3

Table 5. Effect of Spirulina supplementation in the feed of dairy cows on milk production.

Animal number	Before Spirulina		After 3 week of spirulina	
	SCC (×10^3)	Milk production (kg)	SCC (×10^3)	Milk production (kg)
1	5020	23	58	26
2	3339	25	460	27
3	1616	27	588	28
4	8837	20	855	23
5	5043	21	848	23
6	16122	17	129	21
7	1226	25	115	26
8	12215	18	78	22
9	6100	20	430	22
10	6871	21	50	24
Total	66389	217	3611	242
Average±SD	6638.9±4675.9	21.7±3.23	361.1±321.4	24.2±2.39

the experiment which was 31.01±6.3, 30.27±6.2, 30.49±6.6, and 25.3±5.7 at 0, 7, 14, and 21 days, respectively (Table 4).

Milk production of all spirulina-supplemented cows was recorded at 0, 7, 14, and 21 days of spirulina supplementation. The average milk yield increased from 21.7±3.23 kg/day at 0 day (before spirulina supplementation) to 24.2±2.39kg/day after 21 days of spirulina treatment, (Table 5).

DISCUSSION

Mastitis is the most ubiquitous disease among dairy animals in Egypt, (Talaat *et al*., 2023) and it is responsible for huge economic losses especially subclinical mastitis (SCM) which affect directly on milk quantity and quality so, continuous periodic examination in parallel with good hygiene and management is the track to reduce the percent of the infection (Cobirka *et al*., 2020). In light of this study, the prevalence of subclinical mastitis at the animal level was 32.2% using CMT which indicated that the SCC level is more than 400×10^3 cells/ml, so the subclinical mastitis between these cows reflects an important challenge for these farms' development. The prevalence of subclinical mastitis in this study is lower than that reported by Badiuzzaman *et al*. (2015); Bakr, *et al*. (2019); Ndahetuye *et al*. (2019); Pumipuntu *et al*. (2019), and Hussein *et al* (2022) who recorded the prevalence as 72.07%, 84%, 76.2%, 59%, and 60%, respectively, however Mourya *et al*. 2020 reported nearly the same result which was 31.55%. On the other side, Abdel-Rady and Sayed (2009) recorded a lower prevalence than our study (19.24%). Moreover, Zizet (2015) and Ait-Kaki *et al*. (2019) reported 6.2% and 26%, respectively.

In the same spirit, there was variation in the prevalence of SCM between the examined farms (Table 1) 64.4%, 10%, and 19.2% for farm A, farm B, and farm C, respectively. With a comprehensive view of the subclinical mastitis problem in dairy herds, there is no way the end of this crisis, but we can control its prevalence. The variation in the prevalence of SCM between different studies may be attributed to environmental conditions, animals' immune status, milking practice differences, seasonal variation, animal factors (age, parity, and lactation) and hygienic conditions (Helmy *et al*., 2020; Hussein *et al*., 2022).

SCC were detected by BacSomatic and distributed into three levels (Table 1) ($<200\times10^3$cell/ml), ($200-400\times10^3$cell/ml), and (above 400×10^3cell/ml) with a rate of 45.6%, 22.2%, and 32.2%, respectively. Samples with SCC less than 200×10^3cell/ml were considered healthy or negative conversely, infected or positive ones had SCC higher than 200×10^3cell/ml (Ruegg, 2012; Kamal *et al*., 2014). The overall prevalence of SCM according to SCC ($> 200\times10^3$cell/ml) was 54.4%. Zeinhom *et al*. (2013); Jena *et al*. (2015); Kamal *et al*. (2014) and Iraguha *et al*. (2017) recorded higher results which were 87.1%, 74.55%, 73%, and 62%, respectively. The prevalence of SCM among the examined farms was 74.4%, 28.3%, and 52.5% for farm A, farm B, and farm C, respectively.

SCC has a significant effect on milk composition and quality (Pytlewski *et al*., 2010; Talukder and Ahmed, 2017). The mean of fat, protein, casein, SNF, and TS% were 2.94±0.75, 3.48±0.37, 4.82±0.23, 2.58±0.39, 9.06±0.63 and 12.2±1.69, respectively at SCC ($> 200\times10^3$cell/ml) while, at SCC ($200-400\times10^3$cell/ml) were 2.7±0.74, 3.37±0.29, 4.69±0.39, 2.58±0.39, 8.9±0.54, and 11.75±1.12 respectively, and were 2.43±0.95, 3.24±0.33, 4.61±0.22, 2.4±0.38, 8.46±0.81 and 11.21±1.79, respectively at SCC above 400×10^3cell/ml. The examined milk parameters negatively correlated with SCC which was close to the previous results by Hagnestam *et al*. (2007); Sharif *et al*. (2007); Sert *et al*. (2016); Savić *et al*. (2017) and Zecconi *et al*. (2020).

On the other hand, Jia-zhong *et al*. (2010); Cinar *et al*. (2015); Ramos *et al*. (2015), and Silva *et al*. (2018) recorded a positive correlation between SCC and some milk parameters such as fat, protein, TS, SNF, and casein, and a similar negative correlation with lactose content. Moslehishad *et al*. (2010) found no effect of SCC elevation on fat and total nitrogen, but decreased lactose content, TS, SNF, and casein. Processing of such high SCC milk will produce a low quality end product with short shelf life (Le ROUX *et al*., 2003; Sharif *et al*., 2007). The effect of these milk compositional changes will persist during the storage period of the dairy products such as lower sensory acceptance of pasteurized milk and coalho cheeses recorded by Bezerra *et al*. (2020). Bulca and Koc (2020) stated that the proteolytic activity of yoghurts was significantly affected. Moreover, Sert *et al*. (2016) revealed that the protein content of milk powder reduced with SCC increasing. Talukder and Ahmed (2017) showed cheese yield reduction with composition and texture alteration due to proteolytic and lipolytic enzymes from somatic cells which caused degradation of protein and fat respectively (Talukder and Ahmed, 2017).

Spirulina supplementation had a good impact on the SCC of supplemented cow milk and its composition represented by a significant decrease of SCC with a reduced average from $6638.9\pm4675.9\times10^3$cell/ml at day 0 to $361.1\pm321.4\times10^3$cell/ml after 21 days of spirulina treatment. These results were in harmony with Simkus *et al*. (2007) who recorded an SCC reduction of 29.1% and Kulpys *et al*. (2009) who found a decrease in milk SCC and body condition improvement (8.5-11 %) after spirulina additives. Also, Chaves Lopez *et al*. (2016) recorded a significant decrease in SCC of the marine alga-supplemented group half of that of the control group (490 and 272×10^3 cells/mL for the control group and supplemented milk, respectively), while Pajor *et al*. (2021) found a reduction in SCC from 5.73 log cells/mL to 5.34 log cells/ml. The significant decrease in the milk SCC associated with spirulina supplementation will tend to improve the food safety of milk and produce good quality dairy products.

The obtained data in this study highlight a positive correlation between spirulina supplementation and milk composition. The mean of Fat, protein, lactose, and casein% increased from 2.84±0.29, 3.02±0.4, 4.49±0.4 and 2.34±0.28 to reach 3.62±0.16, 3.65±0.43, 4.86±0.41 and 2.59±0.3, respectively after 21 days of spirulina treatment. This may be due to its high nutritive value such as the high amount of protein with all essential amino acids and a wide range of vitamins and minerals (El-Sabagh *et al*., 2014; Jamil *et al*., 2015) which will have a good reflection on milk quality and processing. Simkus *et al*. (2007) reported an increase in the mean of protein, fat, and lactose (9.7% ($P<0.05$), 17.6-25.0 % ($P<0.05$), and 11.7% ($P<0.001$) respectively) in the supplemented group. Kulpys *et al*. (2009) showed an increase in joint yield of milk fat, protein, and lactose subsequent to milk yield increasing after Spirulina additives. Previous studies by Boeckaert *et al*. (2008) and Stamey *et al*. (2012) showed an increase in milk fat while Panjaitan *et al*. (2015) reported an increase in milk protein.

The average urea content in milk was 31.01±6.3mg/l at day 0 and tended to decrease after spirulina supplementation to reach 25.3±5.7 after 21 days of its addition. In agreement with this result, Kulpys *et al*. (2009) found a decrease in urea concentration (22 mg/dl). The lower content of urea in milk after spirulina additives was ascribed to its high protein content which helps avoid cows' health disorders and increasing productivity.

Milk production significantly increased after spirulina supplementation from an average of 21.7±3.23 to 24.2±2.39 kg/day after 21 days of treatment with SCC reduction from $6638.9\pm4675.9\times10^3$ to $361.1\pm321.4\times10^3$. In agreement with these results, Simkus *et al*. (2007) and Piovan *et al*. (2021) recorded an increase in milk yield by 7.6% and 21%, respectively. In a study by Kulpys *et al*. (2009), feeding spirulina additives positively influenced milk productivity and economical indexes with a 21% increase in milk yield of cows receiving dietary Spirulina. They stated that the average milk income for each supplemented cow increased by 378 Lt compared to the herd receiving no spirulina

CONCLUSION

SCC should be periodically scanned and kept as lower as possible (<200,000cell/ml) to avoid the negative effect of its elevation on milk quality and yield. Spirulina supplementation has a good significant effect on milk composition (Fat, protein, lactose, and casein), increase milk productivity and reduce milk SCC. Using spirulina feed additives in dairy cows' feeding will result in improvement of milk quality and yield and production of good quality dairy products with prolonged shelf life.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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