ISSN 2320-1355

JOURNAL OF GLOBAL BIOSCIENCES

Volume 3, Number 7, 2014

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Journal of Global Biosciences
ISSN 2320-1355
Volume 3, Number 7, 2014, pp. 1034-1045
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

BACTERIAL PATHOGENS AND SOMATIC CELL COUNT IN SHEEP AND GOAT MILK

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Abstract

Milk somatic cell count (MSCC) is often used to detect mastitis or problems in udder health in large ruminants and it is also propagated for small ruminants milk , but due to the various factors infleuncing SCC it allows only limited conclusion on the udder health of them. The object of this study was to estimate the losses in chemical constituents content of milk according to different levels of MSCC, and to evaluate the influence of microbial load in MSCC .Seventy (70) raw milk samples (40 ewes milk, 30 goats milk) were collected from El Sharkia Province, Egypt. *Coagulase negative staphylococci (CNS)* were the most prevalent bacteria isolated in both goats and ewes milk samples by percentage of 46.2% and 34.6% respectively. The milk samples with *Staphylocoous aureus (S. aureus)*, constituted about 15.4% of goats milk samples. On the other hand *S.aureus* presents 23.1% of ewes milk samples. *Escherichia coli (E.coli)* was isolated from 7.7% of goats milk. Of ewes milk each of *Yersinia enterocolotica* and *Yersinia frdriksenii (Y. fredriksenii)* were the most prevalent while *Yersinia pseudotuberculosis (Y. pseudotuberculosis)* was the most prevalent in goat. It was concluded that, the presence of bacterial pathogens in ewes and goats milk led to the increase of the total MSCC, however the microbiological analysis showed that the bacterial pathogens were present in about 13.3% of goats milk samples containing low SCC (below 1×10^6/mL) and in 20% of ewe milk samples below 1×10^5/mL.

Key words: SCC-CNS-S.aureus- Ewe-Goat- correlation.

INTRODUCTION

Milk somatic cell count (MSCC) has been considered as the index of glandular irritation in the mammary gland *(Morek-Kopec et al., 2009)* it has been found that infected glands have a high MSCC *(Leitner et al., 2004b & Barrón-Bravoa et al., 2013).* MSCC is widely used for evaluating milk quality and to define milk prices *(Kalantzopoulos et al., 2004 & Raynal- Ljutovac et al., 2005).*

MSCC in small ruminants measures the different cell types present in milk, including leukocytes and epithelial cells. Unlike the merocrine milk secretion that occurs in cow milk secretion in goats and ewes is largely apocrine in nature, and cytoplasmic particles, similar in size to milk somatic cells, are normal constituents of their milk. These particles are not classified as cells because they do not contain nuclei or deoxyribonucleic acid (DNA), although they contain large quantities of ribonucleic acid (RNA) and proteins *(Madureira et al., 2010 & Souza et al., 2012).*

MSCC in milk from healthy goats is higher than the MSCC observed in milk from uninfected cows and ewes. Unlike in milk from cows and ewes, Polymorphonuclear leukocytes (PMNLs) PMNLs comprise the major leukocyte type (40–87%) in goats milk. Because the neutrophils act as the first line of immunological defense against infections, this could explain why goats are more resistant to mastitis *(Tian et al., 2005).*

At present MSCC in milk has been used widely as an indicator for the detection of mastitis in cows and it is also propagated for goats milk *(stuhr et al., 2013)* but the major part of investigations found no significant relationship between a rise in MSCC and the occurrence of intra mammary infection (IMI) *(Kyozaire et al.,2005 & Moroni et al., 2005b).* As there are some physiological factors influence MSCC like bread *(Jendretzke, 2009),* stage of lactation *(Paape et al., 2001)* and estrus *(Moroni et al., 2007& Christodoulopoulos et al., 2008)* as well as hygiene standards *(Delgado-Pertinez et al., 2003)* or milking equipment *(Souza et al., 2009).*

In goats, the physiological factors may account for up to 90% of the variation in MSCC *(Haenlein, 2002 & Raynal-Ljutovac et al., 2007).*

Mastitis in sheep has a large impact on milk production. Significant changes in the protein, fat, lactose, among other components, may occur as well as reduced production levels. *(Oliveira., et al 2013).*

In dairy goats, fat and protein content and milk yield could be affected by daily variations as a consequence of the incidence of non-infectious, genetic, environmental and seasonal factors *(Raynal-Ljutovac et al., 2007& Tangorra et al., 2008).*

The increase in MSCC is associated with a decrease in milk quantity and changes in its composition as there is an increase in albumin content in the milk serum and a reduction of casein, fat and lactose *(Bernacka, 2006).*

The aim of this study was to estimate the total count of MSCC, in relation to bacterial pathogens in sheep and goats milk and to estimate losses in fat, protein and lactose components according to different levels of MSCC.

MATERIALS AND METHODS

Collection of samples (A.P.H.A., 1992):

In the present study, seventy (70) raw milk samples (40 sheep milk & 30 goats milk) were collected from El Sharkia Province, Egypt. Animals were selected to be clinically healthy. Milking was performed after cleaning the teat end with cotton soaked in 70% ethyl alcohol and previous discard of the first three streams of milk.

Milk Somatic cell count

Milk Somatic cell count (MSCC): Milk samples were examined automatically using somatic cell counter MT05 apparatus. The sample was warmed at 40˚C for 5 minutes, and then mixed before reading *(Radostitis et al., 2000).*

Chemical examination: (www.Milkotester.com)

Chemical examination was done by ultrasonic portable milk analyzer (milkotester model-Master Mini) for:

Determination of fat%.
Determination of protein%.
Determination of lactose%.
Determination of total solids%.

Milk samples should be 5-35˚C and mixed well before the examination. Pouring it several times out of one vessel into another and back.

Microbiological examination.

Preparation of decimal dilution (APHA, 2004).
Determination of aerobic mesophilic count (BAM, online 2009).
Isolation of salmonella spp. (APHA, 2003).
Isolation and identification of Yersinia species (Landgraf, 1993).
Isolation and Identification of staphylococcus spp. (BAM, online, 2009).
Isolation of E.coli: (ICMSF, 1978).

RESULTS AND DISCUSSION

Statistical analytical results of ewe in (table1) showed that MSCC mean was 20.2 $x10^6$±15.1 $x10^6$ these results were coordinated with *Gonzalo et al., 2002; Suarez et al., 2002; Lafi et al., 2006; Kern et al., 2013 & Oliveira et al., 2013* while lower results were recorded by *Berthelot et al., 2006; Blagitz et al., 2008; Nunes et al., 2008 & Davasaztabrizi et al., 2013.* For fat results *Morgan et al., 2006; Afolayan et al., 2009; Mioc et al., 2009; Vanderlindenet et al., 2009, ferreira et al., 2011& Kern et al., 2013* found that there's no changes in fat content, but *Jaeggi et al., 2003 & Bianchi et al., 2004* gave similar results.

Results recorded in (table 1) revealed that the protein was in line with that was estimated by *price et al., 2000; Jaeggi et al., 2003; Ferreira et al., 2011 & Oliveira et al., 2013.* Other authors as *Albenzio et al., 2005; Morgan et al., 2006; Afolayan et al.,2009; Kammerlehner, 2009; Mioc et al., 2009 & Kern et al., 2013* found that there are no changes in the protein content. *Nudda et al., 2003; Albenzio et al., 2004 & Bianchi et al.,2004* give somewhat hig-her results.

Regarding lactose results *Nudda et al., 2003; Albenzio et al., 2004; Bianchi et al., 2004; Mioc et al., 2009; Kern et al., 2013 & Oliveira et al., 2013* gave similar results while *Kammerlehner, 2009* reported no changes. On the other hand *Pirisi, 2000* found that there were no changes in the total solids in the examined ewes milk samples, while *Jaeggi et al., 2003 & Oliveira et al., 2013* gave similar results.

Table (1): MSCC and Chemical constituents of milk:

Species	**Goat N=30**			**Sheep N=40**		
Variable	**Mean±S.E**	**Max.**	**Min.**	**Mean ±S.E**	**Max.**	**Min.**
MSCCml^{-1}	40.9x10^6 ±36x10^{6a}	23x10^7	11x10^4	20.2x10^6 ±15.1x10^{6b}	94x10^6	8x10^4
Fat%	3.02±0.42 ^{a}	4.4	1.4	5.24±0.95 ^{b}	8.3	3.2
Protein%	2.62±0.60 ^{a}	4.3	0.9	5.23±0.63 ^{a}	7.8	3.67
Lactose%	2.28±0.85 ^{a}	4.5	0.5	2.47±0.69 ^{b}	5.3	0.6
TotalSolid%	9.95±0.78 ^{a}	14.2	9.0	11.45±1.73 ^{b}	19	7.8

$^{a, b}$ Means within the same row carrying different superscripts are significantly different at ($p<0.05$).

Table (2): Microbial load in relation to MSCC level in goats and ewes milk*:*

Species	**SCC**	**Aerobic mesophilic count**	
		Min.	**Max.**
Goats	**Up to 1x10^6**	11x10^4	52 x10^5
	1x10^6 -1.5x10^6	17 x10^5	20 x10^6
	1.5x10^6 -2x10^6	43 x10^5	91 x10^6
	More than 2x10^6	15 x10^6	23 x10^7
Sheep	**Up to 1x10^5**	13 x10^2	33 x10^3
	1x10^5-5x10^5	71 x10^3	15 x10^4
	5x10^5 -1x10^6	34 x10^4	47 x10^5
	More than 1x10^6	15 x10^4	94 x10^6

Table (3): Incidence of pathogenic bacteria in relation to MSCC level in goats and ewes milk:

species	SCC	Pathogen free		Presence of pathogenes	
		NO.	%	NO.	%
Goat (N=30)	Up to $1x10^6$	9	30	4	13.3
	$1x10^6$ -$1.5x10^6$	4	13.3	2	6.7
	$1.5x10^6$ -$2x10^6$	2	6.7	1	3.3
	More than $2x10^6$	2	6.7	6	20
Sheep (N=40)	Up to $1x10^5$	7	17.5	8	20
	$1x10^5$-$5x10^5$	3	7.5	4	10
	$5x10^5$ -$1x10^6$	4	10	2	5
	More than $1x10^6$	0	0	12	30

Table (4): microbiological composition of examined goats and ewes milk

	Goat (N=13)		Sheep (N=26)	
	N.	%	N.	%
Staphylococcous spp.				
1-CNS	6	46.2	9	34.6
-S. chromogenes	0	0	3	11.5
-S.epidermidis	1	7.7	3	11.5
-S.caprae	4	30.8	2	7.7
-S.capitis	1	7.7	1	3.8
2-S.aureus	2	15.4	6	23.1
Yersinia.spp.	4	30.8	8	30.8
-Y.enterocolitica	1	7.7	3	11.5
-Y.intermediate	0	0	2	7.7
-Y.pseudotuberculosis	2	15.4	0	0
-Y.fredriksenii	1	7.7	3	11.5
E.coli	1	7.7	3	11.5
Salmonella	0	0	0	0

Normal MSCC limits for ewe milk have not been determined, but some authors suggest SCC ≥ 500 × 10^3 cells/ml *(Vivar-Quintana et al., 2006 & Cassius et al., 2007)* For ewes with mammary glands having no clinical abnormalities and giving apparently normal milk, which was bacteriologically positive. Therefore the samples in this case had high MSCC.

Wide variation for milk components of ewe is seen in literature due to differences in age, stage of lactation, breed and nutrition *(Brito et al., 2006& Park et al., 2007).*

Results recorded in (table 1) revealed that MSCC results of goats milk were similar to that of *Luengo et al., 2004 & Hall and Rycroft, 2007*, while lower results were recorded by *Moroni et al., 2005b; Souza et al., 2009; Koop et al., 2010; Persson and Olofsson, 2011 & Oliveira et al., 2011.*

Moroni et al., 2005c; Aulrich; Barth 2008 & Bagnicka et al., 2011 found that 20% of samples below $1x10^6$ and 5% lies between1 $x10^6$ and 2 $x10^6$. On the other hand *Ying et al., 2002 &Chen et al., 2010* found that there are no changes in fat content but *Jaeggi et al., 2003 & Pisoni et al., 2004a, b* gave similar results, while protein was in line with estimates by *Jaeggi et al., 2003; Pisoni et al., 2004a, b; Bernacka 2006 & Chen et al., 2010.* Others gave somewhat higher results *(Ying et al., 2002 & Leitner et al., 2007).*Regarding lactose results *Jaubert et al., 1996b; Zeng and Escobar 1996a; Contreras et al., 1999; McDougall and Voermans, 2003 & Moroni et al., 2005a* gave similar results while *Pasquini et al., 1996* reported no changes.

Pasquini et al., 1996 & Jaubert et al., 1996b found that there were no changes in the total solids content of the examined goat milk samples

In goats, MSCC is more difficult to relate to possible infections than in the case of cow and ewes *(Sánchez-Macías et al., 2013).*

The different results of the MSCC effect on the goat milk composition may be attributable to the individual effect of animal, breed and other variable factors such as flock, year and season of kidding and stage of lactation *(Haenlein, 2002; Raynal-Ljutovac et al., 2007&Leitner et al., 2011b).*
Several factors affect the milk quantity and quality factors such as stage of lactation, age, time of day, lentivirus infection and nutritional management *(Menzies and Ramanoon, 2001)* in dairy sheep and goats, but subclinical intramammary infection (IMI) is the single most important factor *(Leitner et al., 2004a, b).*
Our current study showed that goat milk samples had significant differences in sheep milk samples in MSCC, lactose, fat & total solids while protien showed no significant difference.
In the study, fat was negatively correlated with SCC. r = -0.05(SD= 0.03) lactose, T.S. and SCC showed low correlations for SCC and lactose (r = -0.032, SD= 0.03). SCC and T.S. (r = -0.02, SD = 0.02). finally protien, and SCC (r = -0.41, SD = 0.04) showed non significant correlation as correlation is significant at the 0.05 level.
These results of a low, positive or negative residual correlation near zero were also described in other investigations *(Baro et al., 1994; Fuertes et al., 1998; Bianchi et al., 2004 & Kern et al., 2013)*
It is evident from the result given in (table 2) that the *aerobic mesophilic count*/ml. In ewe milk ranged from 13×10^2 to 94×10^6. Relatively similar results were obtained by *Fotou et al. (2011).*
And for goat milk samples were ranged from 11×10^4 to 23×10^7. These findings substantiate those reported by *Oliveira et al., 2005 & Oliveira et al., 2011* Lower values were obtained by *Yamazi et al., 2013.*
Aside from the public health consequences associated with high bacterial contamination of raw milk, the possible regional economical losses triggered by the contamination are of special concern. *(Oliveira et al., 2011).*
Table (3) discussed the presence of pathogens in milk samples and showed that almost 65% of ewe milk samples contained pathogens with high prevalence to *Coagulase Negative Staphylococci (CNS)*. These results are similar to that reported by *Hariharan et al., 2004 & Kern et al., 2013,* While lower results were presented by *Beheshti et al., 2010; Fotou et al., 2011 & Davasaztabrizi et al., 2013.*
Gonzalo et al., 2002 found pathogens in all examined ewes milk samples. On the other hand, about 43.3% of goats milk samples contained pathogens, the most prevalent pathogen was *CNS*. These results are in accordance with those presented by *Moroni et al. 2005c; Aulrich and Barth, 2008 & Bagnicka et al.,2011,* while lower results were reported by *Stuhr et al., 2013.*
Table (4) cleared that *CNS* were the most prevalent bacteria isolated in both goats and ewes milk samples by percentage of 46.2% and 34.6% respectively. For goats milk samples *Bagnicka et al., 2011* found them, but by lower percentages. Higher percentages were detected by *Contreras et al., 2007; Taponen and Pyorala 2009 & Persson and Olofsson 2011.*
Ariznabarreta et al., 2002 & Oliveria et al., 2013 find *CNS* in ewe milk samples by higher percentages.
Several studies have pointed to *CNS* as the main etiological agent of small ruminant Intra mammary Infections IMIs *(Berthelot et al., 2006; Contreras et al., 2007; Min et al., 2007; Raynal-Ljutovac et al., 2007; Nunes et al., 2008; Della Libera et al., 2010; Cuccuru et al., 2011 & Guaraná et al., 2011).*
Although the *CNS* is traditionally regarded as less pathogenic than *S. aureus*, they are the most prevalent pathogens causing subclinical mastitis in goats. The thermostable staphylococcal enterotoxins and toxic-shock syndrome toxin-1 can be produced by these bacteria isolated from subclinical mastitis as well as chronic or acute mastitis. The *CNS* can persist in the mammary gland and are able to Adhere to bovine mammary gland cells with almost the same, although less invasive, capacity than S. aureus. Moreover, *CNS* could be more resistant to antimicrobial agents than S. *aureus*. They also develop multi resistance easily *(Taponen and Pyörälä, 2009)*

The main species of *CNS* isolated from goats milk samples were *Staphylococcus epidermidis* and *S. caprae* and *S.capitis* while in ewe milk samples were *Staphylococcus epidermidis* , *S.chromogenes*, *S.caprae* and *S. capitis*.

Some authors found that the main species of *CNS* isolated from infected udder halves in small ruminants were *Staphylococcus epidermidis*, *S. chromogenes, S. simulans, S. xylosus* and *S. caprae (Ariznabarreta et al., 2002; Bergonier et al., 2003; Moroni et al., 2005a,b; Contreras et al., 2007; Cuccuru et al., 2011 & Leitner et al., 2011b).*

The milk samples with S*. aureus*, constituted about 15.4% of goats milk samples (Table4). Some authors demonstrated higher results *Foschino et al., 2002; Muehlherr et al., 2003 & Jorgensen et al., 2005.* Our results are similar to that reported by *Moroni et al., 2005c & Contreras et al., 2007.* While lower results were reported by *Bagnicka et al., 2011; Persson and Olfsson, 2011; Rahimi and Alian, 2013 & Stuhr et al., 2013.*

Contreras et al., 2007 did not find *S.aureus* in any of the examined goats milk samples.

On the other hand *S.aureus* presents 23% of ewe milk samples (Table4) these results are in accordance with those presented by *Fotou et al., 2011.*Lower results were represented by *Beheshti et al., 2010; Kern et al., 2013 & Rahimi and Alian 2013* while higher results were reported by *Oliveria et al., 2013.*

Leitner et al., 2004a didn't find *S.aureus* in any of the examined ewes milk samples.

The thermostable enterotoxins produced by *S.aureus* play an important role in foodborne diseases. In addition, *S. aureus* produces the leukotoxins and other virulence factors, such as haemolysins, exfoliative toxins and toxic-shock syndrome toxin. Moreover, these bacteria have the ability to form slime and biofilm *(Taponen and Pyörälä, 2009).* Table (4) declared that *E. coli* was isolated from 7.7% of goat milk samples. Lower results were founded by *Foschino et al., 2002* and higher results were detected by *Muehlherr et al., 2003; Jorgensen et al., 2005 & Yamazi et al., 2013.*On the other hand *E.coli* was isolated from 11.5% of ewe milk samples, higher results were reported by *Muehlherr et al., 2003* and lower results were found by *Beheshti et al., 2010; Fotou et al., 2011& Kern et al., 2013.*

The presence of the *E.coli* in milk indicates possible contamination by manure, soil and contaminated water*. E. coli* and *coliform* bacteria are often used as indicator microorganisms, and the presence of *E. coli* implies a risk that other enteric pathogens may be present in the samples *(Fotou et al., 2011).*

We couldn't find *salmonella* in any of the examined goat or ewes milk samples, while *Muehlherr et al., 2003* isolate *salmonella* from both of them by percentage of 61.6 % and 71.4% respectively.

Fotou et al., 2011 isolate *salmonella* from 5% of examined ewes milk samples. *Oliveira et al., 2011* isolated *salmonella* from only two goats milk samples, while *Morgan et al., 2003 & Moroni et al., 2005b* could not isolate it.

Yersinia spp. were isolated from 30.8% of both ewe and goats milk samples (Table4) and recorded *Y. Fredriksenii and Y. enterocolitica* as the most prevalent in ewes while *Y. pseudotuberculosis* was the most prevalent in goat.

Abd ElAal and Atta, 2009 isolated them from only 20%of ewe and 32% of goats samples. And reported *by. Fredriksenii* as the most prevalent one in both ewe and goat.

Y. enterocolitica is widely distributed through the environment and have been isolated from raw milk, sewage-contaminated water, soil and humans. *Y. enterocolitica* is considered as a, food borne pathogen causing symptoms such as fever, diarrhea, nausea and abdominal pain, the diseases of which range from self-limiting gastroenteritis to fatal septicemia. *Y. pseudo tuberculosis* is associated mainly with mesenteric adenitis. Cases of mastitis caused by *Y. pseudo tuberculosis* have been reported in cattle with clinical or subclinical presentation; lumps, swelling, clotted milk and increased somatic cell counts were the salient features *(Shwimmer et al., 2007)* Food has been proposed to be the main source of intestinal yersiniosis, although pathogenic isolates have seldom been recovered from food samples.The psychrotrophic nature of this organism is a particular significance in milk and milk products that are normally stored at low temperatures. In raw milk *Yersinia enterocolitica* strains were able to survive in the presence of high numbers of competing microorganisms and were able to maintain the virulence plasmid during extended storage at refrigeration temperature *(Larkin et al., 1991).*

CONCLUSION:

In conclusion the present study demonstrated close relationship between presence of bacterial pathogens and total SCC, In most samples the presence of bacterial pathogens in goats and ewes milk caused the increase of the total SCC. For this reason, breeders use the SCC as an indicator of goat subclinical mastitis. However, the microbiological analysis showed that the bacterial pathogens were present in about 13.3% of goats milk samples containing low SCC (below 1×10^6/mL) and in 20% of ewe milk samples below 1×10^5/mL. Therefore, the SCC cannot be the only decisive indicator of bacterial infection of the mammary gland in goats and ewe and it is important to search additional indicators of goat subclinical mastitis.

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