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SOME PHYSIOLOGICAL FACTORS INFLUENCE SOMATIC CELL COUNTS AND MILK CONTENTS ASSOCIATED WITH MASTITIS IN IRAQI BUFFALOES

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ABSTRACT

The aim of this study was design to investigate the prevalence of mastitis in lactating buffaloes and the association with somatic cell count (SCC)and the variation of these cell in relation to parity , stage of lactation and milk yield and on rations of fat and protein in milk, and to estimation the simple correlation coefficient between milk content of SCC and its` components in 80 lactating buffaloes in Babylon province / Iraq for the period from 9/2013 to 13/21/2014 . The results indicates that the prevalence of clinical mastitis was 9.735%, the SCC significantly increased with advanced number of lactation, there was significant increase of SCC in the second and third stage of lactation .There was variations in daily milk yield< 1500 kg may reach 975.63 $\times 10^3$ cells /ML of milk. The parity significantly influenced the SCC in dairy buffalo ($p< 0.01$) and significantly affected fat and protein content of milk ($p<0.05$). Significant changes in fat 7.15% were also observed with increasing SCC $>1000\times 10^3$ cells/ML milk, while NS in protein rations were recorded. The correlation coefficient between SCC content of milk and fat 0.26 was positive and significant ($p<0.05$), and non-significant with protein 0.09, and lactation period and milk yield were -0.38 and -0.41 respectively were negative and significant ($p<0.01$).

KEYWORDS: *Buffalo, Mastitis Somatic cell count ,Parity , Milk contents.*

INTRODUCTION

Somatic cell count (SCC) is a useful measure for detection of subclinical mastitis and milk quality. Guha *etal*. (2012) pointed to the existence of two forms of mastitis the first is clinical mastitis as clear changes which appear in each of the udder and installation of milk and be a high percentage of somatic cells in milk . The second form is mastitis semi- clinical (sub- clinical mastitis)is characterized by changes evident in the milk components and are not accompanied signs of the disease with the daily increase in the number of somatic cells and this may affect the overall milk , leading to lower quality. The causes of mastitis involve a complex relationship of three major factors, that is, host resistance, bacterial agents and the environmental factors (Gera and Guha, 2011). Somatic cell count and bacteriological examination indicate the status of the mammary gland. The secretion of these cells in cow's and goat's milk is influenced by the number and stage of lactation, management practices and intramammary infections(Harmon, 1994; Wilson et al., 1995) However, buffalo milk, number and stage of lactation did not affected. The somatic cells (Singh and Ludri, 2001) .Mastitis affects the milk quality in terms of decrease in milk protein, fat, sugar (lactose) contents and increase in somatic cell count (Urech, *et al.,* 1999). Increased in the leucocyte number in milk and in the mammary gland, as a response to the assaulting pathogens or to their metabolites leads to an increase in SCC (Atasever, 2012). According to International Dairy Federation (IDF, 2005) threshold limit of 200×10^3cells/ ml of milk had been recommended for subclinical mastitis . In EU countries , according to the directive 92/EEC , bulk milk samples with SCC greater than 400×10^3cells/ ml of milk is advised not to be used for human consumption(Atasever and Erdem,2010) .Of modern and documented statistics by the Ministry of Agriculture (2008) indicate that the number of buffalo in Iraq, 285 thousand heads and more than 70% of this number is spreading in the Middle Euphrates region and southern Iraq . Breeders buffalo suffering from high concentrated feed and green feed costs, which constitutes 57.1 % of the buffalo and note the existence of scarcity silo, which is one of the main and handicapping in the prosperity and development of buffalo problems (YOUSAF,*etal* 2010). AS the importance of the subject and the impact of increased somatic cells (SCC) on the milk quality and its components of protein and fat and the scarcity of studies under way in Iraq on the buffaloes on this topic, the current study is aimed to study the effect of some of the factors affecting mastitis (parity, stage of lactation- the level of milk yield) on milk content of somatic cells and the ratios of fat and protein in milk and to estimate the simple correlation coefficient between milk content of somatic cells (SCC) and its components. in a sample of buffalo in the province of Babylon\ Iraq.

MATERIALS & METHODS

This study was carried out in the Hashemiya Justice (25 km south-west of the province of Babylon / Iraq) using animals reared using a loose housing system. We used a total of 80 of the Iraqi female lactating buffalo and their offspring for the period from 01/09/2013 up to 31/12/2014. Are a herd of buffalo management in the Hashemite region according to a program that includes nutrition and reproductive care and sometimes veterinary, and there are no special buildings for the animals, but a simple place in the form of a fence of wood inside the orchard where linking animals, with up to five sheds exploited by animals during precipitation in winter while remaining in the majority of the day as free-roaming, as well as simple sheds for newborns up to weaning. Nutrition vary from chapter to chapter depending on the availability of feed , the animals are grazing on the green fodder in summer and autumn for four hours in the morning, as the animals feed on feed consisting of white corn and yellow jet, and after grazing, especially during the summer animals go to dive in the river adjacent to the farm with an area of up to 40 acres beach, from ten o'clock in the morning until one in the afternoon, and then the animals back to the grazing from one o'clock noon until six pm, but during the winter grazing shall be for a period of only four hours a day (6-10 am), while during the spring grazing shall be 6 hours a day , especially on crops and alfalfa mixtures of barley and alfalfa. It is served daily concentrate fodder includes bran with love cotton and up to 6.7 kg per head per day. The calves are fed only breast milk from the first day and for two months, after which it is providing concentrate fodder has up to 3.4 kg per day, as well as milk for a period of two months (until the age of the animal becomes four months), weaning (just cut milk with him), to give him the green coarse or dry feed and freely while continuing to give concentrate fodder. The breeding season is usually from the month of September of each year and continues until October, while calving usually begin from July until the end of September.

Milk collection models

Milk sample was taken after 2-3 weeks after birth and continued for very dry buffalo , Samples were collected manually as getting two stages the first one at the beginning of milking after rid of the first drops (strips) of front a quarterly udder , and the second phase after the milking process is finished and one quarterly udder rear they were mixed together to represent one sample per buffalo , and preserved milk samples immerse in ice crushed during transport until the holding of the necessary laboratory tests (National mastitis council,1990). California mastitis test was conducted on milk samples following the methods as described by Dhakal (2006). The parity of the buffaloes were II, III, IV, somatic cell in milk was determined by the method of (Das and Singh 2000). In breife10µl of fresh milk was spread on glass slide and was stained using methylene blue dye solution (0.6 gm. methylene blue, 40 ml Tetrachloroethane ,95% ethyl alcohol and 6 ml glacial acetic acid) and the SCC were measured microscopically . The data were analyzed using least analysis of variance.

Method of estimating the proportion of fat and protein in milk

The chemical analysis of milk after the addition of potassium Dichromate by 20 mg / 100 ml milk to save the milk from damage to be used in the estimation of the components of milk protein and fat, and conducted chemical analyzes of milk following method.

A) Estimate the proportion of fat

Estimated percentage of fat in raw milk using the method according to the Babcock method described by American Public Health Association (1978) by separating the fat-mediated concentrated sulfuric acid and then read the result on the tube inserted directly.

B) Estimate the proportion of protein

The percentage of protein estimated using the method According to MicroKjeldahl by the A.O.A.C (1970).

Statistical analysis

Method was used as a model of linear General (General Linear Model -GLM) within the statistical program Statistical Analysis System-SAS (2012)

1-Mathematical model I: to investigate the effect of the studied factors on milk content of somatic cells.

$Yijklm = \mu + Pi + Sj + Lk + Gl + eijklm$

As:

Yijklm: the value of viewing m.

µ: the overall average for the recipe studied.

fourth-Pi: The effect of sequence of parity (second-third).

Sj: the effect of season of calving (April-May-June-July)

LK: the effect of the level of milk production (high of more than 1,500 kg from 1200 to 1500 kg - moderate - low less than 1,200 kg)

Gl: the impact of milk production phase (1 = the first two months, 2= month, the third and fourth, 3 = of the fifth month, up from milk production phase of that season.

eijklm: random error which is distributed naturally at an average zero and variance of ð².

2-Mathematical model II: to study the effect of the extent (Score) milk content of somatic cells on the rations of fat and protein in milk..

$Yij = \mu + Ai + eij$

Ai: the extent of the impact (Score) content of milk somatic cells (1, 2 and 3)

RESULTS AND DISCUSSION

In the present study, the overall prevalence of clinical mastitis in buffaloes was observed (Table 1), Of the 80(9.735%) buffaloes were examined, 36.25% (29) buffaloes were found positive suffering from clinical mastitis while 63.75% (51) was detected as negative. Similar finding 11.7%was reported by Rahman *etal*1983 in Iraq.

Table 1. The overall prevalence of clinical mastitis in buffaloes.

Animal species	Number of animal examined	Prevalence of mastitis %
infected buffalo	29	36.25
Non- infected buffalo	51	63.75
Total number	80	9,735

The CMT is a rapid and inexpensive test to determine the somatic cell concentration in the milk. It is a specific test for deoxyribonucleic acid of somatic cell nuclei. It can be useful in identifying udder quarters that have abnormally high SCC the higher susceptibility of milking buffaloes to mastitis could be due to one of the following reasons. The buffaloes are predicted for water and muddy places. They consistently sit in dirty and unhygienic milking places, sheds etc. There is close contact between healthy and diseased animals in common grazing and wallowing places. Milking procedure is wrong with unhygienic conditions. The teats are exposed to injury with inverted thumbs. In buffaloes, unweaned calves can cause injury and create a focus for infection. The calves when unable to feed cause injury because of biting, pulling and hitting the udder. While taking out for grazing, wallowing and driving the animals are made to run and the large pendulous udder is liable to injury and infection.(Bilal *et al* ,2004 ,Dhakal 2006,Baloch *et al* 2013).

As the somatic cell count (SCC) in the milk is the most used indicator in programs for mastitis control and prevention around the world several factors might influence the SCC variation (Medeiros *et al* 2011) according to the results obtained in current study. the influence of stage of lactation on SCC is given in table 2 shows that Somatic cell count were significant ($p<0.01$)decreased in the second month of lactation 478.12× 10³ cell /ml milk and significant($p<0.01$) increased in the second and third stage of lactation 951.08,743.61× 10³ cell /ml milk respectively. Similar report was cited by Munoz *et al* (2002) in cows. These results could be attributed to the lesions caused by daily milking or even to the progress of bacterial infections over the lactation period or throughout the lactation because of a dilution effect (Reneau, 1986). Result were dealing with other study (Schultz1977, Sharif and Mohammad, 2008, Madouasse, 2009).

Milk yield was negatively related to SCC this was also reported earlier in buffalo (Singh and ludri 2001) might be either to the dilution of somatic cell count by increase milk production or variations of SCC with change of milk yield as shown in table 2.

Table 2. Effect of milk yield and stage of lactation on milk SCC (lease square mean ± SE)

Factors	Number of observations	Least square mean± SE (× 10³ cell /mL)
Milk yield (kg)		
low>1200	28	528.82±36.58 b
Medium(1200-1500)	39	891.43± 62.19a
High< 1500	13	975.63± 52.33a
Significance	-------	**
Stage of lactation		
First (the 1st month of the beginning of production)	80	478.12± 29.66c
Second(the 3rd & 4th month from production	80	951,08± 59.71a
Third (5th month until the end of lactation	80	743.61±40.09b
Significance	---------	**

bac= means differ significantly,($p<0.01$)**

Table 3 shows that the SCC increased($p<0.01$) with parity from 681,23× 10³ cell /mL in parity II to 1093,72× 10³ cell/mL in parity VI which could be due to an increased risk of infectious pathogens entering the udder and causing mastitis another reason for the high somatic cells count SCC with the progress of parity may be due to environmental and physiological factors related to the activity of immune system and working to reduce the animal's resistance to diseases that are accompanied by an increase in the somatic cells count SCC in the milk as the animal's age-exposed to many diseases including chronic with no symptoms and even animal cured from these diseases remain more vulnerable causes increase of white blood cells in the bloodstream and in the mammary tissue this white cells penetrate barrier tissue of the gland and mixed with milk where the number of somatic cells goes up(OBrien *etal* 2009).

Table 3: Influence of parity on milk Somatic cell count (Least square mean ± SE)

Influence factor	Number of observation	Least square mean± SE(× 10^3 cell /mL)
Parity		
II	21	681,23±52,29c
III	35	844,29± 62,79b
VI	24	1093,72± 91,65a
Significance	--------	**

bac= means differ significantly, (p<0.01) **

The data given in table 4 shows an influence over (score) milk somatic cell count on rations of fat and protein It can be seen from the table (4) there was significant differences(p<0.01) in the ratios of fat and protein in milk between the number of cells less than 750 × 10 ³and more than 1000 × 10 ³ cells / ml milk , and matches this result is reached by Vasconcelas,*et al* (2002) , who pointed to there were no significant differences in fat ratio between the number of cells less than 500 × 10 ³ cells / ml and more than 2500 × 10 3 cells / ml milk and Haenlein *et al* (1973) who pointed to the absence of significant differences for the proportion of protein in milk cell count less than 250 × 10 ³ and morethan1000 × 10 ³ cells / ml milk.

Table 4. Shows the effect of extent (score) milk somatic cell count SCC on rations fat and proteins.

Score × 10 ³ cells / ml milk	Least square mean± SE	
	Fat ratio	Protein ratio
<750	5,38±0.62b	4.19±0.48a
750-1000	5.92±0.46b	4.22±0.19a
>1000	7.15±0.52a	4.07±0.25a
Significance	*	NS

(P< 0.05)a b, NS = non –significant *

Correlation coefficient between milk content of somatic cells and its components and some of the studied traits:

It can be seen from the table (5) that there is a positive correlation and increase value (P <0.05) between milk content of somatic cells and ratios of fat in milk reaching 0.26 .this result not agree with those reported by Tripaldi *et al* (2010) , in this study clarify there were no significant differences between milks`SCC and protein rations in milk0.09 And inconsistent as a result of this study in terms of the inverse relationship between milk content of somatic cells and the ratios of fat and protein in milk, David *et al.*(1995) who concluded that there is a negative correlation between the preparation of somatic cells in milk, fat and protein ratios. As it turns out that the link between milk content of somatic cells and total milk yield was negative (-0.41) and significant (p <0.01), It has attributed the cause of low milk production with the high number of somatic cells to the negative impact that these cells have on mammary gland tissue and its 'cells Which is reflected in the decline in milk production and that these cells change the permeability of membranes and gland , which leads to leakage of blood components to milk and filled canals and tanks gland with clots milk and then decrease in milk production Schallibaum (,2001) It has been found that the correlation coefficient between somatic cells and the length of lactation negative and highly significant 0.38- (P <0.01) and that the cause of the Palace milk season with the high number of somatic cells may be due to the infection of the udder and the resulting increase in these cells lead to a decline in milk production sharply , which requires an early time drying the buffalo animals is the fact that production has become useless.(Nickerson 1994).

Table 5. Show Correlation coefficients between milk content of somatic cells (SCC) and some of the physical qualities of productivity.

Physical qualities of productivity	Simple correlation coefficients
SCC and fat ratio	0.26*
SCC and protein ration	0.09 NS
SCC and total milk yield	-0.41**
SCC and palace milk season	- 0.38**

NS= non –significant,(p< 0.05)* , (p< 0.01)**.

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