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Effect of Mastitis on Milk Fat Content

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Abstract: Two different farms were subjected to an investigation for the presence of mastitis. The incidence of the disease was 20.1 and 36.4 percent in the 1st and 2nd farm respectively. Bacteriological analysis revealed that in the 1st farm *Staphylococcus* spp and *Streptococcus* spp were the causative agents of the diseases, these cultures were isolated in a single form. In the 2nd farm, the causative agent was either a single culture of *Staphylococcus* spp or a mixed cultures of *Staphylococci*, *Streptococci* and *Escherichia coll*. The fat content of milk decreased greatly due to the infection. In the 1st farm, the decrease was greater when the infection was due to *Staphylococci* than *Streptococci*, while in the 2nd farm, the results varied greatly, but the overall decrease of the milk fat content of the 2nd farm was significantly greater than that of the 1st farm.

Key words: sub clinical mastitis, milk fat composition

Introduction

Mastitis or udder inflammation is usually a consequence of bacterial infection and is responsible for considerable economic loss to the dairy industry due to the reduction of milk yield. The most prominent phase in the disease is the subacute or subclinical phase in which both milk and udder appear normal and the infection would not be detected without bacteriological and chemical analysis of milk.

Despite the great economic importance of the disease to the dairy industry, reliable estimation of the losses in Egypt are not well indicated. The incidence of mastitis varied greatly due to the effect of several factors such as type of animals (Joshi *et al*., 1976; Pander and Chopra, 1987), age of the animal (Gillamac, 1985), seasonal variations (Weight and Ahlers, 1978; Meany and Egen, 1987), animal sanitation (Gasanov, 1970), hygiene management of the farm (Yosai *et al*., 1980) and the increase in the lactation number together with the geographical origin of the animal (Bakken, 1981). Bacteriological examination of the mastitic milk showed that the main causative agents were different spp of *Staphylococci*, *Streptococci* and *E. coil*, These microorganisms varied greatly being present singly or in a group of two or more.

Since the disease is characterized by changes in the physical and chemical properties of the milk and fat being an important component of milk, any change in its concentration in turn affect the suitability of milk processing and the quality of its products (Ali *et al*., 1980; Kuzmina, 1981; Ragab *et al*., 1980).

The present work deals with the incidence of mastitis in two farms under investigation, the isolation of the causal organisms and the effect of mastitis on the fat content of milk samples under test.

Materials and Methods

Farms and milk sampling: Milk samples from Frisian cows were used in this work. It was obtained from two different farms, the 1st of 328 cows, was located in Menoufia Governorate and was a private farm, The 2nd one belong to Animal Production Research Institute and located in Kafr El Sheikh Government, it contains a herd of 110 cows. Milk samples were collected in 250 ml conical flasks after washing and sanitization-by 4 percent aqueous solution of potassium permanganate-of the udders. Samples were kept in an ice box and examined within 90 mn.

Test for detection of subclinical mastitis: Three different tests were used for the detection of the disease, one of them was carried out in the farm and the other two tests were carried out in the laboratory.

California Mastitis test (CMT) (Auxiliadora *et al*., 1988): This test was carried out in the farm. The positive results were arranged according to the severity of the diseases (1 + ve, 2 + ve, 3 + ve and 4 + ve).

Hotis Test (Maciolek *et al*., 1980):

Bromo thymol Blue Test (BTB) (Hashmi and Muneer, 1981): These tests were carried in the laboratory, a positive result indicates the udder infection.

Isolation of bacteria from milk samples: The different milk samples proven to be mastitic, were subjected to bacterial isolation. For this purpose, different media were used for enumeration of total bacterial count and specific bacterial genera using one ml of milk sample serially diluted in saline solution. Tryptone Glucose yeast Agar (APHA, 1960). Medium for total bacterial count. Violet Red Bile Agar (APHA, 1960). Medium for enumeration of the coliform. Baird Parker Medium (Collins *et al*., 1989) medium for enumeration of *Staphylococcus* spp. Edwards medium (Collins *et al*., 1989) medium or enumeration of *Streptococcus* spp.

Purification and identification of different bacterial isolates: The best grown colonies isolated on different specific media were transferred to nutrient agar slants. They were purified by serial dilution and streak methods. Purity was checked by microscopic examination using Gram's staining method. Identification was then carried out based on the biological and biochemical characteristics of the different bacterial genera under test according to the opinions proposed by Blair *et al*. (1967), Bergy's manual of determinative bacteriology (1975) and Collins *et al*. (1989).

The basic medium used unless otherwise mentioned was the nutrient broth and nutrient agar media (APHA, 1960). The different following tests were carried out concerning the desired tested organisms.

Catalase test, Growth on *Staphylococcus* medium n: 110, Slide coagulase test, Thermonuclease test, Gelatinase test, Sugar fermentation test, Haemolysis of sheep blood cells, Gelatin liquefaction, Bile solubility test, Growth on Eosin Methylene Blue Agar medium, Indole test, Methyl Red test, Citrate utilization, Sugar

fermentation, Voges-Proskauer test, (Collins *et al*., 1989). Growth on Mannitol salt agar medium (APHA, 1960). DNase test (Blair *et al*., 1967). Growth at 10°C and 45°C, Growth in medium containing 6.5 percent NaCl, Growth at pH 9, Growth in the presence of 0.1 methylene blue in milk. Growth at 63°C for 30 mn and Nitrate Reduction (Abd-El-Malek and Gibson, 1948).

Chemical analysis of milk fat: All the mastitic milk samples were subjected to fat analysis together with control samples-according to the method of Ling (1963). The obtained results were subjected to statistical analysis using F-test.

Results and Discussion

The present investigation was restricted to the detection of subclinical mastitis in two different farms. In the 1st farm, 66 mastitic milk samples were collected from 328 cows. The 2nd farm-with 110 cows-36 mastitic milk samples were taken. Ten non mastitic milk samples were collected from each farm and used as control. All these samples were subjected to different chemical and bacteriological analysis.

Incidence of sub clinical mastitis: Tests made to detect subclinical mastitis were recorded in Table 1. The scores obtained from CMT test for the first farm differed from that of the 2nd farm. The severity of the disease was more prominent in the 2nd farm. CMT is considered as a rapid reliable test for detecting mastitis in situ (Campbell and Marshall, 1975).

Concerning the Hotis test, the collected samples reacted differently, in the 1st farm, 59 samples out of 66 samples gave positive result, but in the 2nd farm 19 samples out of 39 samples were positive. Miller (1943) stated that the Hotis test always reflect the presence of *Streptococci* in samples. BTB is considered as confirmatory test for the presence of the disease, so all the samples showed a positive result. From the above mentioned result, the incidence of subclinical mastitis was about 20 percent and 36 percent in the first and second farm respectively.

Table 1: Detection of subclinical mastitis in the different samples collected from the two different farms (Total no of samples in 1st farm = 66 and in the 2nd farm = 36)

Sample no	Tests used for the detection		
	CMT test	Hotis test	BTB test
1st farm			
59 samples	+ve	+ve	+ve
7 samples	+ve	-ye	+ve
2nd farm			
2 samples	+ve	+ve	+ve
8 samples	+ve	-ve	+ve
9 samples	2+ve	+ve	+ve
8 samples	2+ve	-ve	+ve
4 samples	3+ve	+ve	+ve
4 samples	4+ve	+ve	+ve
1 samples	4+ve	-ve	+ve

CMT = California Mastitis test BTB = Bromo thymol blue test

Table 2: Total and differential count from the samples collected from the two farms

Bacterial count	Farms under test	
	1st farm (66 samples) average/ml	2nd farm (36 samples) average/ml
Total microbial count	3400	9100
Staphylococci isolates	2090	6100
Streptococci isolates	1900	2300
E. coli isolates	-	10

Total and differential bacterial count: The total bacterial count of the different samples were calculated and tabulated in Table 2. Since mastitis can be due to several micro organisms, the work was focused only on three genera namely *Staphylococcus* spp, *Streptococcus* spp and *Escherichia coil* (Table 2).

From the results, we could detect that the average total and differential count of the 2nd farm was much more higher than that showed by the 1st farm, taking into consideration that the number of collected samples from the 2nd farm was lesser than that of the 1st farm.

The results of the incidence of mastitis and bacterial count correlated together. Ashworth and Blosser (1964) stated that the severity of CMT depends upon the total count, a fact that was clearly shown by the results (Table 1 and 2). High degrees of CMT may be attributed to poor sanitary conditions which leads to increased somatic cell count (Nenkov, 1979; Yosai *et al*., 1980).

Identification of bacterial isolates:

Identification of *Staphylococcus* spp.: The purified, microscopically examined isolates that showed *Staphylococcal* appearance were 290 isolates, 44 isolates from the 1st farm and 246 isolates from the 2nd farm. They were subjected to different biochemical tests and to grow upon different media, the results were tabulated in Table 3. According to the opinions proposed by Blair *et al*. (1967) 222 isolates were considered *Staphylococcus aureus*, 35 isolates were *S. epidermidis* and 33 isolates were *S. saprophyticus*.

Identification of *Streptococcus* spp.: The different purified isolates (89 from the 1st farm and 47 from 2nd farm) that were primarily identified as *Streptococcal* spp were subjected to different tests to prove their identification (Table 4).

Identification was based on that of Bergey's manual of determinative bacteriology (1975). Accordingly, 90 isolates were considered *Streptococcus* agalacitae and 46 isolates as *S. faecalis* (considered now as *Enterococcus faecalis*).

Identification of *Escherichla coli*: Only 5 isolates obtained from the 2nd farm were suspected to be *E. coll*. Following the tests proposed by Bergey's manual of determinative bacteriology (1975), the isolates proved to be *Escherichia coll*. The identified organisms in the different tested milk samples, formed the major genera detected when ever mastitic milk samples were analyzed as shown by Abdul-Nour *et al*. (1978), Jimenez (1979), Ferreiro *et al*. (1981), Verma *et al*. (1981), Kang *et al*. (1986), Konte *et al*. (1988), Schukken *et al*. (1989) and Bartlett *et al*. (1992).

Influence of subclinical mastitis on milk fat content: Fat is an important component of milk, any variation in its content will be In turn reflected on milk composition. The different isolates detected in milk samples whether present solely or in a mixed culture affected greatly the milk fat content. The obtained data after statistical analysis were tabulated in Table 5 and 6. Due to great no of samples, only the higher and lower significant results of each milk samples were recorded in tables.

Effect of the isolates of the 1st farm: Single cultures only were the causative agent of the disease in this farm, the highest effect on

Table 3: Arrangement of the *Staphylococcus* isolates into different patterns according to their reactions towards the used tests

Reaction pattern	Coagulase	DNase	Thermonuclease	Gelatinase	Sugar fermentation			Haemolysis test			No. of isolates yielding respective pattern	Represented spp.
					Mannitol	Trehalose	Mannose	α	β	γ		
1	+	+	+	+	+	+	+	-	+	-	7	*Staph. aureus*
2	+	+	+	+	+	+	-	-	+	-	93	*Staph. aureus*
3	+	+	+	+	+	+	-	-	-	+	41	*Staph. aureus*
4	+	+	+	+	+	+	+	+	-	-	4	*Staph. aureus*
5	+	+	+	+	+	+	-	+	-	-	65	*Staph. aureus*
6	+	-	+	+	+	-	-	+	-	-	3	*Staph. aureus*
7	+	-	+	+	+	+	-	+	-	-	3	*Staph. aureus*
8	+	-	+	+	+	+	+	+	-	-	4	*Staph. aureus*
9	+	-	-	+	+	-	-	+	-	-	1	*Staph. aureus*
10	+	-	-	+	+	-	-	-	+	-	1	*Staph. aureus*
11	-	-	-	-	-	-	+	-	-	+	24	*S. epidermidis*
12	-	-	-	-	-	+	+	-	-	+	2	*S. epidermidis*
13	-	-	-	-	-	-	+	+	-	-	5	*S. epidermidis*
14	-	-	-	+	-	-	+	-	-	+	1	*S. epidermidis*
15	-	-	-	+	-	+	+	-	+	-	1	*S. epidermidis*
16	-	-	-	-	-	-	+	-	+	-	2	*S. epidermidis*
17	-	-	-	-	-	-	-	-	+	-	1	*S. saprophyticus*
18	-	-	-	-	-	-	-	-	-	+	32	*S. saprophyticus*

Table 4: Arrangement of the *Staphylococcus tococcus* isolated into different patterns according to their reactions towards the used tests

Reaction pattern	Growth at 10°C	Growth at 45°C	Growth in medium with 65 % NaCl	Growth in medium with 0.1% methylene Blue in milk	Growth at pH 9.6	Gelatin liquefaction	Bile solubility	Survival at 63°C for 30 cm	Haemolysis test			No of isolated yielding respective Pattern	Represented spp.
									α	β	γ		
1	-	-	-	-	-	-	+	-	-	+	-	90	*S. agelactiae*
2	+	+	+	+	+	+	+	+	-	-	+	31	*S. faecalis*
3	+	+	+	+	+	-	+	+	-	-	+	5	*S. faecalis*
4	+	+	+	+	+	+	+	+	-	+	-	4	*S. faecalis*
5	+	+	+	+	+	-	+	+	-	+	-	6	*S. faecalis*

Table 5: Effect of bacteria isolated from the 1st farm on milk fat content

Milk-borne samples	Fat content		% decrease
	C 3.732		
Staph. epidermidis			
T.S. = 5	T	3.467	7.13
*S.S. = 2		3.433	8.04
LSD at 5 % = 0.248			
Staph. sapaphyticus			
T.S. =	T	3.567	4.45
*S.S. = 4		3.367	9.80
LSD at 5 % = 0.147			
Staph. aureus			
T.S. = 11	T	3.200	14.28
*S.S. = 10		2.800	24.99
LSD at 5 % = 0.289			
Staph. agalactiae			
T.S. = 36	T	3.500	6.24
*S.S. = 31		3.300	11.6
LSD at 5 % = 0.141			
Staph. faecalis			
T.S. = 9	T	3.500	6.24
*S.S. = 3			
LSD at 5 % = 0.149			

C = control for all the test samples; T = test samples
*S.S. = No. of significant samples; *T.S. = No. of total samples

Table 6: Effect of bacteria isolated from the 2nd farm on milk fat content

Milk-borne samples	Fat content		% decrease
	C 3.800		
Staph. epidermidis			
T.S. = 4	T	3.533	7.03
*S.S. = 4		3.433	9.66
LSO at 5 % = 0.168			
Staph. aureus			
T.S. = 14	T	2.600	31.58
*S.S. = 14		2.200	42.11
LSD at 5 % = 0.165			
Staph. aureus + *Enterococcus faecalis*			
T.S. = 4	T	3.300	13.10
*S.S. = 4		2.600	31.50
LSD at 5 % = 0.165			
Staph. aureus + *Staph. saprophyticus* + *Staph. agalactiae*			
T.S. = 5	T	3.300	13.10
S.S. = 5		2.300	39.40
LSD at 5 % = 0.152			
Staph. aureus + *Staph. epidermidis* + *Staph. saprophyticus* + *E. colt*			
T.S. = 1	T	2.267	40.30
*S.S. = 1			
LSD at 5 % = .0.517			
Staph. aureus + *Staph. saprophyticus* + *Enterococcus. faecalis*			
T.S. = 7	T	3.100	18.42
*S.S. = 7		2.300	39.47
LSD at 5 % = 0.169			

C = control for all the test samples; T = test samples
*S.S. = No. of significant samples; T.S. = No. of total samples

milk fat content was due to *Staphylococcus aureus* which caused a decrease in this content varying between 14.2-24.9 percent in relation to control (Table 6), followed by *Streptococcus agalactiae* which decreased the milk fat content by 6.2-11.6 percent then *Staphylococcus saprophyticus* (4.5-9.8%), *S. epidermidis* (7.1-8.04%) and finally *Enterococcus faecalis* which caused a decrease of 6.2 percent.

Effect of the isolates of the 2nd farm: In this farm, the causative agents of the disease were isolates present in a mixed culture or singly. The potent effect was due to single culture of *Staphylococcus aureus* causing a decrease of 31.5 - 42.1 percent in milk fat content, followed by mixed culture of *Staphylococcus* spp and *E. coli* (40.3%), mixed culture of *Staphylococci* and *Streptococci* (13-39%) and the least effect was due to single culture of *Staphyloccus epidermidis* (7.03-9.6%).

The decrease in fat content was observed in both farm,s we could say that *Staphylococcus aureus* and *Enterococcus faecalis* were the highest and least effective organisms respectively. Generally speaking, the decrease in milk fat content of the 2nd farm was more sever than in the 1st farm even when comparing the same species. This is an expected result since the incidence of the disease together with the total and differential bacterial counts were higher in the 2nd farm compared to the 1st showing the severity of the disease.

The obtained results of the decrease of milk fat content was in accordance with the work done by Northern (1970), Butkus *et al*. (1973), Lampo (1973), Abdel-Galil and Nassib (1980), Ferreiro *et al*. (1981) and Merenyi and Wagner (1985). This decrease in fat content may be due to changes occurring in biosynthesis of milk composition as stated by Singh and Singh (1980). Therefore, we could conclude that mastitis is a disease having different degrees of intensity and variations of duration. Any change in milk fat content cause a considerable loss to dairy industry.

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