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Adverse change in milk composition by subclinical mastitis in cows

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Abstract
The study entitled "Effect of subclinical mastitis on milk composition in lactating cows" was conducted to determine the effect of subclinical mastitis on milk quality. This experiment was conducted during May to June, 2018 on a total of 80 lactating dairy cows. The study was designed with the objective to determine the Milk samples were collected and analyzed using Ultrasonic Ekomilk Milk Analyzer Instrument. The study showed that chemical properties of milk samples were pointed out are pH, fat, protein, lactose, solid not fat (SNF), total solids and acidity were significantly (p>0.05) with severity of mastitis. The pH and acidity significantly increased in subclinical mastitis milk samples as compared to normal milk samples whereas significant decreases were recorded in the fat, protein, lactose, solid not fat (SNF) and total solids percent in subclinical mastitis milk samples as compared to normal milk samples of non- affected cows from mastitis disease. A total of 80 cows were screened against subclinical mastitis in cows at dairy farm, Banaras Hindu University, Varanasi.

Keywords: cow, milk, mastitis, milk quality, Ekomilk milk analyzer

1. Introduction

The mastitis is characterized by biochemical changes in composition of milk. These compositional changes reflecting the degree of physical damage are well marked and can be used as a basic indicator for diagnosis of subclinical mastitis. These changes not only alter nutritional quality of milk but also preservation quality. So, investigation on biochemical profile of subclinical mastitis is important. The quality and quantity of milk is affected to varying degrees. Mastitis is not only responsible for great economic losses to the dairy industry but also acts as one of the biggest obstacles in achieving the "White Revolution". In mastitis, the economic losses are due to reduced milk production, poor quality milk, increased cost of Veterinary services and drugs, shortened productive life of animal and finally its replacement cost. Mastitis can be caused by physical or chemical agents but the majority of cases are infectious and usually caused by bacteria. Over 135 microorganisms have been isolated from bovine inflammatory infections and bacteria are one of the major etiological agents of mastitis [10]. Mastitis is caused when pathogenic bacteria enter the sterile environment of the mammary gland, often as a result of disruption of physical barriers such as the teat, requiring prompt and appropriate host defenses to prevent colonization and subsequent disease pathology. In many countries the most common bacterial species causing mastitis are *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus* [1].
Staphylococcus aureus, Streptococci and members of the Enterobacteriaceae are among the most common etiological agents in cows and in other animal species. Mastitis is derived from "matos" referring to the mammary gland, and its meaning inflammation [12]. Mastitis describes an inflammatory reaction in the mammary gland. It is characterized by physical, chemical and bacteriological changes in milk and pathological changes in glandular tissues of the udder and affects the quality and quantity of milk [23]. It is a complex disease associated with variable origin, severity, and outcome depending on the environment, pathogen, and host [26]. Mastitis in dairy animals leads to economic losses in different forms *viz* reduction in milk production (70%), premature culling (14%), veterinary expenses (9%) and milk discarded or low grade (7%) [19]. Bovine mastitis can be defined as a multi factorial disease and is one of the most costly and difficult to control diseases throughout the world [8]. It affects both quality and quantity of milk, increases rate of culling and veterinary expenses [13]. Bovine subclinical mastitis, which has higher rate of prevalence than bovine clinical mastitis in India [18] is economically destructive [34] and clinically elusive due to its camouflaged clinical manifestations [15].

The various diagnostic tests for detection of subclinical mastitis are CMT, WST, SFMT, electrical conductivity of milk, Cl- estimation in milk, Modified Aulendorfer Mastitis Probe (MAMP) test, somatic cell count and culture [33]. The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell counts (SCC) and microbiological status of the quarter. Though bacteriological culture of milk samples is the standard method for identifying mastitis, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted [28].

California Mastitis Test (CMT) is a simple, inexpensive, rapid and highly sensitive test that accurately predicts the inflammatory cell counts in milk from individual quarters or pooled milk samples [16]. The SCC is account used to screen epithelial cells that have been shed from the lining of the gland and white blood cells (leucocytes) that have entered the mammary glands in response to injury or infection [5]. SCC is a useful predictor of subclinical udder infection, therefore, it is considered as an important component for assessing the quality and milk hygiene for mastitis control protocols [29]. The leucocyte count is the basis for most indirect tests employed for diagnosis. Over135 different microorganisms have been isolated from bovine intra mammary infections (IMI), but the majority of infections are caused by *Staphylococcus* spp, *Streptococcus* spp. and gram-negative bacteria [35]. Keeping in view these facts this research work was conducted to determine the effect of subclinical mastitis on milk quality.

2. Materials and Methods

The present investigation entitled "Effect of subclinical mastitis on milk composition in lactating cows" was carried out at Dairy Farm and Laboratory, Department of Animal Husbandry and Dairying, Banaras Hindu University, Varanasi during the year, 2018.

2.1 Methods adopted; Collection of animals

Lactating cows were selected for the present series of investigations. All animals were kept on uniform pattern for feeding and management throughout of the experimental period.

2.2 Housing and management

All the experimental animals were housed in well ventilated cattle shed of Dairy farm, Banaras Hindu University, Varanasi on the pattern of tail-to-tail system. Proper sanitation of the cattle shed was maintained by cleaning it twice a day. The animals were left out for grazing and exercising during the day for few hours.

2.3 Feeding of animals

Well balanced ration as per their requirement and fresh drinking water was regularly provided to all the animals each day during experimental period.

2.4 Milking of animals

Just after calving the cows were hand and machine mulched twice a day both morning and evening at regular intervals throughout the lactation period. The amount of milk produced by each cow was individually recorded every day in the milk record register. The lactation yield was considered as the milk produced by a cow in 305 days. The service and birth records were also maintained at B.H.U. Dairy Farm.

2.5 Sampling of milk

For analysis 100 ml, freshly drawn milk from each quarter of the cows was collected separately in clean, well sterilized and previously dried sample bottle. The samples were taken from morning and evening milking at regular interval for laboratory analysis. Before withdrawing portion for chemical analysis milk samples were brought to the temperature of 68°F (room temperature) and mixed thoroughly into a clean receptacle in order to get homogenous samples.

2.6 Tests used for detection of subclinical mastitis

Modified California Mastitis Test (MCMT) was used to detect subclinical mastitis. MCMT was performed by [20]. Milk affected with subclinical mastitis shows higher number of polymorph nuclear leucocytes which get degenerated due to chemicals present in MCMT reagent and milk sample shows increase in viscosity. This is the basic principle used in MCMT.

2.7 Preparation of modified California mastitis test reagent

The standard reagent was prepared as per following procedure. 30gm of sodium lauryl sulphate powder was taken in 1000ml volumetric flask. Approximately 900 ml of distilled water was added into volumetric flask. The volumetric flask was then kept in water bath at 50 ^{0}C temperature so as to obtain a clear solution. After cooling the solution 0.1gm of Bromocresol purple indicator was added to a final concentration of 1:1000, turning the color of solution to dark purple. Then the final volume of solution was made up to 1000ml by adding distilled water. The pH of solution was adjusted to 8.0 using pH meter. The solution was stored in dark colored bottles [20].

2.7.1 Procedure

The MCMT was conducted in milking shed at the start of milking of each cow. A plastic paddle with four shallow cups marked as left-fore (LF), left–hind (LH), right-fore (RF) and right-hind (RH) was used to detect the individual quarter's incidence of subclinical mastitis. Approximately 2-3ml of first stripping of milk (foremilk) was taken from individual quarter in the respective cup of paddle. Then equal amount (2-3ml) of MCMT reagent was added to each cup of paddle. The contents were mixed by gentle circular motion of paddle in the horizontal plane. Then they were observed for precipitation or gel formation. If gel like substance was formed MCMT was said to be positive and quarter was noted as affected with subclinical mastitis. But if the solution remains watery the MCMT was negative, indicating the quarter was not infected with SCM. The data regarding subclinical mastitis so each individual quarter were recorded on a data sheet.

2.7.2 Description of the visible reaction interpretation

1. No change in mixture (-) Normal
2. A slime which disappears after continuous (t) Trace Swirling
3. Distinct slime with no tendency towards (+) Weak positive Gelformation
4. Mixture thickness immediately with gel (++) Distinct positive
5. A gel forms with a convex surface (+++) strong positive

2.8 Collection of data
The data regarding subclinical mastitis of each individual quarter were recorded on a data sheet. At the same time information regarding type of animal, stage of lactation and method of milking of each animal was recorded to see the effect of these factors on the incidence of subclinical mastitis.

2.9 Analysis of milk samples
Milk samples were collected from cows of Dairy Farm, Department of Animal Husbandry and Dairying subjected to chemical analysis *viz*., pH, fat, solid not fat (SNF), Protein, Lactose, milk acidity and density. Analyses of milk samples were done at laboratory, Department of Animal Husbandry and Dairying, Banaras Hindu University, Varanasi. The individual milk samples were tested for chemical aspects of these parameters.

2.10 Analysis of chemical property of milk
2.10.1 Estimation of milk pH, fat, solid not fat, protein and lactose.
These parameters were determined by using Ultrasonic Ekomilk Milk Analyzer Instrument.

2.10.2 Determination of titratable acidity of milk
Titratable acidity of milk samples was determined as per the procedure described in [3].

Apparatus
1. White Porcelain Basins - hemispherical, 60 ml capacity.
2. Pippette - 10 ml.
3. Burette - 50 ml
4. Stirring Rods - glass, flattened at one end.

Reagents
1. Standard Sodium Hydroxide Solution (0.1 N): A concentrated stock solution of sodium hydroxide was prepared by dissolving equal part of sodium hydroxide (pellets) in equal parts of water in a flask. The flask was tightly stoppered with a rubber bung and any insoluble sodium carbonate formed was allowed to settle for 3 to 4 days. The clear supernatant liquid was used for preparing standard 0.1 N solution. About 8 ml of stock solution was required per liter of distilled water.
2. Phenolphthalein Indicator Solution: 1 g of phenolphthalein powder was dissolved in 100 ml of 95% ethyl alcohol of 0.1 N sodium hydroxide solution was added until to the faint pink color appears. It was further diluted with distilled water to a final volume of 200 ml.

Procedure
10 ml of milk was pipette into 150 ml porcelain dish and mixed with equal volume of boiled cooled distilled water. To this 0.5 percent Phenolphthalein indicator (3-4 drops) was added and titrated against 0.1 N standard NaOH solution. Titratable acidity was calculated as percent lactic acid by the following formula.

Calculation
Titratable acidity (as lactic acid per 100 ml of milk): $9V_1N_1/V_2$
Where,
V_1 Volume in ml of the standard sodium hydroxide required for titration,
N Normality of the standard sodium hydroxide solution
V_2 Volume in ml of milk taken for the test

2.11 Statistical Analysis
At tabular analysis of collected data was used to accomplish the objectives of study. A Student 't' test used to test the significance between normal milk and subclinical mastitis milk of cows described by [32].

3. Results
The present investigation was conducted to investigate the Effect of subclinical mastitis on chemical composition of milk *viz*, pH, fat and solid not-fat (SNF) during the year 2018 at Animal Farm, Department of Animal Husbandry and Dairying, Banaras Hindu University, Varanasi. The experimental findings as influenced by different parameters are discussed below.

3.1 Chemical composition of milk
Out of 63 positive quarters of subclinical mastitis, 20 milk samples from 20 subclinical mastitis quarters and 20 samples from normal quarters of same 20 cows were analyzed for following parameters.

3.1.1 Milk pH and Acidity

Table 1: pH and acidity of normal and subclinical mastitis milk.

pH			Acidity Content		
Samples	**Normal Milk**	**SCM Milk**	**Samples**	**Normal Milk**	**SCM Milk**
1	6.2	6.5	1	0.12	0.16
2	6.5	6.8	2	0.13	0.16
3	6.5	6.6	3	0.12	0.15
4	6.4	6.7	4	0.14	0.18
5	6.3	6.5	5	0.13	0.16
6	6.4	6.7	6	0.12	0.17
7	6.3	6.6	7	0.13	0.16
8	6.5	6.8	8	0.14	0.15
9	6.2	6.5	9	0.14	0.16
10	6.3	6.8	10	0.13	0.17
11	6.3	6.9	11	0.14	0.18
12	6.4	6.6	12	0.12	0.19
13	6.3	6.6	13	0.13	0.16
14	6.5	6.8	14	0.14	0.18
15	6.5	6.6	15	0.15	0.18
16	6.3	6.6	16	0.13	0.16
17	6.3	6.9	17	0.14	0.15

18	6.5	6.8	18	0.13	0.18
19	6.4	6.7	19	0.14	0.16
20	6.4	6.8	20	0.13	0.19
Average	6.37	6.69	Average	0.13	0.19
Significant at 5% level, 't' cal = -8.30			Significant at 5% level, 't' cal = -10.11		

Table 1, represents the pH values of 20 milk samples for the comparison between normal and subclinical mastitis milk during the study. The pH of normal milk ranged between 6.2 and 6.5 and that of subclinical mastitis milk, it was observed between 6.5 and 6.9. The average pH of normal and subclinical mastitis milk was 6.37 and 6.69, respectively. pH value increased by 0.32 units in subclinical mastitis milk. On an average pH values of subclinical mastitis milk were increased than normal milk pH values but non-significantly. The average percentages of acidity in normal milk were 0.13 percent and that of subclinical mastitis milk was 0.17 percent (table 6). It was reduced in SCM milk by 23.52 percent. The acidity percentage of normal milk ranged between 0.12 and 0.14 percent and that of subclinical mastitis milk was between 0.15 and 0.19 percent. On average lactose percent decreased significantly in all subclinical mastitis milk samples.

3.1.2 Milk fat and SNF percent

Table 2: Fat and Solid not fat percent of Normal and Subclinical mastitis milk (%).

Fat			SNF		
Samples	**Normal Milk**	**SCM Milk**	**Samples**	**Normal Milk**	**SCM Milk**
1	3.8	3.4	1	9.8	7.8
2	3.8	3.1	2	9.9	8.1
3	3.7	3.2	3	10.1	8.3
4	3.9	3.3	4	9.7	7.9
5	4.2	3.4	5	9.5	7.7
6	3.6	3.4	6	9.6	8.5
7	3.9	3.3	7	9.8	8.3
8	3.8	3.2	8	9.9	7.9
9	3.9	3.9	9	9.7	8.2
10	3.9	3.2	10	9.5	7.9
11	4.1	3.2	11	9.7	8.4
12	3.8	3.3	12	10.2	8.3
13	3.6	3.1	13	9.8	7.8
14	3.7	3.3	14	10.1	8.7
15	3.7	3.2	15	9.6	8.2
16	4.0	3.3	16	9.7	7.9
17	3.9	3.1	17	9.8	7.7
18	3.8	2.7	18	9.6	8.4
19	3.5	3.0	19	9.5	7.9
20	3.8	3.2	20	9.4	8.1
Average	3.82	3.21	Average	9.74	8.1
Significant at 5% level, 't' cal = 12.70			Significant at 5% level, 't' cal = 22.2		

Average fat percentage in normal quarter milk and subclinical mastitis milk were observed as 3.82 and 3.21 percent respectively (Table 2). After comparison, it was revealed that fat percentage reduced by 15.96 percent SCM affected milk. The fat percentage of normal milk was ranged between 3.5 to 4.1 percent that of subclinical mastitis milk ranged between 2.7 to 3.4 percent. From the present study of fat percentage, it was observed that the average percentage of fat significantly decreased in subclinical mastitis milk samples. The average percentages of Solid not fat in normal milk were9.74 percent and that of subclinical mastitis milk was 8.1 percent (table 2). It was reduced in SCM milk by 16.83 percent. The solid not fat percentage of normal milk ranged between 9.4 and 10.2 percent and that of subclinical mastitis milk was between 7.7 and 8.7 percent. On an average solid not fat percent decreased significantly in all subclinical mastitis milk samples.

3.1.3 Milk protein and lactose content

Table 3: Protein and lactose content of normal and subclinical mastitis milk

Protein			Lactose Content		
Samples	**Normal Milk**	**SCM Milk**	**Samples**	**Normal Milk**	**SCM Milk**
1	3.57	3.55	1	4.83	4.8
2	3.5	3.42	2	5.69	4.64
3	3.58	3.5	3	4.79	4.72
4	4.17	3.32	4	4.89	4.56
5	3.52	3.44	5	4.66	4.42
6	3.55	3.36	6	4.78	4.66
7	3.54	3.52	7	4.69	4.56
8	3.53	3.45	8	4.8	4.7
9	3.75	3.44	9	4.68	4.66
10	3.35	3.33	10	4.56	4.54
11	3.66	3.5	11	4.82	4.78
12	3.6	3.36	12	4.96	4.64
13	3.65	3.29	13	4.86	4.48
14	3.66	3.49	14	4.65	4.62
15	3.67	3.33	15	4.71	4.63
16	3.65	3.45	16	4.96	4.79
17	3.94	3.46	17	4.86	4.83
18	3.56	3.51	18	4.92	4.55
19	3.69	3.01	19	4.97	4.67
20	3.57	3.34	20	4.68	4.25
Average	3.63	3.40	Average	4.83	4.62
Significant at 5% level, 't' cal = 4.95			Significant at 5% level, 't' cal = 3.96		

The average percentages of protein in normal milk were 3.63 percent and that of subclinical mastitis milk was 3.4 percent (table 3). It was reduced in SCM milk by 6.33 percent. The protein percentage of normal milk ranged between 3.35 and 4.17 percent and that of subclinical mastitis milk was between 3.01 and 3.55 percent. On an average protein percent decreased significantly in all subclinical mastitis milk samples. The average percentages of lactose in normal milk were 4.83 percent and that of subclinical mastitis milk was 4.62 percent (table 3). It was reduced in SCM milk by 4.34 percent. The lactose percentage of normal milk ranged between 4.56 and 4.97 percent and that of subclinical mastitis milk was between 4.25 and 4.83 percent. On average lactose percent decreased significantly in all subclinical mastitis milk samples. 3.1.4 Total solid content of milk

Table 4: Total solid content of normal and subclinical mastitis milk

Total Solid Content		
Samples	**Normal Milk**	**SCM Milk**
1	13.5	11.2
2	13.8	11.3
3	13.7	11.6
4	13.5	11.1
5	13.6	11.1
6	13.2	11.9
7	13.6	11.6
8	13.6	11.1
9	13.6	11.5
10	13.4	11.0
11	13.8	11.5
12	13.9	11.7
13	13.4	10.9
14	13.5	11.8
15	13.3	11.4
16	13.7	11.2
17	13.6	10.3
18	13.3	11.3
19	13.3	11.1
20	13.5	11.5
Average	13.54	11.32
Significant at 5% level, 't' cal = 21.17		

The average total solid content in normal milk was 13.54 percent and that of subclinical mastitis milk was 11.32 percent (table 4). It was reduced in SCM milk by 16.39 percent. The total solid of normal milk ranged between 13.2 and 13.9 percent and that of subclinical mastitis milk was between 11.1 and 11.9 percent. On an average total solid percent decreased significantly in all subclinical mastitis milk samples.

4. Discussion

The normal milk pH values were close to the values reported by [19, 7]. Reported increased pH value in SCM milk (6.7 to 6.9) than normal milk (6.4 to 6.8) [4]. Also reported the pH values as increased in subclinical mastitis. These results are in agreement with the results of present study. Increased in pH of subclinical mastitis milk in the study could be due to increased permeability of the gland tissue to blood components results in higher values in milk. This might be partially due to increased movement of bicarbonate ions into milk, since the lactose production decreased and the alkaline salts from the blood enter in the milk become more alkaline showing pH above 7.0 as indicated by [25]. Similar results were reported by [2] observed that average values of acidity, was decreased significantly in subclinical mastitis milk [14]. Reported a significant decrease in acidity of mastitis in cows [22] reported that the average fat percent in normal milk of Gaolao was 4.26 [30] stated the average fat percentage of normal milk of dairy farm, College of Agriculture, Nagpur was 4.86. Similarly [24] observed the decrease in fat percent of SCM milk.

The decrease in fat content in milk due to impaired synthesis and secretary activity of the udder epithelial cell [27]. These results are in agreement with the findings of the present investigation. Similar results were reported by [21], who noticed decrease in solid not fat content of milk, in yielding reaction trial, also [24], observed the solid not fat content of subclinical mastitis milk reduced to 8.51 ± 0.05 gm/dl and in normal milk 8.57±0.08 gm/dl. Similar findings were reported by [30 32]. The results of these scientists were close with the results of present study. Lactose and Protein were major components of SNF, it appeared that drop in SNF was mainly due to decreased lactose content in mastitis milk studied by [25]. Total solids content of milk was found to be significantly lower in mastitis quarters than normal quarters. This finding is similar to study done by [9]. Similar results were reported by [34] reported that the composition of mastitis cow milk had a higher significant decrease in total proteins and koslter's value increased content. [24] Noticed the fat, protein, lactose and solid not fat content of milk affected by subclinical mastitis were reduced to 2.89 ± 0.78 gm/dl, 3.12 ± 0.55 gm/dl, 4.60 ± 0.70 gm/dl and 8.57 ± 0.08 gm/dl respectively. Similar results were reported by [6] examined the milk lactose level of clinical cases of mastitis (1.10±0.05 g/dl) which was significantly lower than normal (4.86±0.04 g/dl), whereas in subclinical mastitis it was (4.37±0.07 g/dl). They also observed that mean lactose content in acute mastitis (1.03±0.06 g/dl) was lower than chronic mastitis (1.17±0.07g/dl) [34]. Reported that the composition of mastitis cow milk had a higher significant decrease in lactose content of milk [24]. Noticed the fat, protein, lactose and solid not fat content of milk affected by subclinical mastitis were reduced to 2.89 ± 0.78 gm/dl, 3.12 ± 0.55 gm/dl, 4.60 ± 0.70 gm/dl and 8.57 ± 0.08 gm/dl respectively. Similar results were reported by [17] observed that the composition of mastitis cow milk had a higher significant decrease in total solid [24]. Noticed the fat, protein, lactose and solid not fat content of milk reduced by subclinical mastitis. 5. Conclusion It is concluded that a significant increase was recorded in pH and acidity of subclinical mastitis milk samples as compared to normal milk samples whereas significant decreases were recorded in the fat, protein, lactose, solid not fat (SNF) and total solids percent in subclinical mastitis milk samples as compared to normal milk samples of crossbred cows at dairy farm, Banaras Hindu University, Varanasi.

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