Detection of subclinical mastitis using the rennet coagulation test as compared with the traditional methods

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Abstract:

The present study aimed to utilize the rennet coagulation test (RCT) as a simple, applicable and indicator (as innovated method) for detecting the subclinical mastitis in the buffalos and cows farms by using the value of the calculated rennin units (RU /ml rennet). Moreover, chemical composition of milk as well as curd tension, curd synersis and yield of milk were done. Results of RCT were compared with the traditional methods used to detect the subclinical mastitis. Results showed no significant differences between the RCT method and the other methods of detecting mastitis milk. There were a decrease in the total solids content of the milk, the strength of the resulting curd, and the yield of mastitic milks while curd synersis, chloride, sodium and pH values were increased.

This study recommends the possibility of using the method of rennet coagulation test to detect the subclinical mastitis because it was simple, rapid on the farm and less expensive than the other methods. RCT method discovered and directed to mastitis in its initial stages, avoiding the losses on the farm and producing milk with good health and technological specifications.

Keywords: mastitis, rennet, milk yield, economic efficiency.

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Introduction

Bovine mastitis is a common disease in the dairy industry that has serious effects on the health of dairy cows and the economy of the industry (Pedersen et al., 2021). Subclinical and clinical mastitis are two clinical forms of bovine mastitis. Mastitis can be diagnosed by tests, including milk bacterial count, somatic cell count, California mastitis test, milk electrical conductivity, and milk microbiology. Mastitis lowers the milk quality and quantity of its constituents. Clinical mastitis is characterized by obvious changes in the milk, such as discoloration and the presence of clots of the udder, as well as clinical indications of infection and inflammation, such as fever, redness, soreness, and udder and lymph node swelling (Adkins & Middleton, 2018). Bovine mastitis caused a 10-20% reduction in milk yield and changes in milk composition. It has low- fat content (Mahran, 1992) as well as a decrease in non-fat solids (SNF), total solids (TS), protein, and lactose with an increase in serum albumin, chloride, and sodium in milk. Thus, mastitis milk is not suitable for making cheese, butter, and condensed milk. Such unnatural milk containing large gross bacteria poses a public health hazard. On the other hand, it is difficult to differentiate between the healthy and clinical animal types of mastitis and must be used bacteriological tests. The procedures and diagnostic capabilities of mastitis tests and milk quality for the dairy farm were studied by Ruegg and Reinemann (2002) like that, the changes in the activity of milk coagulants measured by rennin units (RU/ ml rennet) were discussed by Hassabo (2004). The field techniques to

diagnose early subclinical mastitis are very important for the production of high-quality milk on a buffalos farm (Saber et al., 2017). The electrical conductivity (EC) in the detection of subclinical mastitis was examined for giving health udder (Galfi et al., 2015). Kobayashi, Y. (1987) detected mastitis using the simplified resazurin rennet test, which differs from the method used in the research that it is a laboratory test, not a culture one, where 10 ml of milk was taken from the teat directly, resazurin and rennet were placed on it and incubated for an hour at a temperature of 37 °C, but the rennet test used in the research depends only on the temperature of the milk directly during milking, which is a faster test.

In the present study, the rennet coagulation test (RCT) was employed as a simple applicable indicator for mastitis in the farm by using the value of the calculated rennin units (RU/ ml rennet). A comparison with other tests used for detecting mastitis was also performed.

Material & Methods

- The present work was carried out at Animal Production Research Station, Mehallet Moussa for buffaloes, and at especially farm, Kafer El-sheikh for cows, in the period from January to April 2019.
- **Materials :**
- Dairy animals

Cow (French Holstein) and local buffaloes were chosen on the basis of examination.

- Spry dried skim milk was supplied by Arla Food Co., Sweden.
- Liquid rennet … a good quality liquid rennet was bought from the local market**.**

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- **Sampling of milk**

During the morning, a milk sample was taken from each animal (cow or buffalo) and its yield was recorded.

- **Methods of analysis**:

The following methods were used as follows: Milk: pH value (determination by using a pH meter (Digital pH meter M41150) equipped with a combined glass electrode; Titratable acidity was evaluated according to (Richardson, 1986) ; total solid, fat, protein , and lactose by milkoScan (Model 133B; N. Foss Electric, Hillerød, Denmark) , Sodium content was determined according to the method of (James 1995) and chloride using an automatic titrator.

Methods (tests) for mastitis detection :

* California mastitis test (CMT): was performed using the approach described by (Quinn et al.,1994), which involved testing the four quarters of individual buffalo and cow.

* Somatic cells: Testing Fossomatic 5000 (Foss Electric A/S 69, Slangerupgade DK 3400 Hilleroed, Denmark Comp. Reg. No.73 39 98 15) was used to count somatic cells (SCC) in the milk samples. Classification of animals into two groups according to SCC cells , normal udder health had less than 400 000 and subclinical mastitis contained more than 400 000 cell/ml **(Rysanek and Babak, 2005**).

* Electrical Conductivity (EC): was measureed in milk of each quarter during milking. The animals were classified into two groups based on electrical conductivity value: normal health udder (more than 300) and subclinical mastitis udder (between 251– 300) (Norberg et al., 2004).

* Rennet coagulation test (RCT): approximately 25 ml of milk were collected from each quarter into a plastic paddle

with four shallow cups similar to those used in the California mastitis test. 10 rennin units (RU) were added to each cup of paddle, equivalent to 0.02 g of powder rennet and 0.4 - 2 ml of liquid rennet, and then the time required for coagulation by the rennet was measured. If the time for coagulation was up to one minute, it means that the RCT was negative and indicating that the quarter was not infected with subclinical mastitis. But if the coagulation time is up to 3 minutes, the RCT was positive, and the quarter was noted as affected with subclinical mastitis. The number of rennin units (RU) was estimated using the following Fahmi and Amer's equation (1962):

RU / ml or gram = D/T X 100 where:-

RU / ml or gram = Rennin units/ ml liquid or gram powder rennet.

D = dilution rate of rennet before addition to milk.

T= clotting time of 25 ml of standard milk in seconds.

*curd tension : was determined according to Chandrasekhara *et al.*, (1957).

* synersis: was determined according to Mehanna and Mehanna (1989).

- Statistical analysis :

Results were analysed statistically using the software program of the SAS system (1999) and differences between means were determined by Duncan's multiple range tests at a level of 0.05 probability , Steel& Torrie (1980).

Results

Figure 1 shows that the yield of milk decreased from 21.8 Kg in negative mastitis milk to 18.7 Kg for mastitic cows milk , and the rate of decreasing of the yield of buffalos milk was from 8.8 to 7.4 Kg for normal and mastitic milk

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respectively. These results were in agreement with Tripaldi et al 2010 who found that the subclinical mastitis caused significant decreased buffalo milk yield.

pH values of Subclinical mastitis milk were increased than the pH values of the normal one . The pH value of normal milk was 6.6 in cow and 6.7 in buffalos milk , increased by 0.4 in both subclinical mastitis milks , in order Table (1) . Increased permeability of the gland tissue to blood components results in higher values of these components in milk, and this may explain the higher pH values of subclinical mastitis milk. This could be attributed , also , to the increase in serum albumin, chloride, and sodium contents and a decrease in the protein, and lactose in mastitis milk, causing the pH to rise over 7.0, as mentioned by Rao (1990). The percentages of acidity in normal milks were 0.16 and 0.15 % and in subclinical mastitis were 0.14 and 0.13 % in cow's and buffalo's milk respectively. Generally, the changes in pH and acidity were found significant and Similar findings were reported by Bagri et al 2018, who noticed that average acidity values in subclinical mastitis milk were decreased significantly.

Table (1) also revealed that the total solids % were reduced in all subclinical mastitis milk samples. Normal milk had a total solid content of 11.8 ± 0.23 and 15.6 ± 0.21 % , while subclinical mastitis milk had a total solid content of 10.1 ± 0.21 and 14.40 ± 0.19 % in cow's and buffalo's milk respectively. Muhamed et al., 1998 found that the composition of mastitis cow milk had a higher significant decrease in total solid than that of normal cow milk. Fat percentage of normal milks were 3.5 ± 0.11 and 6.5 ± 0.10

% , decreased to 2.8 ± 0.10 and 6.0 ± 0.10 in case of subclinical mastitis milk of cow's and buffalo's milk , respectively. Similar results were reported by Swami et al. , 2017 who noticed a decrease in fat content of mastitis milk, due to the impaired synthesis and secretion activity of the udder epithelial cell. Also , the same table (1) revealed a decrease in solid not fat (SNF) content of subclinical mastitis cow's and buffalo's milk . SNF content reduced from 8.5 ± 0.14 in cows normal milk to 7.3 ± 0.14 in subclinical mastitis milk and from 9.0 ± 0.14 in normal buffaloes milk to 8.4 ± 0.14 in subclinical mastitis milk. Mostly , this is due to the decrease in protein and lactose contents , which represent the largest part of the solid not fat . A decrease in protein from 3.5 ± 0.09 % to 2.9 ± 0.09 % was observed in normal and subclinical cow's milk, respectively and from 4.0 ± 0.10 % to 3.6 ± 0.10 % in normal and subclinical buffalo's milk in order . In addition , a decrease in lactose content from 4.9 ± 0.13 % to 4.4 ± 0.10 % in normal and subclinical cow's milk and from 5.0 ± 0.12 % to 4.8 ± 0.12 % in normal and subclinical buffalo's milk , respectively , was noticed also . These results are in agreement with Uallah et al 2005 who found a slight decrease in protein contents in mastitic milk and attributed this to an increase in the activity of plasmin, a proteolytic enzyme, which causes sligh degredation to milk casein. Sodium and chloride contents were increased due to mastitis. The sodium contents in normal milks were 0.06 and 0.05 % and that of subclinical mastitis milk were 0.09 and 0.07 % . Also chloride contents in normal milks were 0.10 and 0.09 % and that of subclinical mastitis milk were 0.14 and 0.13 % in cow's and buffalo's milk , respectively.

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The results of the present study are in line with those mentioned by Westfall et al 2011, who reported that sodium content was increased from 0.029 % in healthy cow to 0.096 % in infected one due to mastitis , respectively.

Mastitis tests :

Various tests have been used to detect subclinical mastitis such as the California mastitis test (CMT), somatic cell counts (SCC), and electrical conductivity (EC). In the current study, the rennet coagulation (RC) test was used to detect the subclinical mastitis and compared with the results of the previous tests to prove its effectiveness. RC test is differ than the CMT one , where milk from animals infected with mastitis does not coagulate by adding rennet in RC test, while it coagulates by placing the reagent in the CMT test . In Table (2), results showed that subclinical mastitis cows in California mastitis Test was 24.71% (17.3/70), somatic cell count was 26.14% (18.3/70) and Electrical conductivity was 26.71%(18.7/70) while the same result of subclinical mastitis cows in RC test was 27.14% (19.0/70) . Subclinical mastitis in buffaloes based on California mastitis test was 9.86% (6.9/70), somatic cell count was 11.43% (8.0/70), and Electrical conductivity was 11.86% (8.3/70) while the same result of subclinical mastitis buffaloes in rennet coagulation test was 10.43% (7.3/70) . These results are in agreement with those of Kala et al 2021, who made a comparison of different diagnostic tests for the detection of subclinical mastitis in buffaloes.

Subclinical mastitis was associated with impaired rennet coagulation properties as shown as Table (3). So the rennet coagulation time (RCT) and curd syneresis were increased,

while curd tension was decreased. The RCT (sec) for cow and buffalo normal milk were 48 and 40 sec , increased to 110 and 105 sec for mastitic milk , in order. This may be due to the increased proportion of chloride and sodium ions in mastitis milk. These results confirmed with that reported by Anis et al., (1983), who revealed that increasing of sodium chlorid of buffalo milk from 0 to 2.5% led to increasing the RCT from 90 to 240 sec respectively.

Physical properties :

It seems from data given in Table (3) that the curd tension (CT) values were found higher in case of no mastitis (normal) cows and buffalos being 31.6 and 43.4 g , and 22.8 and 32.9 g in case of subclinical mastitis ones .The rate of curd syneresis (CS) values (ml/15g curd) in normal cows milk were found higher than that in the subclinical mastitis ones being 4.3 , 5.1, 6.2 and 7.3 ml at 10, 30 60 and 120 min. , respectively , when the prepared curd was kept at room temperature. These values were 4.8, 5.7, 6.8 and 7.6 ml for subclinical mastitis milk , in order. Values of CS for subclinical mastitis buffalos milk behaved the same trend of cow milk and were 3.8, 4.3, 5.2 and 5.9 after 10,30,60 and 120 min. and were 2.8, 3.4, 4.4 and 5.4 at 10,30,60 and 120 min., respectively, for negative subclinical mastitis ones . The rennet coagulation time (RCT) showed higher values in mastitis milk than in the normal one .

Conclusion

In conclusion, it was observed that the use of rennet coagulation test (as an innovative method) gave the same results obtained from the basic and usual tests used in detecting mastitis . It was a quick and accurate test. Chemical composition and physical properties of subclinical

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mastitis milks were found inferior than that in normal ones , in both cows and buffaloes animals.

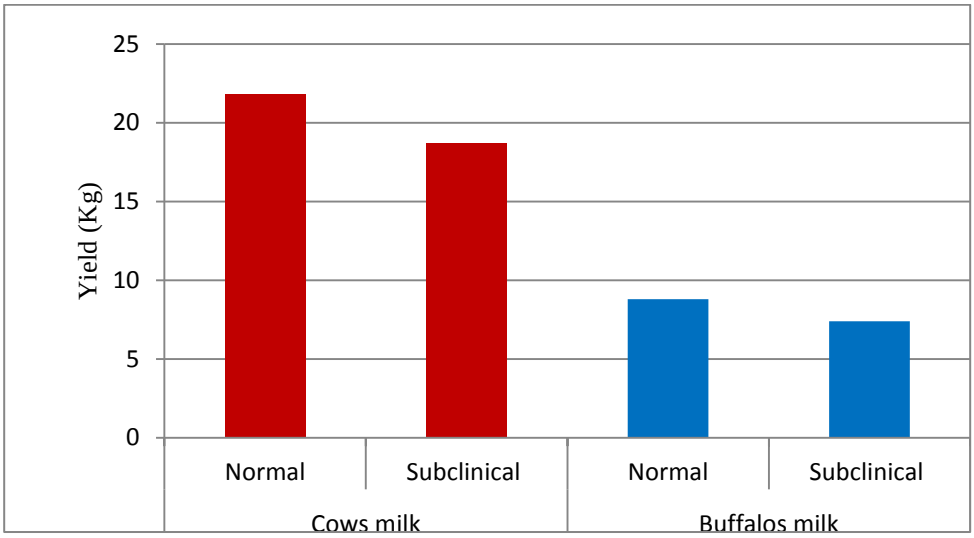

Figure (1) Yield of normal and mastitic milk for lactating buffalos and cows per day

Table (1) pH, acidity and chemical composition of normal (control) and mastitic milks for lactating buffaloes and cows

Variable parameters	Cows milk		Sig.	Buffalos milk		Significant
	Normal	Subclinical		Normal	Subclinical	
pH	6.6 ± 0.03	7.0 ± 0.05	*	6.7 ± 0.03	7.1 ± 0.03	*
Acidity (%)	0.16 ± 0.05	0.14 ± 0.04	**	0.15± 0.04	0.13 ± 0.03	**
Fat (%)	3.5 ± 0.11	2.8 ± 0.10	*	6.5 ± 0.10	6.0 ± 0.10	*
Protein (%)	3.5 ± 0.09	2.9 ± 0.09	**	4.0 ± 0.10	3.6 ± 0.10	**
Lactose(%)	4.9± 0.13	4.4 ± 0.10	**	5.0 ± .12	4.8 ± 0.12	**

Solids not fat (%)	8.5 ± 0.14	7.3 ± 0.14	*	9.0 ± 0.14	8.4 ± 0.14	*
Total solids	11.8 ± 0.23	10.1± 0.21	*	15.6 ± 0.21	14.40 ± 0.19	*
Sodium(%)	0.06 ± 0.01	0.09 ± 0.04	*	0.05 ± 0.06	0.07 ± 0.01	*
Chloride (%)	0.10 ± 0.06	0.14 ± 0.08	*	0.09 ± 0.08	0.13 ± 0.05	*

Means with different superscripts in the same row differed significantly≤.

* Significant at P≤0.05 ** high Significant P≤0.05

Table (2):- Tests for detecting mastitis for lactating buffaloes and cows.

Tests for detecting mastitis	lactating cows		Mastitic cows (%)	lactating Buffalos		Mastitic buffaloes (%)
	Normal	Subclinical		Normal	Subclinical	
CMT	52.7±2.30^{a}	17.3±1.80^{b}	24.71	63.3±2.20^{a}	6.9±2.60^{b}	9.86
SCC	51.7±2.33^{b}	18.3±2.30^{a}	26.14	62.0±1.90^{b}	8.0±2.32^{a}	11.43
EC	51.3±3.20^{b}	18.7±2.94^{a}	26.71	61.7±2.70^{b}	8.3±3.60^{a}	11.86
RCT (control)	51.0±1.90^{b}	19.0±3.80^{a}	27.14	62.7±2.63^{a}	7.3±4.20^{b}	10.43

CMT = California Mastitis Test

SCC = Somatic cell count

EC = Electrical conductivity

RC = Rennet coagulation test (sec.)

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Table (3): Rennet coagulation time (RCT), curd tension and curd syneresis of normal and subclinical mastitic milk for lactating cows and buffaloes

Physical properties		Cow's milk		Buffalo's milk		P value
		Normal	Subclinical	Normal	Subclinical	
RCT (sec.)		48 ± 4.5	110 ± 4.1	40 ± 4.2	105± 3.5	**
Curd tension (g)		31.6 ± 2.0	22.8 ± 1.9	43.4 ± 1.7	32.9 ± 1.64	**
Curd syneresis *AWE after	10 min.	4.3	4.8	2.8	3.8	*
	30 min.	5.1	5.7	3.4	4.3	*
	60 min.	6.2	6.8	4.4	5.2	*
	120 min.	7.3	7.6	5.4	5.9	*

* AWE: amount of whey exuded in (g / 15 g curd)

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