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SOME BIOCHEMICAL CHANGES IN SERUM AND MILK OF MASTITIC BUFFALOES

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Abstract

Total protein and protein fractions were determined in serum and milk samples collected from 60 dairy buffaloes suffering from clinical mastitis. Lactose, calcium, phosphorus, magnesium, potassium, sodium and chloride contents in milk samples were also detected. The results of this study showed that, during progressive mastitis, serum total proteins, albumin and B-globulins were significantly decreased, while, α and δ-globulins were increased. Milk protein was decreased when somatic cell count reached 2.22 ± 0.0045 million cell/ml or more. Casein content was diminished and levels of whey proteins were increased as a consequence of mastitis. Although total casein percentage was dropped, the results diversed in fractions where a marked increase was observed in K casein fraction. The percentage of immunoglobulins and serum albumin in mastitic milk became several times higher in comparison with those in healthy buffaloes milk.

The lactose level in mastitic milk was significantly reduced. sodium and chloride more concentrated in mastitic milk and the amounts of calcium, phosphorus, magnesium and potassium were decreased considerably. Generally, the quantity and quality of milk were greatly affected by the degree of udder inflammation which, in turn, affected the processing properties of milk and its nutritive value.

INTRODUCTION

Despite extensive research and control efforts, mastitis remains a major problem for dairy industry (Alenichkina *et al.*, 1993).

Mastitis is a complicated disease having different causes (microbial, improper nutrition or husbandry, or sudden change in temperature). It also has different degrees of intensity and variations in duration and residual effects. Mastitis exists in different forms with regard to the etiologic agent and mechanism of pathogenesis. Each type of mastitis can result in different gradations of response depending on the severity and the extent of the inflammation, thus, changes from the normal composition of milk may be slight or severe (Shuster, 1991). The economic aspect of this problem is highly important as it causes great losses in dairy products due to diminishing in milk and fat production (Sandholm *et al.*, 1995).

This work was carried out to evaluate the quality of mastitic milk through estimation of some biochemical parameters at different degrees of mastitis.

MATERIALS AND METHODS

Milk samples were collected aseptically together with blood samples from 60 clinically mastitic dairy buffaloes at Ismaillia Province. Blood and milk samples were also collected from ten clinically normal dairy buffaloes and used as control group. Isolation and identification of the bacterial etiological agents were carried out on milk samples according to Cruickshank *et al.* (1975) and Quinn *et al.* (1994). Milk samples were classified according to the degree of inflammation into 3 stages: mild, moderate and progressive stages. Milk samples were also subjected for the following chemical and cytological examinations:

1- Milk total protein was estimated according to Oser (1979).

2- Electrophoretic analysis : serum and milk proteins were electrophoretically fractionated using cellulose acetate membrane and veronal buffer (pH 8.3, ionic strength 0.05) at 200v. for 30 minutes. The strips were stained with ponceau S dye (Mhatre *et al.*, 1962, Henry *et al.*, 1974).

3- Cytological examination : the somatic cell count (SCC) was carried out according to IDF (1984).

4- The sera were separated from blood samples and analysed for total serum protein according to King and Watton (1959).

5- Serum protein fractions were separated and measured by electrophoresis using cellulose acetate strips according to Henry *et al.* (1974).

6- Lactose content in milk determined as described by Ling (1963).

7- Determination of electrolytes : calcium was estimated using the procedure of Glindler and King (1972), and phosphorus according to Kilching and Freiburg (1951). Sodium and potassium were measured using flame photometer, while, magnesium and chlorides were determined according to Oser (1979).

8- Statistical analysis of the data was performed after Milton and Tsokos (1983) to asses the significance of difference between means of controls (normal dairy buffaloes) and mastitic animals using "t" test.

RESULTS AND DISCUSSION

The bacteriological examination revealed isolation of *Staphylococcus aureus* (31%), *Staphylococcus epidermidis* (30%), *streptococcus agalactiae* (15%), *Corynebacterium pyogenes (5%) and Streptococcus dysgalactiae (4%)* for single infection *Streptococcus agalactiae* with *Staphylococcus aureus (10%)* and *Streptococcus agalactiae with Escherichia coli (5%)* for the mixed infection.

Reduction in milk yield was one of the apparent symptoms of mastitis. The reduction in milk yield depends on the degree of inflammation which can be estimated from the somatic cell count in milk (Sandholm *et al.,* 1995). Results of this study showed a significant increase in the somatic cell count as the degree of inflammation increased, where, it was 0.12 ± 0.0024 million cell/ml in mild cases of mastitis, 2.22 ± 0.0045 million cell/ml in moderate cases and 10.51 ± 0.014 million cell/ml in progressive cases of mastitis as compared with 0.11 ± 0.0024 million cell/ml in milk of normal buffaloes. These results agreed with those reported by Andrews (1983) as he mentioned that milk quantity decreased linearly in relation to the logarithmic value of the cell count. He also reported that somatic cell count reflected the changes which occurred in the composition of milk protein, and this agreed with the results of this work as milk protein did not decrease until somatic cell count was 2.22± 0.0045 million cell/ml (Table 1). The significant increase in somatic cell count may be due to influx of neutrophil granulocytes from the circulatory system (Sandholm *et al.,* 1995). Also, the results of this work revealed that the proportion of casein was generally diminished and the proportion of whey proteins increased as a consequence of mastitis (Table 1).

Although the total casein content was significantly decreased, the results for casein fractions showed a significant increase in the percentage of K-casein fraction as the degree of inflammation increased.At the same time, there was a significant decrease in the percentages of α and B casein fractions (Table 1). These results agreed with those reported by Alenchkina *et al.* (1993) and Sandholm *et al.* (1995). The increase in the K casein fraction was a consequence of the decomposition of other caseins (Sandholm *et al.,* 1995). The decomposition of caseins resulted from increased proteolytic activity rather than from disturbances of secreation (Andrews, 1983 and Shuster *et al.,* 1991). The amount of whey proteins was increased considerably in line with the degree of inflammation (Table 1), where, many of whey proteins originated from blood, and their filtration into the mammary gland was increased as the inflammation proceeded (Sandholm *et al.,* 1995).

The percentages of immunoglobulins and serum albumin were several time higher as compared with those in healthy buffaloes milk, while, the percentages of B-lactoglobulin and α-lactalbumin were significantly droped in moderate and progressive cases of mastitis (Table 1). On the other hand, the percentages of proteose peptons and lactoferrin were increased in mastitic milk. These results agreed with those reported by Munro *et al.* (1984) and Shuster *et al.* (1991). These changes in protein composition of milk induced by mastitis could be attributed to the inflammatory reaction, where, the permeability of the blood vessels increased resulting in the passage of ions and proteins from blood to milk. Also, inflammatory cells moved from blood to milk, the epithelial cells which produced milk became less efficient, cells broke down and enzymes were released, leading to interruption of casein synthesis by cells of milk alveoli. On the other hand, leakage of blood plasminogen into milk, and subsequent activation of plasmin into the proteolytically active form which broke down casein gave the serious appearance of milk (Kaartinen *et al.*, 1988, Shuster *et al.*, 1991 and Sandholm *et al.*, 1995).

The significant elevation in the immunoglobulins and serum albumin percentage in milk may be attributed to their infiltration form blood to milk at higher levels due to the increase in the permeability of blood vessels as a consequence of inflammation (Sandholm *et al.*, 1995).

Alenichkina *et al.* (1993) mentioned that, during mastitis immunoglobulins were, not only transferred from blood plasma, but also synthesized in mammary gland tissues. Kaartinen *et al.* (1988) and Knight (1991) mentioned that cytokines are peptide mediators released from activated macrophages, T-lymphocytes and endothelial cells during inflammation. These cytokines regulate the protein synthesis of the liver during the acute phase of inflammation; the liver produces acute phase protein which binds harmful molecules and debris produced after tissue damage (including proteases, haemoglobin and DNA fragments). During acute phase of inflammation, liver increases the production of some proteins at the expense of others including albumin. Liver shifts from production of one protein to another, where the production of the positive acute phase proteins (haptoglobulin, α-protease-inhibitor, fibrinogen, ceruplasmin, α_1-acid glycoprotein and amyloid A) increases, and the production of negative one decreases including prealbumin, albumin and transferrin. These findings agreed with the results of this study, where, serum electrophoresis showed a significant decrease in the albumin and B-globulin. On the other hand, a significant elevation in alpha and gamma globulins was noticed (Table 2).

The results of the present study showed also that the lactose content in mastitic milk was significantly reduced; sodium and chloride became more concentrated. The levels of calcium, phosphorus, magnesium and potassium were significantly reduced (Table 3). The decrease in lactose content may be due to reduction in the concentration of α-lactoalbumin which is necessary for the biosynthesis of lactose (Kaartinen *et al.*, 1988). The decrease in lactose level was leading to disturbance in the somatic balance between milk and blood. To maintain the balance, sodium and chloride ions were filtered from blood to milk and their contents were increased greatly beyond their normal levels (Sandholm *et al.*, 1995).

The mineral and trace elements of milk change during mastitis, and this change affects the processing properties of milk and its nutritive value (Webb *et al.*, 1983). During mastitis, the selective ability of the udder epithelium to concentrate ions is weakened and the passive permeability increases. As a consequence, the salt concentrations in blood and milk balance are in such a way that sodium and chloride become more concentrated, and the amounts of calcium, phosphorus, magnesium and potassium decrease considerably (Sandholm *et al.*, 1995). The curdle ability of milk is weakened by the reduction in the absolute quantity of calcium and phosphorus and the change in their ratios (Munro *et al.*, 1984). The change in lactose and salt balance also reduces heat tolerance of milk and organoleptic properties (Sandholm *et al*, 1995).

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Table 1. Total Protein and Protein fractions in serum of healthy and mastitic buffaloes.

	Total protein	Fraction %			
	gm/dl	Albumin	α globulin	B-globulin	δ-globulin
Healthy buffaloes	7.1±0.35	40.85±1.4	18.01±0.62	14.73±0.33	26.4±0.74
Mastitic buffaloes					
Mild	6.75±0.1	38.91±1.7	18.17±0.58	14.97±0.63	27.95±0.89
Moderate	6.07±0.28*	31.69±1.1***	19.44±0.736	14.38±0.562	34.49±0.68***
Progressive	5.87±0.25**	29.19±1.2***	20.20±0.8*	13.54±0.35*	37.07±0.77***

Significant at: * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Table 2. Total protein and protein fractions percent in milk of the healthy and mastitic buffaloes.

Total protein	Normal milk	Mild cases of mastitis	Moderate cases	Progressive cases
Total protein gm/100 ml	5.1 ± 0.4	4.78 ± 0.3	3.7 ± 0.25 **	3.2 ± 0.21 ***
Casein fractions %				
α	43.05 ± 1.5	41.4 ± 1.31	34.5 ± 1.25 ***	21.13 ± 1.21 ***
B	31.6 ± 0.81	30 ± 0.72	24.3 ± 0.64 ***	19.79 ± 0.554 ***
K or δ	5.38 ± 0.35	6.6 ± 0.55	10.4 ± 0.62 ***	18.9 ± 1.3 ***
Total casein %	80.03 ± 1.8	78.0 ± 1.6	69.2 ± 1.5 ***	59.82 ± 1.2 ***
Whey protein	19.97 ± 0.91	22 ± 0.98	30.8 ± 1.05 ***	40.18 ± 1.2 ***
B-lactoglobulin	11.4 ± 0.34	12.0 ± 0.51	10.44 ± 0.31*	7.7 ± 0.4 ***
a-lactoalbumin (A&B)	3.8 ± 0.21	3.7 ± 0.25	3.16 ± 0.2*	2.8 ± 0.14 ***
Immunoglobulins	2 ± 0.1	2.9 ± 0.13**	7.5 ± 0.2 ***	13.95 ± 0.5 ***
Proteose Peptons	1.5 ± 0.02	1.6 ± 0.03	2 ± 0.06 ***	3.93 ± 0.13***
Serum albumin	0.95 ± 0.09	1.49 ± 0.11 **	5.5 ± 0.2 ***	8.9 ± 0.32 ***
Lacto ferrin	0.32 ± 0.02	0.31 ± 0.07	2.2 ± 0.1 ***	2.9 ± 0.15 ***
Million somatic cell/ml	0.112 ± 0.0024	0.120 ± 0.0036	2.22 ± 0.0045***	10.51 ± 0.014***

Significant at: * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Table 3. Some minerals, electrolytes and lactose level in normal and mastitic milk.

Parameter	Animal	
	Healthy	Progressive masitic
Ca (mg/100ml)	120.8 ± 0.96	78.6 ± 0.4 ***
P (mg/100ml)	107.2 ± 1.05	58.2 ± 0.8 ***
Ca/P ratio	1.27 ± 0.01	1.35 ± 0.015 ***
Mg (mg/100ml)	14.5 ± 0.4	6.8 ± 0.32 ***
K (mg/100 ml)	130 ± 1.2	100 ± 0.9 ***
Na (mg/ 100 ml)	28.4 ± 0.3	62 ± 0.9 ***
Cl (mg/ 100 ml)	90.5 ± 1.1	148 ± 1.3 ***
Lactose (gm/ 100ml)	3.7 ± 0.14	2.4 ± 0.11 ***

Mean ± SE.

Significant at:

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

REFERENCES

1. Alenichkina, G.E., V.M. Sevast-Vanova and A.D. Belov. 1993. The cytomorphology of milk and some metaboilites in buffaloes with mastitis. Voprosey Vet. Biol., 24:35-37.

2. Andrews, A.T. 1983. Breakdown of casein by proteinase in bovine milk's with high somatic cell counts arising from mastitic or infusion with bacterial endotoxin. J. Dairy Res., 50: 57-66.

3. Cruickshank, R., J.P. Duguid B.P Marmion and R.H. Swain. 1975. Medical Microbiology. 12th Ed. Vol. II Churchill Living Stone, Edinburgh, London and New York.

4. Glindler, M. and J.D. King. 1972. Determination of calcium. Am. J. Clin. Path., 58: 376.

5. Henry, R.C., D.L. Canron and J.W. Winkelman. 1974. Clinical chemistry _ Principals and Techniques. pp. 437-440, Harper Row, Hagerstown.

6. International Dairy Federation (IDF). 1984. Recommended methods for somatic cell counting in milk. Bull. IDF. 168.

7. Kaartinen, L., K. Veijalainen., P. L. Kuosa S. Pyorala and M. Sandholm. 1988. Endotoxin-induced mastitis: Inhibition of casein synthesis and activation of the caseinolytic system. J. Vet. Med. B., 35:353-360.

8. Kilchling, H. and B. Freiburg. 1951. Determination of alkaline phosphatase and inorganic phosphorus. Klin. Photometric 3 rd Ed. Wiss. Verl. Gesmbh Stuttgart.

9. King, E.J. and I.D.P. Watton. 1959. Microanalysis in Medical Biochemistry. PP. 58, Churchill Ltd. London.

10. Knight, C.H. 1991. Mammary gland biology. Flem. Vet. J. 64:33-41.

11. Ling, E.R. 1963. A Textbook of Dairy Chemistry. 3rd Ed., pp. 412 Chapman and Hall Ltd., London.

12. Mhatre, N.S., J.G. Leeder and G.N. Wogan. 1962. Cellulose acetate electrophoresis of milk serum proteins. J. Dairy Sci, 45 (6) : 717 - 723.

13. Milton, J.S. and J.O. Tsokos. 1983. Statistical Methods in Biological and Health Sciences. McGraw-Hill Book Company.

14. Munro, G.L., P.A. Grieve and B.J. Kitchen. 1984. Effects of mastitis on Milk yield, milk composition, processing properties and yield and quality of milk products. Aust. J. Dairy Technology, 39:7-16.

15. Oser, B.L. 1979. Hawk's physiological chemistry. 14th Ed., pp. 1111-1112, 1137-1140 , TATA McGraw Hill publishing Company Ltd., New Delhi.

16. Quinn P.J., M.E. Carter, B.K. Markey and G.R. Carter. 1994. Clinical Veterinary Microbiology pp. 90-100. WOLFE, USA.

17. Sandholm M., T. H. Buzalski L. Kaartinen and S. Pyorala. 1995. The bovine udder and mastitis. Gummerus Kirjapaino Oy, Jyvaskyla, Finland.

18. Shuster, D. E., R.J. Harmon, J.A. Jackson and R.W. Hemken. 1991. Suppression of milk production during endotoxin-induced mastitis. J.Dairy Sci., 74:3763-3774.

19. Webb, B., A.H. Johnson, J.A. Alford. 1983. "Fundamentals of dairy chemistry." 2nd Ed., P. 87-124 The Avipublishing Company, INC Westport, Connecticut.

بعض التغيرات البيوكيميائية فى مصل ولبن الجاموس المصاب بالتهاب الضرع

روضات على مطاوع ، أميمة محمود

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تم تقدير البروتين الكلى وأجزائة فى مصل ولبن ٦٠ جاموسة حلوب مصابة أكلينيكيا بالتهاب الضرع. أيضا تم تقدير كمية سكر اللبن الكالسيوم ، الفسفور ، الماغنسيوم ، الصوديوم والكلوريد فى لبن الجاموس المصاب بالتهاب الضرع ومقارنتها بمثيلاتها من لبن الجاموس السليم اكلينيكيا.

وقد أوضحت نتائج هذا البحث إنخفاض قيمة الألبيومين والبيتاجلوبيولين وأرتفاع قيمة الجاما والألفاجلوبيولين فى مصل الحيوانات المصابة بالتهاب الضرع كما ان بروتين اللبن قد انخفض عندما وصلت عدد الخلايا الجسدية فى لبن الجاموس المصاب بالتهاب الضرع الى ٢,٢٢± ٤٤٠٠٠ مليون خلية / ملليميتر . وقد انخفضت أيضا نسبة الكازين الكلى وأرتفعت نسبة البروتين بمصل اللبن نتيجة لألتهاب الضرع بالرغم من قلة الكازين إلا أن النتائج كانت عكسية بالنسبة لأجزاء الكازين حيث ظهر إرتفاع ملحوظ فى جزء الكازين (K).

هذا وقد تضاعفت نسبة جلوبيولينات المناعة والبيومين المصل فى لبن الجاموس المصاب بالتهاب الضرع عدة مرات عند مقارنتها بميثيلاتها فى لبن الجاموس السليم . وقد إنخفض مستوى سكر اللبن إنخفاضا معنويا فى إلتهاب الضرع وأصبح الصوديوم والكلوريد اكثر تركيزا فى لبن الضرع المصاب ، بينما قلت كمية الكالسيوم ، الفوسفور ، الماغنسيوم والبوتاسيوم الى حد بعيد .

وعموما فإن كمية ونوعية اللبن تتأثر تأثراً كبيراً بدرجة التهاب الضرع ومن ثم فإنها تؤثر على تصنيع اللبن وعلى قيمته الغذائية.