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THE EFFECT OF SEVERITY OF MASTITIS ON PROTEIN AND FAT CONTENTS OF BUFFALO MILK

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ABSTRACT

The present study was conducted under field conditions to determine the effect of severity of mastitis on the milk protein and fat contents. For this purpose, 150 buffaloes, having almost same stage of lactation (third to fourth month post calving) and parity (second to third), were selected randomly from District Jhang. Milk protein and fat contents were determined by using Formol and Gerber methods, respectively. The severity of mastitis was graded as P1, P2 and P3 by surf field mastitis test. Average protein (3.85 ± 0.76%) and fat (5.01 ± 0.19%) contents were maximum in the milk of buffaloes that were negative for mastitis. However, these contents decreased with the severity of mastitis from 3.56 ± 0.10 to 3.14 ± 0.10% for protein and 4.91 ± 0.17 to 4.39 ± 0.15% for fat.

Key words: Mastitis, fat, proteins, buffalo, milk.

INTRODUCTION

Milk is an important part of the diet of human beings. It contains a wide range of dietary components of vital importance like water, proteins, lactose, minerals and vitamins. The exact composition of milk varies with the breed, species, feeding regimes, the stage of lactation and udder health. The nutritional significance of milk is indicated by the fact that daily consumption of a quart (1.14 liters) of milk furnishes approximately all the fat, calcium, phosphorus, riboflavin, one half of the protein, one third of vitamin A, ascorbic acid, thiamine and one fourth of calories needed daily by an average individual (Bilal and Ahmad, 2004). Milk is an important source of dietary calcium, which is a key factor in determining healthy bone and teeth development in the young ones and an adequate intake is essential to support optimum skeletal development.

Milk available to our masses is lower in food value due to a higher prevalence of mastitis in dairy animals (Allore, 1993). Mastitis lowers the milk quality and quantity. The most important changes in milk include discoloration, presence of clots and swelling of udder in clinical mastitis, which not only causes loss of production but also lowers the quality of milk. Another misfortune is that risk of mastitis is greater in high producer animals as compared to average and low producers (Oltenacu and Ekesbo, 1994). In addition to causing colossal economic losses to the farmers, the disease is important from consumers and milk processor's point of view. This is because the milk from the affected animals may harbor the organisms potentially pathogenic for human and processing of such milk results in sub-optimal out put of substandard finished fermented products like yogurt and cheese (Barbano, 1989).

In certain parts of the country, milk is being sold on the basis of fat percentage. The animals positive for mastitis have lower fat contents in the milk (Mahran *et al.,* 1992), resulting in a loss for the farmer. The informations about the effects of mastitis on milk composition are very limited in Pakistan. Therefore, the present study was conducted to determine the effect of mastitis on milk protein and fat contents in buffaloes under field conditons.

MATERIALS AND METHODS

This study was undertaken to investigate the effect of mastitis on milk protein and fat contents. Milk samples were collected from 150 buffaloes selected from the field, keeping in view the stage of lactation and lactation number. Before collecting the milk samples, teats were thoroughly washed by tap water and first two streaks of milk were discarded. About 100 ml of milk sample was collected from each teat. Two drops of formaline (10%) were added to each sample as preservative and they were stored at -4°C till further analysis.

Surf field mastitis test (SFMT), as described by Muhammad *et al.* (1995), was used for grading the severity of mastitis. Severity of mastitis was graded as: no visible change in appearance, negative; mild clumping (P1); rapid/moderate clumping (P2) and rapid/heavy clumping (P3). Milk protein contents were determined according to the method described by Davide (1977) and for milk fat determination the method of Aggarwala and Sharma (1961) was used. For protein determination, 10 ml of well mixed milk was poured into Erlenmeyer flask, 10 ml of phenolphthalein indicator, followed by 0.4 ml of neutral saturated potassium oxalate solution, were added and mixed. These were set aside for 2 minutes. Titration with standard 0.1N sodium hydroxide solution was carried out to a faint pink colour. The value of alkali used was noted. Then 2 ml of 40% formalin solution and 10 ml distilled water were taken in a flask and titrated with 0.1N sodium hydroxide solution to the same pink shade and the volume of alkali used was recorded. The percentage of protein present in buffalo milk was obtained by multiplying the volume of 0.1N sodium hydroxide used (subtracted the both volume) by the formal factor 1.91.

However, for milk fat determination, 11 ml milk sample and 1 ml amyl alchohal were gently added to 10 ml of sulphuric acid already poured in each Gerber's acidobutyrometer and were carefully mixed after properly closing the butyrometers with rubber stoppers. The butyrometeres were then centrifuged for 3 to 4 minutes and percentage of fat was recorded.

Statistical analysis

The mean values (±SE) of milk protein and fat contents in respect to various groups i.e. mastitis grade (control, P1, P2 and P3), side of the quarters (right and left) and location of quarters (front and rear) were computed. In order to ascertain the magnitude of variation in these parameter among various groups, the data were analyzed by using two-way analysis of variance technique (Steel and Torrie, 1980). Means were compared by Duncan Multiple Range Test (Montgomery, 1997), where necessary.

RESULTS AND DISCUSSION

Milk protein contents decreased due to mastitis (Table 1). Mean milk protein concentration was 3.85 ± 0.07% in negative quarters but 3.56 ± 0.10, 3.26 ± 0.06 and 3.14 ± 0.10% in quarters with mastits of P1, P2 and P3, grades respectively. Maximum protein contents were found in negative and the minimum in P3 grade mastitic quarters. Statistical analysis indicated a significance difference in protein contents due to the severity of mastitis. However, non-significant differences were found in milk protein contents between left/right and fore/rear quarters (Table 2).

The results of the present study are in line with those of Mert *et al.* (1992), who reported that milk protein contents decreased from 0.92 to 0.82 gm due to mastitis. Similarly, Hortet *et al.* (1998), Urech *et al.* (1998) and Rawdat and Omaima (2000) found a decrease in protein contents in mastitic milk.

In this study fat contents were also affected due to mastitis (Table 3). Mean milk fat concentration was 5.01 ± 0.19% in negative quarters but 4.91 ± 0.17, 4.46 ± 0.19 and 4.39 ± 0.15% in P1, P2, P3 grade mastitic quarters, respectively. Maximum milk fat contents were found in negative and minimum in P3 grade mastitic quarters. Statistical analysis indicated a significant difference in fat contents due to the severity of mastitis. However, a non-significant difference in milk fat extents was found between left/right and fore/rear quarters (Table 4).

These results are in agreement with those of Abdel-Galil and Nassib (1980), who found a slight decrease in fat contents in mastitic milk. Mandal and Raheja (1985) reported that fat contents decreased from 5.18 to 5.06% with severity of mastitis. Shahin and Haggag (1987) collected milk samples from 45 healthy and 45 mastitic buffaloes and found average fat contents of 7.28 ± 0.14 and 4.02 ± 0.8% in the two groups, respectively. Similarly, Mahran *et al.* (1992) indicated 18% less fat in mastitic milk as compared to normal. Hortet *et al.* (1998) reported that there was a slight reduction in milk fat contents in dairy cows due to mastitis.

As observed in the present study, decrease in protein and fat contents in mastitic milk may be attributed to breakdown of milk protein and/or milk fat resulting from mastitis. Milk from clinically or subclinically affected mastitic animals had very high increase in the activity of a proteolytic enzyme (plasmin). This enzyme can cause extensive damage to the milk casein in the udder prior to milk collection from the animal. Similar to protein breakdown, milk fat breakdown that results from mastitis is also caused by enzyme called lipase. The predominant type of fat in milk is in the form of triglycerides (Barbano, 1989). Lipases attack the triglycerides and release free fatty acids. At very low concentrations, free fatty acids produce off-flavours in milk and dairy products that are characterized as rancid off-flavours (Schmidt *et al.*, 1988).

Conclusion

Based on the findings of the present study it can be concluded that severity of mastitis decreased the food

Table 1: Mean milk protein concentrations (%) of normal and mastitic buffaloes

Groups	Right		Left		Overall mean
	Front	Rear	Front	Rear	
Control	3.87 ± 0.16	3.73 ± 0.09	3.99 ± 0.16	3.78 ± 0.13	3.85^{a} ± 0.07
P1	3.53 ± 0.21	3.64 ± 0.21	3.51 ± 0.20	3.55 ± 0.20	3.56^{b} ± 0.10
P2	3.20 ± 0.12	3.18 ± 0.11	3.36 ± 0.12	3.26 ± 0.12	3.26^{c} ± 0.06
P3	3.05 ± 0.17	3.17 ± 0.23	3.10 ± 0.17	3.20 ± 0.21	3.14^{c} ± 0.10

Overall mean values with different superscripts differ significantly (P<0.01).

Table 2: Analysis of variance for the effects of various factors on milk proteins in mastitic buffaloe

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F-value
Grading (G)	3	12.159	4.053	13.5671^{**}
Side (S)	1	0.075	0.075	0.2500^{NS}
G × S	3	0.156	0.052	0.1740^{NS}
Front & rear (FR)	1	0.008	0.008	0.0264^{NS}
G × FR	3	0.528	0.176	0.5892^{NS}
S × FR	1	0.032	0.032	0.1062^{NS}
G × S × FR	3	0.004	0.001	0.0050^{NS}
Error	144	43.018	0.299	
Total	159	55.979		

**Significant at P≤0.01, NS = Non-significant.

Table 3: Mean milk fat concentrations (%) of normal and mastitic buffaloes

Groups	Right		Left		Overall mean
	Front	Rear	Front	Rear	
Control	5.11 ± 0.40	5.12 ± 0.40	5.05 ± 0.40	5.12 ± 0.41	5.01^{a} ± 0.19
P1	4.96 ± 0.37	4.92 ± 0.36	4.90 ± 0.35	4.88 ± 0.35	4.91^{ab} ± 0.17
P2	4.65 ± 0.11	4.47 ± 0.38	4.46 ± 0.38	5.01 ± 0.40	4.46^{ab} ± 0.19
P3	4.36 ± 0.31	4.67 ± 0.32	4.47 ± 0.26	4.00 ± 0.31	4.39^{b} ± 0.15

Overall mean values with different superscripts differ significantly (P<0.05).

Table 4: Analysis of variance for the effects of various factors on milk fat in mastitic buffaloes

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F-value
Grading (G)	3	11.478	3.826	2.8209^{*}
Side (S)	1	0.066	0.066	0.0484^{NS}
G × S	3	0.906	0.302	0.2226^{NS}
Front & rear (FR)	1	0.053	0.053	0.0388^{NS}
G × FR	3	0.336	0.112	0.0827^{NS}
S × FR	1	0.008	0.008	0.0056^{NS}
G × S × FR	3	2.561	0.854	0.6294^{NS}
Error	144	195.306	1.356	
Total	159	210.712		

*Significant at P≤0.05, NS = Non-significant

value of milk in terms of reduced protein and fat contents.

REFERENCES

Abdel-Galil, H. and T.A. Nassib, 1980. Mastitic milk and its effect on white cheese manufacture. Assiut Vet. Med. J., 7: 13-14.

Aggarwala, A.C. and R. M. Sharma, 1961. A Laboratory Manual of Milk Inspection. Asia Publishing House, Bombay, India.

Allore, H.G., 1993. A review of the incidence of the mastitis in buffaloes and cows. Pakistan Vet. J., 13: 1-7.

Barbano, D.M., 1989. Impact of mastitis on dairy product quality and yield: Research update. 28th Annual Meeting, National Mastitis Council, Inc. Tampa, Florida, USA.

Bilal, M.Q. and A. Ahmad, 2004. Dairy Hygiene and Disease Prevention. Usman and Bilal Printing Linkers, Faisalabad, Pakistan.

Davide. C.L., 1977. Laboratory Guide in Dairy Chemistry Practical. FAO Regional Dairy Development and Training Center for Asia and the Pacific, Phillipines. Dairy Training and Research Institute, Univ., Phillipines Los Bamos College, Langnene.

Hortet, P., H. Seegers, P. Hortet and H. Seegers, 1998. Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Prev. Vet. Med., 37:: 1-20.

Mahran, G. A., L.F. Hamzawi, H.F. Haggag and M. M. Ali, 1992. Chemical composition of buffalo colostrum and buffalo milk in mastitic animals. Egyptian J. Food. Sci., 20(1): 67-83.

Mandal, P. C. and R. K. Raheja, 1985. Influence of mastitis on the milk lipids in Indian buffaloes (*Bubalus bubalis*). Arch. Vet., 17: 99-103.

Mert, N., M. Tayar, M. Ogan, A. Muftuoglu and M. Yavuz, 1992. Biochemical changes in the milk of cows with sub-clinical mastitis. Uludag Vet. J., 11(2): 13-18.

Montgomery, D.C., 1997. Design and Analysis of Experiments. John Wieley and Sons, New York, USA.

Muhammad, G., M. Athar, A. Shakoor, M.Z. Khan, F. Rehman and M.T. Ahmad, 1995. Surf Field Mastitis Test: An inexpensive new tool for evaluation of wholesomeness of fresh milk. Pakistan J. Food. Sci., 5: 91-93.

Oltenacu, P.A. and I. Ekesbo, 1994. Epidemiological study of clinical mastitis in dairy cattle. Vet. Res., 25(2-3): 208-212.

Rawdat, A. M. and N. M. Omaima, 2000. Some biochemical changes in serum and milk of mastitic buffaloes. Egyptian J. Agri. Res., 78(4): 1737-1747.

Schmidt, G.H., L.D. Vleck, and M.F. Hutjens, 1988. Principles of Dairy Science, 2nd Ed. Prentice Hall, England.

Shahin, Y. and H.F. Haggag, 1987. The free fatty acids content in buffalo's milk as affected by mastitis. Assiut J. Agri. Sci., 18(4): 335-341.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach, McGraw Hill, Publ. Co. Inc., New York, USA.

Urech, E., Z. Puhan and M. Schallibaum, 1998. Changes in milk protein in fractions as affected by subclinical mastitis. J. Dairy Sci., 82: 2402-2411.