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RESEARCH ARTICLE

Therapeutic Effectiveness of Zinc and Copper Alone and in Combination with Enrofloxacin for the Treatment of Sub-Clinical Mastitis in Dairy Buffaloes

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ABSTRACT

The present study was designed to compare the therapeutic effectiveness of zinc (Zn) and copper (Cu) alone and in combination with enrofloxacin for the treatment of sub-clinical mastitis in dairy buffaloes. Out of 152 lactating buffaloes, 45 suffering from sub-clinical mastitis were selected on the basis of positive Surf Field Mastitis Test (SFMT). These buffaloes were randomly divided into five equal groups (A-E). Buffaloes of group A were supplemented with $ZnSO_4$ @ 1 g per animal per day, Group B $CuSO_4$ @ 1 g per animal per day for 14 days. Group C was injected with enrofloxacin @ 2.5 mg/kg B.W through intramuscular route for 5 consecutive days. Buffaloes of group D were administered with intramuscular injection of enrofloxacin @ 2.5 mg/kg B.W for five consecutive days along with the supplementation of $ZnSO_4$ (1 g per day) and $CuSO_4$ (1 g per day) for 14 days. Group E was kept as untreated control. Milk and blood samples were collected at day 0, 7 and 14 post treatment for various tests like somatic cell count (SCC), SFMT based and bacteriological based cure rates, total leukocyte count (TLC), lymphocyte percentage, serum zinc and copper concentration. The SCC in milk decreased significantly ($P<0.01$) in combined therapy group D at day 14 post treatment. Serum zinc and copper concentrations increased significantly ($P<0.01$) in supplemented groups at day 7 and 14 post treatment. TLC and lymphocytes percentage was significantly higher ($P<0.01$) in combined therapy at day 7 and 14 post treatment. Percent cure rate on the basis of SFMT and bacteriological based cure rate was higher in group D. Milk yield of affected quarter was improved significantly ($P<0.01$) in the group D and other groups showed non-significant ($P>0.05$) improvement at day 14 post treatment. It may be concluded that the combined therapy was better than alone therapy of zinc sulphate, copper sulphate and enrofloxacin.

INTRODUCTION

Pakistan is gifted with huge livestock population. The livestock sector shares 55.1% of the agricultural GDP. There are nearly 31.7 million buffaloes in Pakistan which produces 28.694 million tons of milk annually. Buffaloes share 61.8% of the total milk production in our country (Anonymous, 2013). Buffalo is called 'the black gold' of Pakistan (Bilal et al., 2006). Among the dairy diseases, mastitis is one of the most important dairy diseases in Pakistan (Akram, 2002). Mastitis occurs in two forms viz., clinical and sub-clinical (Radostits et al., 2007). Mastitis leads to huge economic losses in Pakistan (Ahmad, 2001). Economical loses are in the form of decrease in milk production and increase in cost of medication (Degraves and Fetrow, 1993).

In sub-clinical mastitis, the apparent signs are absent but somatic cell count (SCC) is high (Radostitis et al., 2007). Sub-clinical mastitis results in culling of large number of animals, increased treatment cost, decreased milk production and poor quality milk (Dobbins, 1997). Sub-clinical mastitis causes more economical losses because it is 3 to 4 times more prevalent than the clinical mastitis (Jasper et al., 1982). Mastitis leads to reduction in the number of B- lymphocytes in the blood which indicates immunosuppression (Ishikawa and Shimizu, 1982). The subclinical mastitis also resulted in increased level of serum haptoglobin and serum amyloid A (Nazifi et al., 2011).

Zinc plays a vital role in different functions of immune system. It directly induces monocytes to produce interleukin 2, 6 and tumor necrosis factor alpha (TNF α) which act as the pro-inflammatory mediators (Rink and Kirchner, 1998). Zinc is a cofactor for many proteins and enzymes which take part in the initial response of body to any infection or inflammation (Prasad, 1979). Superoxide dismutase which requires Zn for activation is an enzyme which protects the cellular membranes of udder and teat against the activity of superoxide radicals (Moynahan, 1981). Deficiency of Zn causes decrease in basal metabolic rate of ruminants and also leads to weaken skin and epithelium (Harmon and Torre, 1994). The cows which receive Zn supplementation are comparatively healthier than those animals which are not receiving Zn supplementation (Chirase et al., 1991).

Copper is also an important element for the proper functioning of immune system. (Failla, 2003). This element plays a vital role in the formation of neutrophils and also influences the phagocytic activity of macrophages (Linder, 1991). Deficiency of Cu causes decrease in number of circulating neutrophils, B cells and T cells. Animals which are deficient in Cu are highly prone to infections (McDowell, 2005).

When Cu is given along with Zn, it plays an important role in the activity of superoxide dismutase and also helps in excretion of free radicals. Ceruloplasmin has an anti-inflammatory activity, thus may be helpful to control mastitis (Tomlinson et al., 2008). Excessive supplementation of Zn results in secondary Cu deffeciency. So, dietry Zn must not be more than five times the dietry Cu (National Research Council, 2001).

Pakistani soil is deficient in available elements like Zn, phosphorus and nitrogen (Alam et al., 1997). Majority of the forages available at livestock station Khizerabad, Sargodha, Pakistan are deficient in Zn, Cu and cobalt (Khan et al., 2009). Deficiency of Zn, Cu, iron and selenium has been reported in Nili-Ravi buffaloes at Buffalo Research Institute, Pattoki, District Kasur, Pakistan (Akhtar et al., 2009).

Enrofloxacin (Encure-10^{TM}, Nawan Laboratories Pvt. Limited, Karachi, Pakistan) showed remarkable decrease in SCC and total bacterial count in bovine sub-clinical mastitis treatment (Reena and Dash, 2003). Enrofloxacin had 83.3% recovery rate in bovine sub-clinical mastitis.

As there is immunosuppressive effect of mastitis and immunomodulatory effect of Zn and Cu, therefore, present study was planned with objective to Compare the therapeutic efficacy of Zn and Cu alone and in combination with enrofloxacin in the treatment of sub clinical mastitis.

MATERIALS AND METHODS

Out of 152 lactating buffaloes, 45 suffering from sub-clinical mastitis were randomly selected on the basis of positive SFMT (Muhammad et al., 2010) from three dairy herds having almost similar management conditions with slight variation in feeding regime in Faisalabad. Animals of each dairy herd were further divided into five equal groups (A, B, C, D and E). Guidelines of International Dairy Federation (Thorburn, 1990) for mastitis therapy were followed for assigning study animals in different groups.

Milk samples were collected aseptically from all the experimental animals 3 times i.e. at day 0, day 7 and 14 of treatment plan. The milk samples were cultured on blood agar for bacteriological examination and SFMT performed and the reactions generated were interpreted as described by Schalm et al. (1971).

A quarter was considered to be infected if 5 or more similar colonies were present on the plate. The representative colonies were isolated and purified by streaking on fresh blood agar plates. After isolation, Gram staining was performed. Confirmation was done by catalase test and coagulase test. Catalase positive, coagulase positive and gram positive coccal isolates were presumptively identified as *Staphylococci* or *Micrococci*. Organisms other than *Staphylococcus* were identified by routine biochemical tests (National Mastitis Council Inc., 1990).

Gram staining was performed as (Cruickshank et al., 1975) and slides were examined under oil immersion lens of microscope. Gram positive *cocci* were tested for catalase production and coagulase test was performed to divide *Staphylococcal* isolates into coagulase positive *Staphylococci* (CPS) and coagulase negative *Staphylococci* (CNS). Procedure described by National Mastitis Council Inc. (1990) was followed.

From milk samples of all the experimental animals SCC determined before treatment and on day 14 post treatment. Newman's Lampert staining was used for SCC in milk and Stained slides were examined under an oil immersion lens. It was carried out by adopting the technique described by Schalm et al. (1971).

Copper and zinc concentration in the serum of all the experimental animals were determined before treatment, on day 7 and 14 post treatment. Wet digestion of serum samples was carried out as described by Richard (1969).

Total leukocyte count (TLC) and lymphocyte percentage was carried out at day 0 (control) and day 14 post treatment by Medonic M series (MERCK, Stockholm, Sweden). Daily milk yield of every animal was calculated from day 0 (control) today 14 post treatment. Effect of treatments on milk production was compared.

Statistical analysis

The data thus obtained was analyzed by randomized complete block design (RCBD) and means were compared by Duncan's multiple range test (DMR) and Least significant difference test (LSD) using SAS statistical software (SAS, 2004).

RESULTS

Effect of treatments on somatic cell count in subclinically mastitic buffaloes

Mean somatic cell count of zinc sulphate, copper sulphate and enrofloxacin combined therapy (group D) was significantly lesser than that of group A, B and C. Cumulative mean of zinc sulphate was nonsignificant (P>0.05) with the enrofloxacin treated. Decrease in somatic cell count is an indicator of good health of the mammary glands. Maximum decrease in SCC was seen in the group which was treated with combined therapy of zinc sulphate, copper sulphate and enrofloxacin (group D) Table 1.

Effect of treatments on bacteriological cure rate in subclinically mastitic buffaloes

Cure rates based on bacteriological examination at day 14 post treatment is given in Table 1. The combined therapy of all three zinc, copper and enrofloxacin (group D) had highest bacteriological cure rate of 80%, followed by zinc sulphate (group A) with 65%, enrofloxacin (group C) 53% and copper sulphate (group B) 40%. So the combined therapy of all three (group D) was superior to the zinc sulphate alone and copper sulphate alone therapy Table 2.

Effect of treatments on Surf Field Mastitis Test based cure rate

Mean Surf Field Mastitis Test based cure rate has given in Table 2. Mean SFMT score of combined treatment (group D) was significantly lesser than the mean SFMT score of zinc sulphate (group A), copper sulphate (group B) and enrofloxacin (group C). Among the different treated groups, the group D showed the highest cure rates percentage on both days of performing SFMT Table 3.

Effect of treatments on milk production of affected quarter

Effect of the treatment on milk yield of the affected quarter was recorded from day 0 to day 14 post treatment. Milk yield of zinc sulphate, copper sulphate and enrofloxacin combined therapy (group D) was significantly higher (P<0.01) than the zinc sulphate alone (group A), copper sulphate alone (group B) and enrofloxacin alone (group C). At day 14 post treatment, total milk production of copper sulphate treatment (group B) was non-significant (P>0.05) with the enrofloxacin treatment (group C) Table 4.

Table 1: Percentage decrease of Somatic cell count in sub-clinically mastitic buffaloes

Groups	Number of buffaloes	SCC ($\times10^5$ cells/ml) Day 0 Mean ± SE	SCC ($\times10^5$ cells/ml) Day 14 PT Mean ± SE	Percent (%) decrease in Somatic Cell Count
Group A	9	10.06±0.182	4.10±0.099	59.3
Group B	9	10.12±0.193	5.50±0.181	45.7
Group C	9	10.06±0.185	4.60±0.140	54.3
Group D	9	10.13±0.189	2.77±0.169	72.7
Group E	9	10.26±0.139	10.27±0.177	1% increase in SCC

(PT)= Post Treatment Group A= Zinc sulphate @ 1 g per animal per day for 14 days. Group B= Copper sulphate @ 1 g per animal per day for 14 days. Group C= Enrofloxacin (Encure-10™, Nawan Laboratories Pvt. Ltd. Karachi, Pakistan) @ 2.5 mg/kg B.W for 5 consecutive days.Group D= Zinc sulphate @ 1 g per day for 14 days, copper sulphate @ 1 g per day for 14 days and enrofloxacin (Encure-10™, Nawan Laboratories Pvt. Ltd. Karachi, Pakistan) @ 2.5 mg/kg B.W for 5 consecutive days.Group E= Untreated control

Table 2: Bacteriological cure rates percentage of sub-clinically mastitic buffaloes

Groups	Total number of cured quarters at day 14 post treatment	Cure rate percentage % at day 14 post treatment
Group A	18	65
Group B	13	40
Group C	16	53
Group D	21	80
Group E	2	7

DISCUSSION

Higher somatic cell count showed the infection of udder and lead to deterioration of milk quality (Berguland et al., 2007). Normal SCC in buffaloes varies from 0.5×10^5 cells/ml to 3.75×10^5 cells/ml with a mean of 1.4×10^5 cells/ml (Silva and Silva, 1994). At the end of experiment, lowest SCC is seen in the group received the combined therapy of zinc sulphate, copper sulphate and enrofloxacin.

At day 14 post treatment, there was no significant difference in the serum zinc concentration of combined therapy of zinc sulphate and copper sulphate (group D) and zinc sulphate alone therapy (group A).

Due to sub-clinical mastitis in buffaloes, there is decrease in the total leukocyte count as reported by Zaman et al. (1997) and Knight (1983). Similar result of total leukocytes count (4.08×10^3 cells/mm^3) was found in sub-clinically mastitic buffaloes selected in this study at day 0 (before treatment). Normal total

Table 3: Comparison of mean±SE Surf Field Mastitis Test score in sub-clinically mastitic buffaloes

Group	Days			Mean
	0	7	14	
A	3.44±0.18 a	1.56±0.44 c	0.67±0.33 d	1.89±0.29 CB
B	3.56±0.18 a	1.89±0.45 d	1.22±0.40 b	2.22±0.28 B
C	3.33±0.17 ab	1.33±0.47 b	1.00±0.41 c	1.89±0.29 CB
D	3.44±0.18 a	0.89±0.35 a	0.11±0.11 e	1.48±0.31 D
E	3.56±0.18 a	3.56±0.18 e	3.56±0.18 a	3.56±0.10 A
Mean	3.47±0.08 A	1.84±0.22 B	1.31±0.22 C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4: Comparison of mean±SE milk yield of affected quarter in sub-clinically mastitic buffaloes

Groups	Days		Mean
	0	14 (post treatment)	
A	1.20±0.023 d	1.75±0.025 b	1.48±0.035 B
B	1.17±0.017 d	1.45±0.023 c	1.31±0.026 C
C	1.18±0.018 d	1.70±0.020 c	1.44±0.024 B
D	1.19±0.015 d	1.95±0.022 a	1.57±0.046 A
E	1.19±0.019 d	1.19±0.019 d	1.19±0.013 D
Mean	1.19±0.008 B	1.38±0.021 A	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

leukocyte count in buffalo is 9.1×10^3 cells/mm^3 as reported by Hagawane et al. (2009) and Sulong et al. (1980). These treatments help to increase TLC towards normal as at day 14 post treatment.

Due to sub-clinical mastitis in buffaloes, there is decrease in the lymphocyte percentage as reported by Zaman et al. (1997) and Knight (1983). Similar result of lymphocyte percentage 47.7% was found in sub-clinically mastitic buffaloes selected in this study at day 0 (before treatment). Normal lymphocyte percentage in buffalo is 56.7% as reported by Hagawane et al. (2009). At day 14 post treatment, lymphocyte percentage is increased towards normal.

Surf Field Mastitis Test is a precious tool for semi-quantitative evaluation of milk cell concentration and a good indicator of udder infection (Fazal-ur-Rehman, 1995; Busato et al., 2000). The cure rate of SFMT was nearly similar to the results of Upadhayay et al. (2008) who reported 75% cure rate in zinc sulphate supplementation and 41% cure rate in copper sulphate supplementation.

Milk production was almost always lower in sub-clinical mastitis. Buffaloes treated with the combined therapy of zinc sulphate, copper sulphate and enrofloxacin showed maximum recovery in milk yield. These findings are in line with that of Gray and Schalm (1992) who also reported a significant improvement in milk production when cows were recovered from sub-clinical mastitis.

Zinc sulphate supplementation had better results than copper sulphate supplementation. Highest increase in the combined therapy of zinc sulphate, copper sulphate and enrofloxacin (group D) was due to the synergetic effect of zinc sulphate and copper sulphate in the production of lymphocytes and neutrophils (Tomlinson et al., 2008).

It can be concluded from the findings of present study that the combined treatment of zinc sulphate, copper sulphate and enrofloxacin was better than zinc sulphate alone, copper sulphate alone and enrofloxacin alone therapy in the treatment of sub-clinically mastitic buffaloes. It may be considered as future line of treatment for mastitis in Pakistan.

REFERENCES

Ahmad R, 2001. Studies on mastitis amoung dairy buffaloes. Pakistan Veterinary Journal, 21: 220-221.

Akhtar SM, AA Farooq and M Mushtaq, 2009. Serum concentrations of copper, iron, zinc and selenium in cyclic and anoestrous Nili-Ravi buffaloes kept under farm conditions. Pakistan Veterinary Journal, 29: 47-48.

Alam SM, A Latif and Z Iqbal, 1997. Response of rice to nitrogen and zinc fertilization with or without copper. Pakistan Journal of Soil Sciences, 13: 1-4.

Anonymous, 2013. Pakistan Economic Survey. Economic Advisor's Wing, Finance Division, Govt. of Pakistan, Islamabad, Pakistan.

Berglund I, G Pettersson, K Ostensson and K Svennersten-Sjaunja, 2007. Quarter milking for improved detection of increased somatic cell count. Reproduction in Domestic Animals, 42: 427-432.

Bilal MQ, M Suleman and A Razia, 2006. Buffalo: Black gold of Pakistan. Livestock Research and Rural Development. 18: 1-4.

Busato A, P Trachsel, M Schallibaum and JW Blum, 2000. Udder health and risk factors for sub-clinical mastitis in organic dairy farms in Switzerland. Preventive Veterinary Medicine, 44: 205-220.

Chirase NK, DP Hutcheson and GB Tompson, 1991. Feed intake, rectal temperature and serum mineral concentration of feedlot cattle fed zinc oxide and zinc methionine and challenged with infectious bovine rhinotracheitis virus. Journal of Animal Sciences, 69: 37-41.

Criuckshank L, JP Duguid, BP Marmion and RHA, Swain. 1975. Medical Microbiology, 12th Ed., Churchill Living Stone, Edinburg, London.

Degraves FJ and J Fetrow, 1993. Economics of mastitis and mastitis control. Veterinary Clinics of North America. Food Animal Practice, 9: 421-434.

Dobbins CN, 1997. Mastitis losses. Journal of American Veterinary Medical Association, 170: 1129-1132.

Failla ML, 2003. Trace elements and host defense: Recent advances and continuing challenges. Journal of Nutrition, 133: 1443S.

Frake PJ, LE King, T Laakkoo and TL Volimer, 2000. The dynamic link between the integrity of the immune system and zinc status. Journal of Nutrition, 130: 1399-1406.

Gray DM and OW Schalm, 1992. The mastitis variable in milk yield as estimated by the California Mastitis Test. American Journal of Veterinary Research, 23: 541-543.

Harmon RJ and PM Torre, 1994. Copper and zinc: do they influence mastitis? Proceedings of National Mastitis Council, Orlando, Florida, USA, pp: 54-65.

Ishikawa H and T Shimizu, 1982. Depression of B-Lymphocytes by mastitis and treatment with levamisole. Journal of Dairy Science, 66: 556-561.

Jasper DE, JS McDonald, RD Mochrie, WN Philpot, RJ Farworth and SB Spencer, 1982. Bovine mastitis research needs funding and sources of support. In: Proceedings of 21st Annual Meeting, National Mastitis Council, Louisville, Kentucky, USA, pp: 182-193.

Khan IZ, M Ashraf, N Ahmad, K Ahmad and EE Valeem. 2009. Availability of nutritional minerals (cobalt, copper, iron and zinc) in pastures of central Punjab for farm livestock. Pakistan Journal of Botany, 41: 1603-1609.

Knight AP, 1983. Blood values of six years old Holstein cows in mastitis. Journal of American Veterinary Medical Association. 182: 126-127.

Linder MC, 1991. Biochemistry of Copper. Earl Frieden (eds), Plenum Press, New York, USA.

McDowell LR and JD Arthington, 2005. Minerals for Grazing Ruminants in Tropical Regions, 5th Ed., University of Florida, Gainesville, Florida, USA, pp: 82-86.

Moynahan EJ, 1981. Acrodermatitis enteropathica and the immunological role of zinc. Safai B and RA Good (eds), Immunodermatology. Plenum Medical Book Co., New York, USA.

Muhammad G, A Naureen, MN Asi, M Saqib and Fazal-ur-Rehman, 2010. Evaluation of a 3% surf solution (Surf Field Mastitis Test) for the diagnosis of subclinical bovine and bubaline mastitis. Tropical Animal Health and Production, 42: 457-464.

National Mastitis Council Inc. 1990. Microbiological Procedures for the Diagnosis of Udder Infection. National Mastitis Council Inc, Arlington, USA.

National Research Council, 2001. Nutrient Requirements of Dairy Cattle. National Academies Press, Washington DC.

Nazafi S, M Haghkhah, Z Asadi, M Ansari-Lari, MR Tabandeh, Z Esmailnezahd and M Aghamiri, 2011. Evaluation of sialic acid and acute phase proteins (Haptoglobin and Serum Amyloid A) in clinical and subclinical bovine mastitis. Pakistan Veterinary Journal, 31: 55-59.

Prasad AS, 1979. Clinical, biochemical and pharmacological role of zinc. Reviews in. Pharmacology and Toxicology, 19: 393.

Radostits OM, CC Gay, KW Hinchcliff and PD Constable, 2007. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Ed, WB Saunders Co, Philadelphia, USA.

Reena M and PK Dash, 2003. Immunomodulatory and therapeutic potential of enrofloxacin in bovine sub clinical mastitis. Asian Australian Journal of Animal Sciences, 16: 889-893.

Richard LA, 1969. Diagnosis and improvements of saline and alkali soils. Department of Agriculture, Washington, DC.

Rink L and H Kirchner, 1998. Zinc-altered immune function and cytokine production. Proceedings of the International Workshop on Zinc and health: Current status and future directions. Bethesda, Maryland, USA.

SAS, 2004. SAS, Statistical Software Version 9.1. SAS Institute Inc., Cary, North Carolina, USA.

Schalm OW, EJ Carol and NC Jain, 1971. Bovine Mastitis. Lea and Febiger, Philadelphia, USA.

Silva ID and KFST Silva. 1994. Total and differential cell counts in buffalo milk. Buffalo Journal, 2: 133-137.

Sulong A, M Hilmi and MR Jaimudeen, 1980. Haematology of the Malaysian swamp buffalo. Pertanika, 3: 66-70.

Thorburn MA, 1990. General principles for the design of clinical trial with special reference to mastitis therapy. Bulletin of International Dairy Federation, 247: 39-48.

Tomlinson JD, MT Socha and JM DeFrain, 2008. Role of Trace Minerals in the Immune System. Proceedings of Penn State Dairy Cattle Nutrition Workshop. Grantville, Pennsylvania, USA, pp: 39-52.

Upadhayay KA, P Gangwar and M Kumar, 2008. Supplementation to prevent subclinical mastitis. Vet World, 1: 40-41.

Zaman F, MS Khan, K Pervaiz, AG Khan, IG Ahmad and M Shafique, 1997. Estimation correlation of protein, total leukocyte count and differential leukocyte count in blood and milk of sub-clinically mastitic buffaloes. Proceedings of 9th International Congress in Animal Hygiene, Helsinki, Finland.