AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 1, № 2, pp 1-3, 2009

Product Quality and Safety

Lysozyme content in buffalo colostrum

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(Manuscript received 10 June 2009; accepted for publication 14 July 2009)

Abstract. *The lysozyme content in buffalo colostrum from Bulgarian Murrah breed was studied. The experiment was conducted during the summer of 2007 at the buffalo farm of Dimitrievo (Haskovo area), Bulgaria. The buffaloes selected for evaluation in this study were clinically healthy. They were selected by age, body weight, and expected date of calving. All animals were on their first lactation. The diet of the experimental buffaloes was formulated to meet the nutritional requirements. It was established that the highest lysozyme content in colostrum was presented immediately after parturition (1,15 μg/ml, P<0,001). Twenty-four hours later, lysozyme content was reduced by 50% (0,74 μg/ml), after 2 days they were 0,30 μg/ml and by post parturient day 7 0,10 μg/ml. Authors recommended newborn buffalo calves to suckle colostrum as early as possible after parturition, in order to receive higher concentrations of lysozyme that would protect them against pathogenic microflora.*

Keywords: buffalo colostrum, lysozyme, natural immunity

Introduction

Lysozyme is an important humoral factor of animal innate immunity. It cleaves the β-(1-4), and probably, the β-(1-2) glycoside bonds in the cellular wall of microorganisms. Its substrates are co-polymer of N-acetylglucosamine-N-acetylmuramic acid as well as oligosaccharides with the same structure; it also acts on polymers containing only N-acetylglucosamines (Pollock and Sharon, 1970). Lysozymes originating from various sources from egg white to bacteriophage, are basic low-molecular weight proteins. They are called muramidases or N-acetylmuramide glucanohydrolases. The N-acetylmuramic bond is cleaved by lysozyme 20 to 50 times more rapidly than the glycoside one (Zehavi et al., 1968). This lytic property of lysozyme allows it to protect animals from various infectious agents. It is most effective against Gram-positive bacteria (Buharin and Vasilev, 1974) but under some conditions, it could work together with the complement system and enhance the lysis of *Escherichia coli* (Pellegrini et al., 1992). Some substances (aprotonin, lactoferrin and nisin) being of a similar structure and activity, increase the bactericide effect of lysozyme (Chun and Hancock, 2000; Ellison and Giehl, 1991). In infected sheep from 4 breeds (Karabakh, Soviet merino, Red Samukh and Azerbaijan merino) Aliev et al. (1973) have shown that lysozyme concentration decreased by 23,3% in Karabakh and Soviet merino sheep and remained unchanged in the other two breeds. In this case, lysozyme served as an index for the resistance of the Red Samukh and Azerbaijan merino breeds.

Shabanov et al. (1973) established that after infection of sheep with *Mycoplasma agalactiae,* lysozyme concentrations increased during the course of infection. They assumed that probably this is one of the causes for the chronic course of the infection. Mutovin and Mamatov (1978) reported that in 10% of mastitis-free sheep the concentration of lysozyme was low, and that 70% of animals suffering from subclinical mastitis also presented low values. This evidences that this humoral factor of natural immunity participates actively in the formation of systemic resistance against the causative agent *Staphylococcus aureus*. The anti-bacterial activity of buffalo milk lysozyme against *Micrococcus luteus, Bacillus subtilis, Enterococcus faecalis* and *Lactococcus lactis ssp. lactis* has been demonstrated by Priyadarshini and Kansal (2002). They performed a biochemical characterization of lysozyme in buffalo milk (Priyadarshini and Kansal, 2003). Because of the immaturity of the immune system of newborns, they absolutely require transfer of maternal immunity for infection resistance via the colostrum. If immunoglobulin concentrations in the colostrums are relatively well known, poor information about colostrum lysozyme concentrations is available. The primary importance of lysozyme for animal health and the few studies upon its content in buffalo milk motivated the present investigation.

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Materials and Methods

Animals and feeding

The study was conducted with clinically healthy pregnant buffaloes, on their first lactation, at buffalo farm Dimitrievo (Haskovo area), Bulgaria. The animals were housed in free stalls with permanent straw bedding. The farm was free of mastitis, brucellosis, tuberculosis, and leucosis. The buffaloes selected for evaluation in this study were clinically healthy. They were selected by age, body weight and expected date of calving. The diet of the experimental animals consisted mainly of fresh grass (daytime grazing) and a concentrate supplement, depending on their daily milk yield. The concentrate was equally provided at every morning and afternoon milking. The diet of the experimental buffaloes was formulated to meet the nutritional requirements.

Colostrum sampling

Colostrum was obtained immediately after parturition, at 24 h post partum and on days 2, 3, 4, 5, 6 and 7. Samples were collected from the morning and evening milkings. They were taken from healthy quarters. None of the buffaloes studied had evidence of clinical mastitis at the time of sampling. Teat ends were cleaned with chlorhexidine before sampling. First streams of colostrum were discharged, and then 10 ml was collected aseptically from each teat into sterile vials. After the collection, individual colostrum samples were cooled at 4?C and transferred to the laboratory of the Department of Animal Genetics, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria where the lysozyme assay test was performed.

Lysozyme assay

Just prior to the analyses of buffalo colostrum, both morning and evening samples were combined and centrifuged at 2000 g. The lysozyme content was assayed by the method of Lie (1985). Twenty ml of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0,07 M Na_2HPO_4 and NaH_2PO_4, pH = 6,2) were mixed with 20 ml suspension of 24 hours culture of *Micrococcus lysodecticus* at 67°C. This mixture was poured out in Petri's dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera were poured out in each well. Eight standard dilutions (from 0,025 to 3,125 g/ml) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were used in the same quantity and were dropped in 8 wells. The samples were incubated for 20 hours at 37°C and lytic diameters were measured. The final lysozyme concentrations were calculated using special computer program developed at Trakia University.

Statistical analysis

Data were analysed using ANOVA model (STATISTICA Software, StatSoft, Inc., USA). Differences between means were tested using *t*-test, and were considered as significant when p values were less than 0,05.

Results

The variations of lysozyme concentrations in buffalo colostrum during post partum period are presented in Figure 1. The enzyme content in colostrum was the highest immediately after parturition, then it markedly and gradually decreased from the 1st day to the 4th day after parturition and finally remained constant (about 10 µg/ml) from the 5th day to the 7th day. Values recorded immediately after parturition and on the 1st day were significantly more elevated than those observed in the other days following post partum ($P < 0.001$ and $P < 0.05$, respectively). The data showed that it is extremely important for newborn buffalo calves to suckle colostrum as early as possible after parturition in order to receive higher concentrations of lysozyme and thus to achieve a defense against the pathogenic microflora.

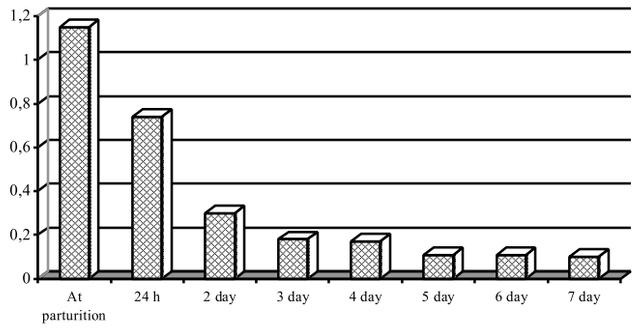

Figure 1. Lysozyme concentration in buffalo colostrums from the parturition to the 7th post parturient day (µg/ml)

Discussion

According to Priyadarshini and Kansal (2002) lysozyme activity is higher in buffalo milk (60 ± 3,9 x 10^{-3} units/ml) than in bovine milk (29,1 ± 1,5 x 10^{-3} units/ ml) and lysozyme in buffalo milk is more stable than in cow milk during storage and heat treatment. Buffalo colostrum contains five times more lysozyme activity than mature milk. Lysozyme activity in buffalo milk is not influenced by the parity of animal or stage of lactation, but it increases during extreme weather (winter and summer). The molecular weight of buffalo milk lysozyme is 16 kDa as determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SIDS-PAGE). The sequence of 23 amino acid residues at the N-terminal end shows 56,5% homology with bovine milk lysozyme and 30,4% with equine milk lysozyme. The specific activity of buffalo milk lysozyme is 10 times that of bovine milk lysozyme. Buffalo milk lysozyme is active over a wide range of pH and its activity is strongly influenced by the molarity of the medium. The anti-bacterial activity of buffalo milk lysozyme against *Micrococcus luteus, Bacillus subtilis, Enterococcus faecalis* and *Lactococcus lactis spp. lactis* has been demonstrated and a sharp increase in milk lysozyme has been observed in buffaloes with sub-clinical mastitis (Priyadarshini and Kansal, 2002, 2003). The lysozyme concentration in cow's colostrum fluctuated within the range of 0.15 to 0.65 µg/ml, with an average of 0.30 µg/ml. In the serum of dairy cows lysozyme concentration was on fourth day post partum as much as ten times higher than in the serum of their progenies 48 hours after parturition (Paulik et al., 1985). Goudswaard et al. (1978) show that lysozyme level in colostrum and mastitis milk is definitely higher than in normal milk. Riedel-Caspari et al. (1991) found out that calves that received complete colostrum (COL+) had a significantly lower neutrophils in their blood on day 2 and a significantly higher activity of lysozyme during their first three weeks of life as compared to the COL- animals. A postnatal increase in the number of ingested Streptococcus agalactiae test bacteria per

100 phagocytic cells occurred later in the COL+ calves than in the COL-. Gueorguiev et al. (1996) consider that there is no relation between serum and colostrum lysozyme concentrations in cows.

It is generally known that susceptibility to udder inflammation is of a polygenic nature. Scientists from many research centres all over the world are carrying out extensive research to detect genetic mechanisms of susceptibility to this disease in various cows. A relationship between the polymorphism of marker 513, genes DRB3, CD18, lactoferrin, lysozyme and the frequent occurrence of mastitis among cows has been established. The above mentioned results make it possible to use marker 513 and genes DRB3, CD18, lactoferrin and lysozyme as candidate genes for mastitis resistance. However, a more comprehensive and complex research is needed to breed cows with better developed resistance to udder inflammation (Sender et al., 2003). Sahoo et al. (1998) reported that lysozyme activity was significantly higher ($P < 0,01$) in polymorphonuclear cells of buffaloes than in cattle or goat (polymorphonuclear) cells. After activation these cells released elastase in all the species studied, while lysozyme was secreted only in buffalo polymorphonuclear cells. Irwin (2004) reported that the expansion of the lysozyme gene family is associated with the evolution of the ruminant lifestyle in ruminant artiodactyls such as the cow. Gene duplications allowed recombination between stomach lysozyme genes that may have assisted in the evolution of an enzyme adapted to survive and function in the stomach environment. Despite the amplification of the lysozyme genes, cow tears, milk, and blood are considered to be lysozyme deficient. They have identified 2 new cow lysozyme cDNA sequences and report that at least 4 different lysozymes are expressed in cows in non-stomach tissues and probably function as antibacterial defence enzymes. These 4 lysozyme genes are in addition to the 4 digestive lysozyme genes expressed in the stomach, yielding a number of expressed lysozyme genes in the cow larger than that found in most non-lysozyme-deficient mammals. In contrast to expectations, evidence for recombination between stomach and non-stomach lysozyme genes was found. Recombination, through concerted evolution, may have allowed some lysozymes to acquire the ability to survive in occasional acidic environments.

In conclusion, colostrums lysozyme concentrations in buffalo were the highest immediately after parturition suggesting the importance for newborn buffalo calves to suckle colostrum as early as possible after parturition, for achieving protection against pathogenic microflora.

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