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ORIGINAL ARTICLE

EVALUATION OF VITAMIN E, SELENIUM, COPPER AND ZINC SUPPLEMENTATION IN TRANSITIONAL PERIOD ON THE MASTITIS IN DAIRY CATTLE

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ABSTRACT: Mastitis is one of the important diseases of dairy herds that lead to enormous economic losses to the livestock industry. The aim of the present study was to evaluate the use of supplements containing copper, zinc, and vitamin E with selenium in a transition period on the incidence of mastitis in livestock. The study was conducted on 151 dairy cows within a period of 4 weeks before calving to 60 days postpartum. Animals were divided into four groups: the first group received a supplement of copper and zinc. The second group received vitamin E and selenium. The third group received copper, zinc, selenium and vitamin E and the fourth group, i.e. the control group didn't get any of the supplementation. The results of the present study have been demonstrated that the incidence of mastitis in the first, second and third groups did not significantly reduced (P = 0.365). Number of cows affected by mastitis in the third group was lower than all other groups and had a significant difference with other groups (P = 0.021). Results from this study indicate that the separate use of supplements has a large impact on the incidence of mastitis however; the combined use of supplements can lead to a reduced incidence of mastitis.
KEYWORDS: Mastitis, Copper, Zinc, Vitamin E, Selenium.

INTROUDUCTION

The transition or preparturient period, from 3 weeks before to 3 weeks after parturition, is a stressful time for dairy cows (Drackley, 1999). During the transition period, immunosuppression commonly occurs and cows exhibit great susceptibility to a number of diseases (Mallard *et al.*, 1998). A number of components of the host defense system are altered during this period including neutrophil function, lymphocyte responsiveness to mitogen stimulation, antibody responses, and cytokine production by immune cells (Mallard *et al.*, 1998; Kehrli *et al.*, 2006). Metabolic demands associated with late pregnancy, parturition, and initiation of lactation would be expected to increase the production of reactive oxygen species (ROS) (Sordillo, 2005). Oxidative stress in transition cows may also be a contributory factor to increased disease susceptibility (Miller *et al.*, 1993). High producing dairy cows are more prone to oxidative stress and can undergo severe stress in certain environmental, physiological, and dietary conditions (Vazquez-Anon *et al.*, 2008). Trace minerals that are a part of enzymes and some vitamins are key components of the antioxidant system (Weiss, 2009).

Impaired neutrophil function prior to parturition has been linked to the occurrence of mastitis, metritis, and retained placenta in dairy cows (Cai *et al.*, 1994; Kimura *et al.*, 2002). The aim of this study was to evaluation of VitE+Se and copper + zinc on the mastitis in the 1month before parturition until 2 month after parturition period.

1.1. Mastitis

Mastitis is an inflammation of the mammary gland in response to injury for the purpose of destroying the infectious agents that have entered the udder and it's a costly and common disease in the dairy industry (Viguier *et al.*, 2009). Many of organism can cause mastitis that including fungi, yeasts, bacteria, virus and mycoplasma (Hortet and seegers, 1998; Bennedfgaard *et al.*, 2003) witch bacteria is the most important (Malinowski *et al.*, 2006; Chaneton *et al.*, 2008).

1.2. Vitamin E

Vitamin E is an important antioxidant that has been shown to play an important role in immunoresponsiveness and health in dairy cows (Weiss and Spears, 2006). Vitamin E is an important lipid soluble antioxidant that protects against free radical-initiated lipid peroxidation (Halliwell and Gutteridge, 1999). The lowest plasma α-tocopherol concentrations are generally observed between 1 week pre-partum and 2 weeks post-partum. The major impact of vitamin E on immunity appears to relate to enhanced neutrophil function. Also It is well documented that the functions of vitamin E and

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selenium (Se) are related together (Weiss and Spears, 2006).

1.3. Copper

Copper is involved in the antioxidant system through its presence in several significant proteins. Copper is present most commonly in the proteins ceruloplasmin and superoxide dismutase (SOD). Ceruloplasmin functions include transportation of copper in the blood to various tissues, oxidizing minerals most notably iron and manganese, and scavenging oxygen radicals to protect cells. As a modulator of the inflammatory process, ceruloplasmin serves as an acute-phase protein. Acute phase proteins rise in the blood with infection and other inflammatory events (Gropper *et al.,* 2005c). The enzyme SOD, which is found both in the cytosol of cells and extracellularly, is copper and zinc dependent. Without the presence of SOD, superoxide radicals can form more destructive hydroxyl radicals that damage both unsaturated double bonds in cell membranes, fatty acids, and other molecules in cells. Therefore, SOD assumes a very important protective function (Gropper *et al.,* 2005c). A study was conducted on first lactation Holstein heifers to assess a potential role for dietary copper in enhancing resistance to E. coli mastitis. Conclusions were made that copper supplementation reduced the severity of clinical signs during experimental E.coli mastitis but the duration of mastitis was unaffected (O'Rourke, 2009). An average Holstein cow producing 50 or 100 pounds of milk needs to consume 225 mg or 300 mg of copper per day, respectively (NRC, 2001).

1.4. Zinc

Functions of zinc include tissue or cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity, and generalized host defense (Gropper *et al.,* 2005b). The mammary gland is an organ that is derived from the skin, thus making zinc necessary to maintain the integrity of the keratin that lines the streak canal. Zinc has a significant effect on gene expression and cellular growth. Supplementing zinc resulted in a 33% reduction in somatic cell count. However, not all forms of organic zinc showed a positive effect on mastitis (Van Saun, 1991). Cell mediated immunity has also been found to be altered by zinc deficiency. Zinc deficiency has been associated with reduced formation of both T and B lymphocytes and phagocytes (Sherman, 1992). T and B cells are the major cellular components of the adaptive immune response. Once they have recognized an invader, the cells generate specific responses that work to eliminate pathogens or pathogen infected cells. Zinc is also involved in the removal of free radicals by SOD. Extracellular and cytosolic SOD require both zinc and copper (Gropper *et al.,* 2005a). Zinc is necessary for the hepatic synthesis of retinol binding protein, which transports vitamin A in the blood (Gropper *et al.,* 2005b). Zinc requirement is about 900 mg/day for a cow producing 50 pounds of milk and 1400 mg/day for a cow producing 100 pounds of milk (NRC, 2001).

1.5. Selenium

One of the most clearly established functions of selenium is that it is a cofactor for the enzyme glutathione peroxidase. Glutathione peroxidase (GPX) is mainly found within the cytosols (70%) of cells and the mitochondrial matrix (30%) (Gropper *et al.,* 2005d). The enzyme, GPX, is responsible for catalyzing the removal of hydrogen peroxide and the reduction of other peroxides from tissues, which is an essential process of the immune system. The enzyme requires selenium as a cofactor or else its activity is impaired. Malbe *et al.,* (1995) showed the relation between selenium and udder health. The percentage of quarters harboring mastitis dropped along with the somatic cell count in the milk. Requirement of selenium for lactating and dry cows is .3 ppm (NRC, 2001).

MATERIALS AND METHODS

This study was done on the 151 dairy cattle that fostered in the same climate and nutrition situation. The study was started on dry cattle (4 weeks before parturition) and continued 8 weeks after parturition and lasted for 3 month. We examined all of dray cattle about the exists clinical mastitis 15 day after draying then selected that animal without mastitis and any other disease. All of cattle were divided inn four groups. First group n=35) were that animals received Vitamin E (3000IU/day/animal) plus Selenium (0.3ppm/day/animal) and continued until 8 weeks after calving. Second group n=40) were that animal received copper (Cu=225mg/ day/case) and zinc (Zn=1000mg/day/case) supplementation. Third group (n=38) were that animal witch treated with (VitE+Se and Cu+Zn) of supplemental. Forth group (n=38) were control group that didn't received none of this supplementation. All of cattle that get mastitis before calving were recorded. After parturition, every day we use California mastitis test (CMT) to discovering that animal had mastitis. Formation of jells or coagulation in the CMT cup were showed this animal are positive in CMT and that animal had mastitis (clinical or subclinical). This exam continued for 8 weeks

after calving and the result of that were recorded every day.

RESULTS

The results of this study were showed in first group we had 6 (17.14%) case that had mastitis and using of VitE and Se supplementation reduced incidence of mastitis in compare to control group but it was not significant. In second group 10(25%) cattle had mastitis but had no significant different between these group base on statistics analyses. In third group we had 6(15.78%) cattle that had mastitis that were shown using both of these supplements can help to prevent the incidence of mastitis. In the control group 12 cattle (26.13%) had mastitis. According to the result of this study, however the incidence of mastitis in group one and third decreased but there was no significant different between groups. The number of animal and quarters that had mastitis is on the following table.

Table 1: number of cattle and quarters that had mastitis

Group	Group1 (VitE+Se)	Group2 (Cu+Zn)	Group3 (VitE+Se and Cu+Zn)	Group4 (control)
Number of animal with mastitis	6	10	6	12
Number of quarters with mastitis	16	21	7	24

The number of quarters that had mastitis were different that include 16 in first group, 21 in second group, 7 in third group and 24 in latest group. There were significant different between third group and control group that show the effectiveness of using both of VitE+Se and Cu+Zn on the decreasing if incidence of mastitis.

DISCUSSION

Many vitamins and minerals are involved in the antioxidant defense system and the lack of any of these substances weakens the immune system in the transition period. Many studies on the effects of minerals on the incidence of livestock diseases in the transitional period after birth have been conducted and their useful effects on mammary gland immune function and the incidence of swelling have been noted. The numbers of phagocytic cells are not important, but the phagocytic ability of neutrophils is important in the disease control (Barbano *et al.*, 2006).

Vitamin E and selenium are two important nutrients that enhance an animal's resistance to mastitis. It seems that vitamin E effects on the immune function via neutrophils (Kimura *et al.*, 2002). Some studies have reported that the oral or parenteral use of vitamin E has no effect on the rate of neutrophils phagocytosis prior parturition, but increases the phagocytic ability of neutrophils to kill bacteria in the blood (Hogan *et al.*, 1990). Another role of vitamin E is its antioxidant effect that has an important protective role against free radicals produced in the lipid peroxidation (Halliwell and Gutteridge, 1999). Due to the increased metabolic demands associated with late pregnancy, parturition and early lactation, production of reactive oxygen species (ROS) is increased (Sordillo, 2005). So, the use of antioxidant substances may be useful to suppress ROS and to boost the immune system (Weiss and Spears, 2006). The results of this study demonstrate that the use of selenium and vitamin E supplementation are useful in boosting the immune system and result in reduced mastitis incidence in animals (6/17%) compared with the use of copper and zinc (25%). Some researchers also reported that vitamin E supplementation reduced the incidence of mastitis (Allison and Laven, 2000; Weiss *et al.*, 1997); however Smith *et al.*, (1984) reported that supplementation 3 weeks before calving had no effect on reducing the incidence of clinical mastitis but reduced clinical mastitis signs and duration of clinical disease.

Smith has also shown that the use of 740 IU of vitamin E in the dry period has decreased mastitis incidence by 37% (Smith *et al.*, 1984) that, compared with the results of the present study, the number of animals with mastitis in the first decreased by half of the affected animals in the control group. Though this difference was not statistically significant, the number of affected cows in the third group was significantly lower compared with the control group. In another study using 4000 IU vitamin E has decreased mastitis incidence rate by 63 percent (Weiss *et al.*, 1997); however, Batra used 100 IU of vitamin E and didn't observe any change in the incidence of mastitis (Batra *et al.*, 1992).

Copper and zinc are minerals that included in the structure of many enzymes, which have an important role in the immune system. Many studies have been conducted on the effects of antioxidants such as vitamin C, beta-carotene, vitamin A, copper, and zinc on the incidence of mastitis (Goff, 2006; Scaletti *et al.*, 2003; Spears, 2000; Wilde, 2006). Some investigators reported that administrating oral copper supplementation was effective in experimental E. Coli mastitis and caused a decreased somatic cells as well as

clinical signs of mastitis (Scaletti *et al.,* 2003). Zinc is involved in the keratin structure as the first barrier against bacterial penetration into breast Channel as well as increased E. Colie and Staphylococcus aureus mastitis has been reported followed by zinc deficiency (Erskine and Bartlett, 1993; Middleton *et al.,* 2004). A few Quantitative studies have been done on the effect of zinc supplementation use on the rate of mastitis. In a similar study conducted by Whitaker in 1997 on Holstein cows in a period from 3 weeks before calving to 100 days after delivery it was concluded that the use of natural zinc resources decreased the number of animals with clinical and subclinical mastitis, significantly (Whitaker *et al.,* 1997) that differs from the results obtained from the present study.

Many studies using different methods have been conducted on the Copper role in strengthening the immune system, but there is no study about the effect of copper on the rate of mastitis (Spears, 2000). Engle conducted a study in 2001 and reported that copper supplementation has no effect on the rate of mastitis (Engle *et al.,* 2001). Often near the time of delivery the rate of mastitis and somatic cell count (SCC) increases (Dang *et al.,* 2008; Spain, 2005). Similarly, near the time of delivery, the amount of vitamins and minerals decreases naturally because of metabolic stress, that results in a reduced immune system and increased mastitis risk (Goff and Stable, 1990; Meglia *et al.,* 2001). In a study by Harmon and Scaletti on experimental mastitis, it was concluded that the use of copper had a protective effect against mastitis (Scaletti *et al.,* 2003; Harmon, 1998). However, the results of the present study have shown that copper and zinc supplementation has reduced the number of animals with mastitis, but the difference has not changed significantly and the use of the two micro-elements does not prevent mastitis; while, the combined use of vitamin E, selenium, copper and zinc significantly reduce the rate of mastitis cows.

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