The Effect of Selenium, Vitamin E and Copper Injection on the Somatic Cell Count and Milk Compositions in Dairy Cows

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ABSTRACT

This study was conducted to evaluate the impact of Se+Vit E and Cu injection on milk somatic cell count and milk composition of dairy cows. Eighty Holstein cows, with approximately the same milk production and parity were randomly assigned to one of the 4 experimental treatments including: control group (saline: 5 ml/day/cow), Se+Vit E (10 ml/day), Cu (2 ml/day) and Se+Vit E + Cu. These vitamin and minerals were intramuscularly injected daily, from one week prior to the predicted parturition date to one week after parturition. Milk samples were taken 3 times: in the beginning, at the end of the first week and at the end of the second week of the trial to analyze the concentration of fat, protein, lactose and solid not fat (SNF); and four times on days 3, 7, 11 and 14 of the trial for somatic cell count (SCC) determination. Cu injection resulted in higher milk production and fat content than other treatment groups. However protein, lactose and SNF concentrations were elevated in Se+Vit E treated cows, milk production wasn’t significantly influenced. Se+Vit E + Cu injection markedly reduced SCC as compared to other experimental treatments. These findings indicated that Se+Vit E and Cu have a favorable effect on udder immune system and milk compositions.

Keyword: Dairy cow, Se, Vitamin E, Cu, Somatic cell count

INTRODUCTION

Vitamin E and Se are among the most stabilized antioxidants in tissues and cells. Vitamin E is the most important fat soluble antioxidant, the most active biological form of α-tocopherol and the constructional part of lipid membrane structure of the cells which has basic functional importance in the maintenance of membrane integrity in virtually all cells of the body against oxygen free radicals (Qureshi et al., 2010). Selenium is an essential trace element found in all tissues throughout the body and physiologically is important since Se is a constructional part of glutathione peroxidase structure. This enzyme converts O₂ to H₂O₂ and consequently to H₂O thus, preventing the generation of other highly reactive oxygen species and protecting cells against lipid peroxidation (Weiss et al., 1997). Also, the key role is in oxidative stress which can play Fenton and Fenton-like reactions. In Fenton reaction, H₂O₂ produces hydroxyl radical (OH) in the presence of Cu+. Similar processes can be observed in the Fenton-like reactions with
different metal ions (Miroslaw et al., 2008). Deficiencies of either vitamin E or selenium have been associated with increased incidence and severity of intra-mammary infections (IMI), increased clinical mastitis cases and higher milk somatic cell counts (Qureshi et al., 2010). The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction (redox) reactions and in scavenging free radicals. The copper-dependent enzyme, cytochrome c oxidase, plays a critical role in cellular energy production. Cytochrome C oxidase generates an electrical gradient by catalyzing the reduction of molecular oxygen ($O_2$) to water ($H_2O$), then this gradient is used by the mitochondria to create the vital energy-storing molecule ATP (O’Rourke, 2009). Copper is also a constructional part of blood cells and plays an important role in hematopoiesis. Thus copper deficiency alters the activity of several enzymes which mediate antioxidant defenses and ATP formation. These effects may impair the cell immune functionality, affecting the bactericidal capacity and making the animals more susceptible to infection and anemia. Recent studies have demonstrated the role of Cu on both incidence of IMI and response to infections during experimentally induced E. coli endotoxin mastitis of dairy cows (Scaletti et al., 2003). On the other hand, clinical mastitis resulted to decreased milk production, increased numbers of leukocytes in milk, altered milk composition and appearance, increased body temperature, and red, warm and swollen mammary quarters (Gunay and Gunay, 2008). Although previous finding show that Se+Vit E and Cu may have favorable impact on antioxidant system but, the objective of this study is to evaluate the impact of Se+Vit E and Cu injection on somatic cell count and milk compositions in Holstein dairy cows.

**MATERIALS AND METHODS**

**Animals and Management**

The present study was performed in one of the commercial dairy cattle farms located in outskirts of Esfahan (city of Iran). Cows were housed in free stall and were offered fresh feed thrice daily as total mix ratio (TMR). They also had free access to water. Anionic salts were used in the diet during the preparturition period for the prevention of hypocalcaemia. After parturition, calves were separated from their mothers and kept in a special compartment and cows were checked in the light of extraction of placenta and uterine involution as daily. Dairy cows also were milked three times a day and were fed after milking.

**Experimental Design**

Total of 80 Holstein dairy cows, with the similar milk production (~30±2 kg) and parity (2-3), were randomly divided into 4 groups of 20 cows to receive IM injections: control group (saline: 5 ml/day/cow), Se+Vit E (10 ml/day/cow, 50 mg Vit E/ml and 0.5 mg Na selenite/ml, Nasr Fariman Co, Iran), Cu (2 ml/day/cow, 50 mg Cu/ml, Parnell Co, Australia) and Se+Vit E + Cu (10+2 ml/day/cow). Dosage of drugs in this study was based on the manufacturer's recommendation. The vitamins and minerals were applied daily as treatments and were intramuscularly injected to the cows, from one week prior to the predicted parturition date to one week after parturition. In order to evaluate the effects of the experimental treatments on
milk composition, milk samples were taken three times at the beginning, at the end of the first week and at the end of the second week of the trial. Samples were also analyzed to determine the percentage of fat, protein, lactose and SNF of milk with the use of MilkoScan 4000 (Combifoss, Denmark). Milk samples also were taken 4 times on days 3, 7, 11 and 14 of the trial to determine the somatic cell counts (SCC) by fossomatic 5000 (Combifoss, Denmark).

Statistical Analysis

Data were subjected to the analysis of variance appropriate for a completely randomized design (SPSS Ver. 1.2). Duncan’s Multiple Range Test (DMRT) was applied to separate means. Statements of statistical significance are based on a probability of ($P < 0.05$).

RESULTS

In this study, the impacts of Se+Vit E and Cu injection were evaluated on milk production, fat, protein, lactose and SNF, as well as somatic cell count of milk. Table 1 shows that Cu injection resulted in a favorable increase in milk production compared to control and other treatments but wasn’t significant in each sampling. In contrast to Cu group, Vit E + Se had the least impact on milk production in total sampling and it wasn’t significant compared to other treatment groups in each sampling.

Table 1. The effect of Se+Vit E and Cu injection on milk production

<table>
<thead>
<tr>
<th>Groups</th>
<th>Milk Production (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S_1</td>
</tr>
<tr>
<td>Control</td>
<td>26.88</td>
</tr>
<tr>
<td>Vit E + Se+Cu</td>
<td>28.80</td>
</tr>
<tr>
<td>Cu</td>
<td>29.28</td>
</tr>
<tr>
<td>Vit E + Se</td>
<td>25.44</td>
</tr>
</tbody>
</table>

**Means with different superscript within a column are significantly different ($P < 0.05$).

1 control (5 ml/day/cow), Se+Vit E (10 ml/day /cow), Cu (2 ml/day /cow) and Se+Vit E +Cu (10+2 ml/day /cow)

Time of sampling: S1: beginning S2: end of the first week and S3: end of the second week of the trial

As Table 2 shows, Cu treatment group non-significantly increased fat percentage of milk compared to other groups. Se+Vit E significantly enhanced milk protein compared to Se+Vit E + Cu but no significant differences were found between control, Cu and Se+Vit E treatment groups. Se+Vit E treatment group also increased milk lactose and SNF compared to control and other treatment groups but no significant difference was found.

Se+Vit E + Cu group had the most efficient impact on the reduction of milk SCC (Figure 1). Se+Vit E + Cu significantly affected SCC ($p<0.05$) compared to control and Cu treatment groups but it wasn’t significant compared with Se+Vit E treatment group on sampling day 3.
Se+Vit E + Cu group decreased SCC (p<0.05) compared to control and other treatment groups on sampling day 7.

Se+Vit E + Cu treatment group reduced milk SCC on day 11 of sampling but it wasn’t significant in comparison to control and other groups. Also, Se+Vit E treatment group was significantly decreased SCC (p<0.05) compared to control and Cu treatment groups but it wasn’t statistically significant with Se+Vit E + Cu treatment group. On day 14 of the experiment, Se+Vit E + Cu treatment group was significantly decreased SCC (p<0.05) compared to control and other treatment groups and there wasn’t any significant difference between the control, Se+Vit E and Cu treatment groups.

Table 2. The effect of Se+Vit E and Cu injection on milk composition

| Groups | Fat (%) | Protein (%) | Lactose (%) | SNF (%) |
|--------+--------+------------+-------------+---------|
|        | S_1    | S_2        | S_3         | S_1    | S_2        | S_3         | S_1    | S_2        | S_3         |
| Control| 3.29   | 3.48       | 3.49        | 3.17_a | 3.09_b     | 3.04_b     | 4.98   | 4.98       | 4.98        | 9.25   | 9.20       | 9.15        |
| Vit E + | 3.31   | 3.28       | 3.62        | 3.24  | 3.17_a     | 3.17_a     | 5.00   | 4.99       | 5.01        | 9.36   | 9.28       | 9.28        |
| Se     |        |            |             |       |            |            |        |            |             |       |            |             |
| Cu     | 3.55   | 3.33       | 3.64        | 2.98_ab| 3.87_d     | 3.05_b     | 4.97   | 4.94       | 4.98        | 9.08   | 8.95       | 9.18        |
| Vit E + | 2.95   | 3.21       | 3.28        | 2.90  | 2.89_c     | 2.97_n     | 4.96   | 4.99       | 4.99        | 8.98   | 8.99       | 9.08        |
| Se+Cu  |        |            |             |       |            |            |        |            |             |       |            |             |

*a,b,c,d* Means with different superscript within a column are significantly different (P < 0.05).

1control (5 ml/day/cow), Se+Vit E (10 ml/ day /cow), Cu (2 ml/ day /cow) and Se+Vit E +Cu (10+2 ml/ day /cow)

Time of sampling: S1: beginning S2: end of the first week and S3 end of the second week of the trial

Figure 1. Effect of Vitamin E + Se and Cu injection on somatic cell count (SCC)
In this study, the effects of Se+Vit E and Cu on milk production were investigated. It revealed that Cu treatment group had the best effect on milk yield. However, Cu treatment group did not significantly increase fat percentage of milk compared to the control and other treatment groups. Furthermore, Cu plays an important role in oxidation-reduction (redox) reactions by scavenging free radical mediates antioxidant defenses. Cu can prevent the oxidation of unsaturated fatty acids which are highly oxidative, consequently leading to the elevation of milk VFAs content and milk production (Qurshi et al., 2010).

Milk fat has been criticized because it contains a less desirable balance of fatty acids than vegetable fat or fish oil (Sol Morales et al., 2000). Milk fat has a large concentration of short chain fatty acids (C4 - C16) and relatively low concentrations of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. A higher proportion of long chain fatty acids (C18) and unsaturated fatty acids are desirable for human health. However, an increasing degree of unsaturation increases the likelihood of oxidized milk. Rations with over 35-40 mg copper/kg have long been associated with increased incidence of oxidized flavors in milk (Sol Morales et al., 2000). Inhibition of fatty acid biohydrogenation induced by ionophores resulted in a decrease in C18:0 and an increase in C18:1 due primarily to an increase in the trans C18:1 isomer in ruminal cultures. These alterations in rumen lipid profile were also reflected in the milk, although the magnitude of change was smaller. Copper decreased the concentrations of the C18:1 trans isomer as well as the C18-conjugated dienes, both of which are intermediaries in the biohydrogenation process. If milk fatty acid profile is a good indicator of ruminal biohydrogenation, it seems that Cu may have had an impact on the microbial population in the rumen, thereby altering the overall process of fatty acid biohydrogenation. The studies indicated that ruminal biohydrogenation of unsaturated fatty acids was less complete when dairy cows were in a depletion of Cu. In another study, lower C18:1 trans isomer was reported in ruminal fluid of steers supplemented with 20 mg of Cu/kg of DM compared with nonsupplemented control steers. The research showed that approximately 40% of conjugated dienes in milk are produced by the endogenous Δ9-desaturase enzyme with the major substrate being the C18:1 trans isomer (Sol Morales et al., 2000). This may explain the lack of an observed increase in trans isomer in the milk of Cu supplemented cows. Microorganisms can utilize Cu by changing its valence via reducing systems of the cells. Enzymes responsible for this reduction require NADH or NADPH as electron donors. This is also a primary route by which microorganism detoxify excess Cu in the environment. Because Cu has a high reducing potential, it may interfere with the reduction process during ruminal biohydrogenation and divert the flow of hydrogen transfer. In contrast, Cu has a stimulating effect on the desaturation of C18:0 in adipose as well as the mammary tissues. This makes it difficult to separate the ruminal effects of Cu from postruminal changes (Sol Morales et al., 2000).

Se+Vit E group enhanced protein (3.24%), lactose (5.01%) and SNF (9.36%) of the milk and it had the least impact on milk production in comparison with other treatments. Selenium and vitamin E can prevent the occurrence of mastitis and subsequently improve milk compositions and quality (Erskine et al., 1990; Erskine et al., 1989; Weiss, 2002). Changes in milk yield and composition accompanying mammary gland inflammations have been studied
extensively (Oliver and Calvinho 1995). However, milk from cows with subclinical mastitis; which is the most common form of the disease, appears normal but milk yield is reduced and milk composition is altered. The relationship of mammary gland inflammation to milk yield and milk composition has received considerable research interest because of tremendous economic implications. Mastitis in dairy cows results in significant losses to the dairy industry and is likely the most expensive disease affecting dairy cattle throughout the world. Results of numerous studies have shown clearly that inflammation of the mammary gland reduces milk yield and alters milk composition. The magnitude of reduced milk yield and alterations in milk composition is influenced by the severity of the inflammatory response, which in turn is influenced by the mastitis pathogen which causes intramammary infection. One feature of mammary glands inflammation is an elevation in the number of somatic cells in milk. Somatic cells in milk primarily consist of neutrophils, macrophages, and lymphocytes. The rate and magnitude of change in somatic cell numbers in milk depends on the pathogen causing intramammary infection and the severity of infection. Consequently, the number of somatic cells in milk, more commonly is known as SCC, has been used extensively to study the inflammatory process and to determine changes in milk composition.

From a pathophysiological perspective, virulence factors produced by mastitis pathogens may influence mammary epithelial cell proliferation in vivo, which could be important during the periparturient period, when mammary tissue undergoes rapid differentiation and growth. From a dairy manufacturing perspective, mammary gland inflammation decreases concentrations of desirable components of milk and increases concentrations of undesirable components. Inflammation of the mammary gland also results in marked alterations in the composition of minerals in milk. Several studies have shown that major components of milk such as lactose, fat, and casein are decreased during inflammation, indicating that cellular synthesis has been altered (Oliver and Calvinho, 1995). Results of studies published thus far support the contention that alterations in milk composition associated with mammary gland inflammation are likely due to several factors, including impaired cellular synthesis, impaired cellular secretion, cellular degeneration, and paracellular transport of molecules from blood to milk and from milk to blood (Oliver and Calvinho, 1995).

In the relation, selenium is an essential micronutrient present in tissues throughout the body. Selenium is important physiologically because it is an integral component of the enzyme glutathione peroxidase. Tissue concentrations of Se are highly correlated with glutathione peroxidase activity and directly related to Se intake. During the metabolism of oxygen within cells, large quantities of superoxide and hydrogen peroxide are produced and these reactive oxygen species can severely damage membrane lipids, DNA, cellular proteins, and enzymes. The specific function of glutathione peroxidase is the conversion of hydrogen peroxide to water and lipid hydroperoxides to the corresponding alcohol. When the concentration of hydrogen peroxide is low, there is less chance that the hydroxyl radical will be formed. The hydroxyl radical is a reactive oxygen species (ROS) that is extremely damaging to cells.

Vitamin E and glutathione peroxidase function at two locations within the cell. Glutathione peroxidase functions in the cytosol of the cell and vitamin E within lipid membranes. An important function of both systems is the protection of membrane PUFA. The PUFA are present in all cellular membranes, but their concentration varies considerably from tissue to tissue. Membranes PUFA are extremely susceptible to attack from reactive oxygen species and
the more concentration of PUFA is, the more cell and tissue are susceptible to oxidant damage. An important PUFA in cellular membranes is arachidonic acid (AA). Arachidonic acid can be metabolized to prostaglandins, thromboxanes, and prostacyclin by the enzyme complex cyclooxygenase and to the leukotrienes by the lipoxygenase enzyme complex. Evidence suggests that AA metabolism is altered in animals deficient in vitamin E, Se, or both of them (Smith et al., 1997). Glutathione peroxidase participates directly in AA metabolism and vitamin E may take part to control peroxidation of AA or its unstable metabolites. The AA metabolites are important for PMN function and the amplification of the inflammatory response following pathogen invasion of tissues including the mammary gland. Pathogen invasion of the mammary gland triggers an influx of PMN and other white blood cells. The production of leukotriene B4 by macrophages and PMN is important for the initiation and amplification of this response. Phagocytosis of the invading pathogen results in a respiratory burst within the PMN. During the respiratory burst there is increased oxygen metabolism within the cell and increased production of ROS. The ROS are produced to kill the engulfed pathogen. The phagocytic process is accompanied by an intracellular increase in peroxides that is necessary to kill the pathogen but potentially dangerous to the cell and surrounding tissue. Accumulation of hydrogen peroxide in PMN is generally associated with reduced intracellular kill of pathogens.

Clearly, the speed which PMN can be mobilized following pathogen invasion and the efficiency of intracellular kill are events of critical importance to protection of the mammary gland from infection. Vitamin E and selenium play essential roles in these events and dietary deficiencies of either leads to impaired PMN function and increased incidence of intramammary infection of dairy cows. According to above mechanism, Vit E and Se injections accelerated the decrease in mammary gland inflammation and milk SCC in quarters. Overall, Vit E and Se resulted in reduction of mastitis prevalence, herd bulk milk somatic cell count and improved milk composition and present finding are in agreement with the report of Smith and coworker 1997).

The Se+Vit E + Cu treatment group resulted in low SCC of milk. These findings might be due to an increase in the activity of polymorphonuclear neutrophils (PMN), immune potency and resistance of the animal against infectious diseases. Also, Se is a constructional part of glutathione peroxidase structure and along with Vit E acts as an important biological antioxidant, which prevents the activity of free radicals leading to the udder health and milk quality (Erskine et al., 1990; Erskine et al., 1989). However, the studies demonstrated a negative correlation between percentage of quarters infected with major pathogens and mean herd glutathione peroxidase activity in whole blood (Erskine et al., 1990). Also, vitamin E in plasma and milk was lower in mastitis cows than in healthy cows and that mastitis cows and dairy goats had lower erythrocyte glutathione peroxidase than healthy cows and goats. Challenge trials have revealed positive effects of vitamin E and Se (Erskine et al., 1990). In the research, Escherichia coli infused into mammary quarters of cows fed diets supplemented (0.14 ppm) or unsupplemented (0.04 ppm) with Se. Vit E was considered adequate in both groups. The influx of polymorphonuclear neutrophils (PMN) into challenged quarters was more rapid in the Se-supplemented cows and resulted in lower E. coli cfu per milliliter of milk (Erskine et al., 1989). As a result, infections were less severe and were eliminated more rapidly, and milk loss was reduced in the Se-supplemented cows.
Pennsylvanians researchers showed mammary PMN function in cows deficient in Se but adequate in vitamin E. The PMN from Se-deficient cows had increased accumulation of hydrogen peroxide, decreased viability, and reduced ability for intracellular kill of mastitis pathogens. Selenium status did not influence the ability of PMN to phagocytes bacteria. Vit E and glutathione peroxidase have sparing effects on the requirements for one another relative to intracellular killing of bacteria. The protection afforded cellular membranes by Vit E may spare the requirement for glutathione peroxidase by reducing free radicals at the membrane, thereby preventing leakage of free radicals into the cytosol and maintaining intracellular killing capacity of the cell. Conversely, glutathione peroxidase activity in the cytosol may spare the requirement for Vit E in the membranes. Injection of Vit E during late gestation was tested as a means of maintaining plasma a-tocopherol during this critical time. Cows were injected with 3,000 IU of Vit E at 10 and 5 d before anticipated calving. Cows injected with Vit E had greater plasma a-tocopherol concentration 5 d after the first injection, at calving, and 1 wk after calving than cows injected with placebo. Neutrophils from injected cows had greater intracellular killing of bacteria at calving than neutrophils from control cows and the injection negated the suppression of in vitro intracellular killing of E.coli by neutrophils at calving (Qureshi et al., 2010). Also, it is concluded that Vit E supplementation around parturition prevented the suppression of blood neutrophil and macrophage function during the early postpartum period. On that basis, those cows with clinical mastitis were reported to have lower plasma a-tocopherol, a trial was conducted to determine whether a subcutaneous injection of 3,000 IU of Vit E at the time of initiation of antibiotic therapy would improve cure rates and reduce SCC in naturally occurring clinical quarters. The injected Vit E had no effect on bacteriological cure rates. However, Vit E injections accelerated the decrease in milk SCC in quarters that were bacteriologically cured, but not in quarters in which the infection persisted (Smith et al., 1997).

Furthermore, copper is a co-factor of superoxide dismutase enzymes, which prevents the detrimental peroxidative impact of free radicals on defensive and immune system of udder gland. Dairy calves fed Cu-sulphate have been reported to develop some sort of udder resistance against E.coli endotoxin and in herds infected with this endotoxin, mastitis severity reduced in Cu-sulphate treated calves resulting in an improvement of milk quality and decrease of SCC (Qureshi et al., 2010).

In conclusion the current study shows that the injection of Se + Vit E and Cu in precalving and postcalving period improved milk yield and fat, protein, lactose and SNF of milk SCC also reduced by Se + Vit E and Cu injection.

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