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THE EFFECTS OF ADMINISTRATION OF SELENIUM AND VITAMIN E ON BLOOD CELL COUNT AND PLASMA ANTIOXIDANT CAPACITY IN HOLSTEIN CALVES UNDER STRESS

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ABSTRACT

The aim of this study was to investigate the effect of Vitamin E and selenium on white blood cellcounts and plasma antioxidant status in Holstein calves under weaning and vaccination stress.In a completely randomized design, twentyweaned Holstein calves were assigned to four treatments and five replicates. Treatments included of control, Vitamin E (12 IU/calf), selenium (1.5 mg/kg BW sodium selenite) and vitamin E + selenium. There were differences among treatments for white blood cells. The highest number of white blood cells was for calves received vitamin E and the lowest one was for control group. Administration of selenium alone and in combination with vitamin E increased white blood cell number as compared to the control group.The highest count of lymphocytes was related to calves received vitamin E, selenium or both of them and the lowest mean was for the control group. In an adverse manner, the highest count of neutrophils was related to the control group and the lowest count was for calves received vitamin E, selenium or both of them. There were significant differences among treatments for serum IgG and IgM concentrations. The highest levels of IgG and IgM were related to calves received vitamin E plus selenium and the lowest levels were for the control group. The lowest glutathione peroxidase level was related to the control group and the highest

level was for calves received vitamin E plus selenium. Administration of vitamin E and selenium alone increased antioxidant enzyme levels significantly as compared to the control group.

Key words: Vitamin E, Selenium, Stress, White blood cell, Calf

INTRODUCTION

Vaccination program against brucellosis coincides with the age of weaning and it has a negative effect on the growth and general health of calves. Weaning singly is one of the most stressful events in the life of calves, moreoverin this age; calf must transfer to the mass rearing house, adapt to distinct diets and vaccinate whichexposed animal to severe stressful conditions known as oxidative stress[1,2,3]. Researchers[2,4] revealed that the oxidative stress induced by weaning significantly decreased plasma protein concentration and the ratio of neutrophils to lymphocytesin calves, which results infailure of the immune system. This event eventually leads to increase susceptibility of calf to infectious agents and diseases.

Oxidative stress occurs as a result of an imbalance between pro-oxidants and antioxidants [5]. This imbalance is due to increased levels of reactive oxygen species, nitrogen species or decreased antioxidant defense system occurs [6,7]. If the production of reactive oxygen species be more than usual, it can damage the cells, including damage to DNA, lipid membranes, and proteins. In order to ameliorate theeffects of reactive oxygen species, antioxidant defense mechanisms designed to neutralize or minimize its harmful effects on body.

Selenium is a mineral needed to maintain the structure and function of antioxidant enzymes and vitamin E has an antioxidant protective role for biological membranes. Previous researches [8,9] have shown that diets supplemented with selenium and vitamin E increased tissue antioxidant capacity. In the literature, there was limited information regarding the effect of administration of selenium and vitamin E, in combination, on white blood cell count, immune response and antioxidant status of calves under weaning and vaccination stress. Therefore, the aim of this study was to determine the effects of selenium and vitamin E administration, individually or in combination, on hematological parameters, plasma immunoglobulin level and antioxidant status in Holstein calves exposed to severestress.

MATERIAL AND METHODS

Animals and experimental design

Twentyhealthy male Holstein calves were weaned simultaneously at day 90 of age and selected for this study. Calves were checked for health and shiped to clean, comfort and closed stall. The environment temperature was maintained at 20 ± 3 °C and humidity at 55-60%. The calves were kept under a 12 h lighting program and had a free access to fresh water, hay and concentrate. Starter was formulated to meet animal requirements for energy, protein, vitamins, and minerals for calves [10]. In a completely randomized design, calves wereassigned to four treatment groups and five replicates. Treatments included of control (withoutsupplementation, but under stress), injection of vitamin E (12 IU per calf), sodium selenite(0.5 mg/kg body weight), and injection of vitamin E + sodium selenite. Vitamin E and sodium selenite were administrated in two periods. Vitamin E and selenium were administrated at the first day and two days later, and then repeated one injection each week for four weeks.Stress challengingwas consisted of weaning at day 90 of age, shippingto new stall at day 95 of age and vaccination against brucellosis (strain RB51) at day 100 of age.

Blood sampling

Blood samples were collected from calves before stress challenging and another one at the end of the experimental period (day 118 of age). Blood samples were collected by jugular venipuncture into evacuated tubes without EDTA and also tubes containing EDTA. Serum was separated by centrifugation (3000 × g for 15 min) of tubes without EDTA.Collected sera were frozen at -20 °C prior to further analysis.Immediately after collection of blood in tubes containing EDTA, it was mixed and transferred to veterinary laboratory for counting total and differential white blood cells.

Measurements

Counting of white blood cell, heterophil and lymphocyte were done with automated counters. The concentrations of serum level of IgG and IgM were determined by ELIZA plate reader usingcommercial kit. The glutathione peroxidase enzyme concentration in the serum of calves was measured using RANSEL kit (Randox Laboratories Ltd. Crumlin, UK).

Statistical Analysis

Data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS software. Birth body weight was evaluated in the model as a covariate, but was not statistically significant ($P > 0.05$). Therefore, unadjusted means are presented. Mean comparison was done using the Duncan`s Multiple Range Test at $P<0.05$.

RESULTS AND DISCUSSION

The effect of different treatments on total and differential white blood cells counts in the final of study is presented in **Table1**. The data collected for these parameters before stress challenging showed non-significant differences, thus it was not report. There were differences among treatments for white blood cells. The highest number of white blood cellwas for calves received vitamin E and the lowest one was for the control group. Administration of selenium alone and in combination with vitamin E increased white blood cell number as compared to the control group.

In the control group, calves challenged with the severe stress like other treatments and in this condition, white blood cell number decreased. It isobviousthatplasma cortisol tended to increase when animal exposed to severe stress. Cortisol has the negative effect on immune organs, especially bone marrow and thymus, hence the white blood cell production decrease [11]. Moreover, during severe stress, level of reactive oxygen species increases dramatically as a result of oxidative stress [12]. Oxidative stress occurs when the concentration of reactive oxygen species generated exceeds the antioxidant capability of the cell. Environmental and management stress generate reactive oxygen species and could induce the lipid peroxidation [13] and disturb production of white blood cells[14].

The highest count of lymphocytes was related to calves received vitamin E, selenium or both of them and the lowest mean was for the control group. In an adverse manner, the highest count of lymphocytes was related to the control group and the lowest mean was for calves received vitamin E, selenium or both of them.

Thechanges in lymphocyte and neutrophilnumbers observed in calves received supplements maybe related to theirantioxidant properties. Selenium improves immune system and as an antioxidant prevents the outbreak of disease [8]. This element has special functional in seleno-enzymes [15], that some of them act as antioxidant enzymes. There are studies [16,17] that showed selenium deficiency in cows reduces the ability of blood and milk neutrophils to kill mastitis causing pathogens. Addition of selenium to neutrophils in *vitro*, however, was effective at enhancing the chemotactic migration and increased the production of superoxide needed for bactericidal activity [18]. Neutrophils obtained from cows with higher blood concentrations of selenium also had a greater potential to produce superoxide and kill bacterial pathogens [11].

Calves under stress, which is common in practice, might require higher dose of vitamin

E to augment the immune system. With respect to immune-competency, higher levels of vitamin E in the diet of calves have been found to influence both cellular [19] and humoral immune responses positively [20]. The protective effects of vitamin E on animal health may be involved with its role in reduction of glucocorticoids, which are known to be immune-suppressor [21,22], inhibiting the production and actions of antibodies, lymphocyte function, and leukocyte population [22,23].It has been reported that white cell function from cows with higher blood selenium concentrations have a better killing ability and therefore may be better able to resist disease [11]. There was no significant difference among treatments for numbers of eosinophils and monocytes.

There were significant differences among treatments for serum IgGand IgMconcentrations (**Figure1**). The highest levels of IgG and IgM were related to calves received vitamin E plus selenium and the lowest means were related to the control group. In the line with our finding, significant immunoenhancing effect of vitamin E has been reported against *Brucellaovis* [24]. In contrast to our finding, there are a number of studies suggesting no effect with vitamin E or selenium supplementation on immunity [9,25]. Others [26] did not observe any significant beneficial effect of vitamin E on humoral immune response against *Pasteurellamultocida* type 6B in buffalo calves. Effects are likely to be farm specific and depend largely on the existing trace element status of the herd.

There was significant difference among treatments for serum glutathione peroxidase level **(Figure 2)**. The lowest glutathione peroxidase level was related to the control group and the highest level was for calves received vitamin E plus selenium. Administration of vitamin E and selenium alone increased antioxidant enzyme levels significantly as compared to the control group. The increase in glutathione peroxidase activity by administration of selenium is in agreement with other studies [27,28]. Selenium functions as a component of at least 25 different selenoproteins (Andrieu, 2008). In these proteins, sulfur is replaced with selenium, which allows the proteins to donate hydrogen and take part in reduction reactions. Seleno proteins include the enzyme iodo thyronine deiodinase which is important in regulating metabolism and glutathione peroxidase and thioredoxin-reductase which are important components of antioxidant and immune systems [10,29].

Table 1: The effects of zearalenone on total and differential blood cells counts

Treatments	W.B.C x1000/µl	Lymphocytes %	Neutrophils %	Eosinophils %	Monocytes %
Control	7.4[c]	35.3[b]	56.2[a]	2.36	4.68
Vitamin E	10.5[a]	55.4[ab]	38.4[b]	1.69	3.12
Selenium	8.8[b]	55.0[ab]	38.0[b]	1.32	4.35
Vit E + Se	9.2[b]	64.6[a]	31.1[b]	1.09	3.63
SEM	0.90	6.36	6.84	1.22	1.5

[abc] Means in the same column with different letter are significantly different ($P \leq 0.05$)

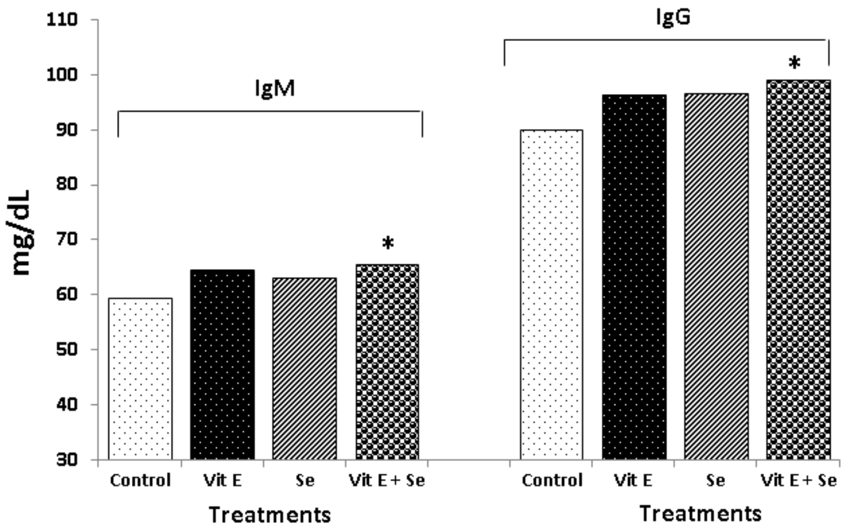

Figure 1: Plasma IgG and IgM levels of stressed calves received no supplement (control), vitamin E (vit E), selenium (Se) and combination of vitamin E and selenium (vit E + Se).

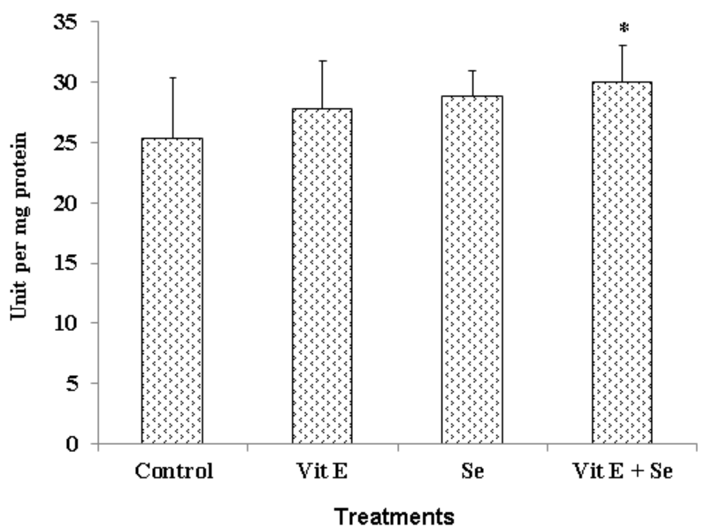

Figure 2: Plasma glutathione peroxidase level of stressed calves received no supplement (control), vitamin E (vit E), selenium (Se) and combination of vitamin E and selenium (vit E + Se).

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