VITAMIN E AND SELENIUM DECREASED CORTISOL IN CATTLE

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ABSTRACT

Twenty dairy cows with left abomasal displacement were used to investigate the effects of vitamin E and selenium treatment on blood cortisol in dairy cows stressed by omentopexy. The cows were randomly divided into two groups. Ten hours before surgery 6 g of DL- α -tocopheryl acetate (6 mg.kg⁻¹) and 67 mg of natrium selenite (0.1 mg.kg⁻¹) in a volume of 40 ml were administered subcutaneously to 10 cows; the control animals (n = 10) received an equivalent volume of injectable water (40 ml). The injection of vitamin E and selenium produced a rapid rise ($P < 0.05$) in blood α -tocopherol and selenium concentrations. The serum vitamin E increased several times ten hours after vitamin E and Se injection and raised continuously to the highest average concentration of 21.6 mg.l⁻¹ at hour 24 after the surgery. The highest selenium concentration was seen ten hours after selenium administration with holding the increased concentrations in comparison to initial ones during the whole study. Serum cortisol increased in both groups after surgery. The highest cortisol concentrations

were reached at one hour after surgery in the experimental and control group (56.7 ± 28.8 and 65.3 ± 26.1 μ g.l⁻¹, respectively). A return to the levels similar to the initial ones was recognized 24 hours after the surgery. The ANOVA revealed a significant effect of vitamin E and selenium injection on serum cortisol ($P < 0.05$). In conclusion, we have demonstrated that abdominal surgery resulted in typical stress changes with a weaker cortisol response to the abdominal surgery in animals treated with vitamin E and selenium.

Key words: cortisol; dairy cows; selenium; surgical stress, vitamin E

INTRODUCTION

Peroxidative processes of lipids, proteins, carbohydrates as well as nucleic acids and their products can exert severe negative effects on cell membranes as well as biochemical pathways. Peroxidative processes and their products may regulate and influence the efficiency of an antioxidative

system. An antioxidative system represented by enzymes and non-enzymatic antioxidants is responsible for maintaining the balance between production and neutralization of reactive oxygen species [12]. Lack of antioxidants can result in various pathological processes like myopathy, cardiomyopathy, embryonic degeneration, liver necrosis, and encephalomalacia [17]. Increased health problems around parturition are particularly problematic as they may greatly impact the productivity of dairy cows in the ensuing lactation [9]. Therefore, it is not surprising that considerable research efforts have focused on defining factors that may contribute to immune dysfunction during this critical period in the production cycle of dairy cows [26].

It has been demonstrated that a number of manipulations including transport [10], therapeutic manipulation [22], and surgery [2], increased secretion of cortisol from the adrenal cortex in cattle. Several studies demonstrated that the stress reaction has enhancing effects on free radical production, thus contributing to an increased lipid peroxidation in animals [21]. Although stress reactions are organised to protect the homeostatic state of animals, they contain elements that may either enhance or diminish susceptibility to the disease process; in many instances, however, stress reactions themselves may induce pathological change [3].

The present trial was aimed to study the effects of vitamin E and selenium treatment on blood cortisol in dairy cows stressed by omentopexy.

MATERIALS AND METHODS

Twenty Holstein-Frisian lactating dairy cows, mean age 4.41 ± 1.34 years ($x \pm SD$), admitted for treatment of left abomasal displacement, were used in the study. All of them were within first six weeks after calving. Their mean body weight was 586 ± 65 kg ($x \pm SD$). They were randomly divided into two equal groups ($n = 10$) according to the order of admission to the clinic. The surgery followed on the subsequent day to correct the abomasal displacement. Ten hours before surgery 6 g of DL- α -tocopheryl acetate ($6 \text{ mg} \cdot \text{kg}^{-1}$) and 67 mg of sodium selenite ($0.1 \text{ mg} \cdot \text{kg}^{-1}$) in a volume of 40 ml were administered subcutaneously to 10 cows; the control animals ($n = 10$) received an equivalent volume of injectable water (40 ml). Abdominal surgery (omentopexy) was performed in a standing position 16–

24 hours after admission. The mean duration of preparation for surgery lasted approximately 30–40 minutes, and the surgery approximately 40 minutes. Procaine-hydrochloride (2 %) was used for local anaesthesia. All experimental animals were housed in pens with straw bedding and were fed hay *ad libitum*. Concentrates were offered on the day after the surgery according to milk yield and had free access to water. No additional health disorders were diagnosed in the experimental animals and no supportive treatment was necessary. All cows recovered and left the clinic on day 4 or 5 after the omentopexy.

Blood samples were drawn from the jugular vein before vitamin E/Se injection, just prior to surgery, immediately after surgery, then 15, 30, 60 minutes, and 2, 5, 10, and 24 hours after surgery. The blood samples were stored at 4°C maximally for two hours before centrifugation. The serum samples were obtained and then stored frozen at -80°C until analysis. The α -tocopherol concentrations in the serum were determined in saponified samples by high performance liquid chromatography (HPLC) using a fluorescent detector. The concentrations of selenium in the serum were measured using the fluorimetric method of Rodríguez et al. [25]. The serum cortisol concentrations were determined by chemiluminescent enzyme immunoassay (Immulate®/Immulate® 1000 Cortisol immunoassay, DPC, L.A., USA).

Statistical analysis was carried out by a two-factorial analysis of variance (one repeated factor: time, one grouping factor: treatment) with the *post hoc* Bonferroni test (IBM SPSS Statistics 23, 2015). Significance was declared at $P < 0.05$.

RESULTS

The subcutaneous injection of vitamin E and selenium resulted in a rapid increase ($P < 0.05$) in blood α -tocopherol and selenium concentrations (Table 1). The serum vitamin E increased to sixfold values ten hours after administration in the treated animals and rose continuously to the highest average concentration of $21.6 \text{ mg} \cdot \text{l}^{-1}$, 24 hours after the surgery. There were no changes in serum Vitamin E concentration in the control group during the study. The serum selenium concentrations of the experimental group showed a similar dynamic like α -tocopherol (Table 1). The highest selenium concentration was seen ten hours after

Table 1. Concentrations of blood α -tocopherol, selenium, and cortisol in operated dairy cows after vitamin E/Se or placebo treatment (mean $\pm$ SD)

Sampling time	Group	α -tocopherol [mg.l ⁻¹]	Selenium [mmol.l ⁻¹]	Cortisol [mg.l ⁻¹]
Before injection	T	2.38 $\pm$ 1.71	0.75 $\pm$ 0.19	8.13 $\pm$ 4.60
	C	2.27 $\pm$ 1.28	0.91 $\pm$ 0.22	7.87 $\pm$ 4.14
Before surgery	T	13.4 $\pm$ 5.16*	1.25 $\pm$ 0.20*	10.9 $\pm$ 7.10
	C	2.33 $\pm$ 1.23	0.91 $\pm$ 0.23	13.7 $\pm$ 11.6
Immediately AS	T	15.9 $\pm$ 3.85*	1.12 $\pm$ 0.20*	48.0 $\pm$ 24.8
	C	2.02 $\pm$ 0.85	0.88 $\pm$ 0.21	58.9 $\pm$ 41.0
15 min AS	T	15.2 $\pm$ 4.15*	1.10 $\pm$ 0.20	43.8 $\pm$ 20.7
	C	2.01 $\pm$ 0.90	0.89 $\pm$ 0.19	59.7 $\pm$ 30.6
30 min AS	T	16.5 $\pm$ 3.60*	1.18 $\pm$ 0.22*	44.5 $\pm$ 19.4
	C	2.05 $\pm$ 0.79	0.86 $\pm$ 0.18	61.1 $\pm$ 28.7
60 min AS	T	16.7 $\pm$ 3.81*	1.16 $\pm$ 0.22*	56.7 $\pm$ 28.8
	C	2.18 $\pm$ 1.00	0.84 $\pm$ 0.17	65.3 $\pm$ 26.1
2 hours AS	T	17.6 $\pm$ 3.18*	1.09 $\pm$ 0.26*	26.6 $\pm$ 16.5*
	C	2.13 $\pm$ 0.84	0.90 $\pm$ 0.21	49.9 $\pm$ 21.7
5 hours AS	T	19.0 $\pm$ 3.08*	1.08 $\pm$ 0.19*	15.8 $\pm$ 7.97
	C	2.11 $\pm$ 0.91	0.88 $\pm$ 0.21	21.8 $\pm$ 11.1
10 hours AS	T	20.6 $\pm$ 2.53*	1.06 $\pm$ 0.16*	9.04 $\pm$ 5.19
	C	2.66 $\pm$ 2.53	0.95 $\pm$ 0.25	12.6 $\pm$ 8.02
24 hours AS	T	21.6 $\pm$ 2.60*	1.10 $\pm$ 0.15	9.41 $\pm$ 9.70
	C	2.51 $\pm$ 1.57	0.98 $\pm$ 0.25	10.7 $\pm$ 5.43
Group effect		P < 0.05	P < 0.05	P < 0.05
Time effect		P < 0.05	P < 0.05	P < 0.05

T —Treatment group (Vit E/Se); C—Control group (placebo); AS—after surgery;
 *—means within sampling times differ significantly (P < 0.05) (Bonferroni test)

selenium administration with holding the increased levels in comparison to initial ones during the whole study.

Serum cortisol concentrations increased in both groups after surgery (Table 1). The highest values were reached at one hour after surgery in both groups. A return to concentrations similar to the initial ones were observed 24 hours after the surgery. The ANOVA revealed a significant effect of vitamin E and selenium injection on plasma cortisol concentrations (P < 0.05).

The initial serum concentrations of vitamin E were low in both groups but not clearly deficient [29]. The concentrations of vitamin E measured in our study were similar to those found by P o n t e s et al. [23] in dairy cows around calvings. The serum concentrations of α -tocopherol found ten hours after vitamin E and selenium injection were approximately as high as those in cattle reported by H i d i r o g l o u and L a f l a m m e [14] who used DL- α -tocopheryl acetate for intramuscular injection in

a similar dosage (4500 IU per 250–30 kg body weight). Thus, it can be assumed that concentrations of vitamin E, reached in the experimental animals within the surgical procedure, were high enough to be effective on a lipid peroxidation or other physiological reactions associated with the stress response in animals.

Similarly, the subcutaneous administration of selenium elevated the blood selenium in the experimental dairy cows within the complete experimental period (24 hours) which could create a different metabolic condition in animal tissues affecting multiple biochemical processes and reactions. These dynamics were similar to that previously reported by Chorf et al. [7] after subcutaneous Se injections of 0.13 mg.kg⁻¹ in feedlot heifers. In dairy cows receiving Se injections in our study, the mean concentrations of serum Se observed prior to the injection in the experimental and control group (0.75 and 0.91 µmol.l⁻¹, respectively) were in the range of reference intervals for dairy cows [13]. Selenium is well known to be effective in reducing oxidative stress and the severity of several proinflammatory-based dairy cattle diseases such as mastitis and metritis [18]. Most of the antioxidant functions of Se were based on the reducing capacity of selenoproteins including the glutathione peroxidase (GPX) and thioredoxin reductase (TrxR) families [26].

Hopster et al. [15] concluded that 69.4 % of 307 baseline cortisol samples had concentrations below 3 µg.l⁻¹, whereas 13.7 % of the samples contained 6 µg.l⁻¹ cortisol or more. The relatively higher mean cortisol values prior to surgery in our study (higher than 7.5 µg.l⁻¹) may be due to sickness and transport stress of the animals. The effect of surgery on plasma cortisol was significant, however, cortisol concentrations fell near to pre-surgery values by the end of the trial. Thus, the pattern of cortisol response to surgery was similar to the pattern seen in 5–6 month-old cattle after amputation dehorning [27]. Comparable to some other studies, a significant effect of vitamin E and selenium administration on cortisol levels could be observed in our study. The reduction effect of vitamin E on the production of cortisol has been shown in calves [24], cattle [6, 11], and mice [16]. Dietary compounds with antioxidant activity reduce activation of the hypothalamic-pituitary-adrenocortical axis in animals [8]. This may be due to an effect of vitamin E suppressing phospholipase A2 activity [5]. The enzyme phospholipase A2 is involved in ACTH secretion [1]. Taniguchi et al. [28] observed

that broiler chickens supplemented with vitamin E and injected with ACTH had reduced corticosterone content in the adrenal glands. However, there was no inhibiting effect of vitamins E and C on the release of cortisol from bovine adrenocortical cells when stimulated with ACTH *in vitro* [19]. In addition, maternal Se supplementation of dams resulted in lower circulating cortisol concentrations in lamb offspring compared with lambs born from non-supplemented ewes [4]. In an experiment with a transportation stress in sheep, a depressive effect of trace element supplementation, including selenium, could be seen on cortisol levels in ewe lambs [20]. It was speculated that Se can act directly on blood cortisol by affecting the free radical-antioxidant capacity balance.

In conclusion, we have demonstrated that the single injection of 6 g of DL- α -tocopheryl acetate and 67 mg of sodium selenite significantly increases the serum vitamin E and Se within 10 hours. In addition, the blood cortisol was reduced in the treated cows. This decrease in blood cortisol may suggest that vitamin E and selenium supplementation may be an effective method to minimize the stress response in dairy cows.

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