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EFFECTS OF A-TOCOPHEROL AND SELENIUM INJECTION ON SERUM CORTISOL IN DAIRY COWS UNDERGOING ABDOMINAL SURGERY

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

Twenty dairy cows with left abomasal displacement were used in this study. The cows were randomly divided into two groups. Ten hours before surgery 6 g of DL-α-tocopheryl acetate (6 mg/kg) and 67 mg of sodium selenite (0.1 mg/kg) in volume of 40 ml (Vitaselen®) were administered subcutaneously to 10 cows; the control animals (n=10) received an equivalent volume of injectable water (40 ml).

The serum vitamin E increased several times ten hours after vitamin E and Se injection and rose continuously to the highest average concentration 21.6 mg/l 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. 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 μg/l, respectively). The ANOVA revealed a significant effect of vitamin E and selenium injection on plasma cortisol (P<0.05).

The decrease of blood cortisol in our study may suggest that vitamin E and selenium supplementation may be an effective method to minimize stress response in dairy cows.

Keywords: SURGICAL STRESS, VITAMIN E, SELENIUM, CORTISOL

It has been demonstrated that a number of manipulations including transport [6], feed deprivation [7], therapeutic manipulation [14], 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 [13]. 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 pathologic change [3]. Elevated lipid peroxidation can play an important role in the pathogenesis of many health disorders in animals [16]. The lipid peroxidation can be monitored by analysing of oxidative status by assessment of pro-oxidants, oxidative damaged molecules (i.e. lipid peroxidation products), and antioxidative status of organism [1]. Biological systems contain powerful enzymatic (glutathione peroxidase, catalase, and superoxide dismutase) and nonenzymatic (tocopherol, b-carotene, ascorbic acid, and glutathione) antioxidant systems protecting organic compounds against the harmful effects of free radicals.

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±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±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 mg/kg) and 67 mg of sodium selenite (0.1 mg/kg) in volume of 40 ml (*Vitaselen®*) were administered subcutaneously to 10 cows; the control animals (n=10) received an equivalent volume of water for injections (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. Pro-

casel (2 % procaine-hydrochloride) was used for local anaesthesia. All experimental animals were housed in pens with straw bedding and were fed concentrates and hay. 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 plasma and serum were obtained and then stored frozen at –80 °C until analyses. The α-tocopherol concentrations in serum were determined in saponified samples by high performance liquid chromatography (HPLC) using a fluorescent detector. The concentrations of selenium in serum were measured using the fluorimetric method. 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 and discussion

The subcutaneous injection of vitamin E and selenium resulted in a rapid increase (P<0.05) in blood α-tocopherol and selenium concentrations (table).

The serum vitamin E increased to six-fold value ten hours after administration of *Vitaselen®* and rose continuously to the highest average concentration 21.6 mg/l 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. The highest selenium concentration was seen ten hours after selenium administration with holding the increased levels in comparison to initial ones during the whole study.

Table

Concentrations of blood α-tocopherol, selenium, and cortisol in operated dairy cows after vitamin E/Se or placebo treatment (mean±SD)

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

Note: E — experimental group (Vit E/Se), C — control group (placebo), AS — after surgery, NS — not, * means within sampling times differ (P<0.05) (Bonferroni test).

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

The concentrations of vitamin E measured in our study were similar to those found by [15] 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 [10] who used DL-α-tocopheryl acetate for intramuscular injection in a similar dosage (4500 IU per 250–300 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 stress response in animals. Similarly, the subcutaneous administration of selenium elevated blood selenium in experimental dairy cows within the whole experimental period (24 h) what could create a different metabolic condition in animal tissues affecting multiple biochemical processes and reactions. This dynamics are similar as previously reported by [5] after subcutaneous Se injections of 0.13 mg/kg in feedlot heifers. In dairy cows receiving Se injections in the present study, mean concentrations of serum Se observed prior to the injection in the experimental and control group (0.75 and 0.91 μmol/l, respectively) were in the range of reference intervals for dairy cows [9].

[11] concluded that 69.4 % of 307 baseline cortisol samples had concentrations below 3 μg/l, whereas 13.7 % of the samples contained 6 μg/l cortisol or more. The relatively higher mean cortisol values prior to surgery in the present study (higher than 7.5 μg/l) are suggested to be results of both 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 [17]. Comparable to some other studies, a significant effect of vitamin E and selenium administration on cortisol levels could be observed in the present study. The reduction effect of vitamin E on the production of cortisol was shown in cattle [8]. 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 transportation stress in sheep a depressive effect of trace element supplementation, including selenium, could be seen on cortisol levels in ewe lambs [12]. It is speculated that Se can act directly on blood cortisol by affecting the free radical-antioxidant capacity balance.

Conclusion

In conclusion, we have demonstrated that the single injection of α-tocopheryl acetate and sodium selenite significantly increases the serum vitamin E and Se within 10 hours. In addition, a significant reduction of blood cortisol was found in treated cows. These decreases in blood cortisol may suggest that vitamin E and selenium supplementation may be an effective method to minimize stress response in dairy cows.

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