Secondary Antibody Response to *Haemophilus somnus* Antigen in Breeding Japanese Black Cattle Fed Selenium-Deficient and α -Tocopherol-Fortified Diets

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ABSTRACT. The cattle with adequate α -tocopherol (Vit E) and marginally deficient selenium (Se) status manifested significantly lower anti-*Haemophilus somnus* antibody titer than the cattle supplemented with Se in the later stage of an 8-week trial. However, in the early stage no difference was observed in magnitude of anti-*H. somnus* antibody development between them. These results suggested that Se may contribute to anti-*H. somnus* antibody production, and that Vit E can make up for Se deficiency to a certain degree.—**KEY WORDS:** anti-*Haemophilus somnus* antibody, cattle, α -tocopherol and selenium.

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Selenium (Se) and α -tocopherol (Vit E) were found to have complementary antioxidant activities in several species of animals. As Se and Vit E are required for the optimal immune system function [2, 4, 11, 13], Se- and Vit E-adequate diets are reasonable feedstuff for cattle. Most cattle in Japan, fed Vit E-adequate forages, are at risk of developing Se deficiency. This is because most forages fed to cattle are from Se-deficient soils. Considering complementary antioxidant activities of Se and Vit E, the possibility rises that sufficient Vit E may make up for Se deficiency. However, Larsen *et al.* [7] reported that dietary Vit E supplementation exerted no stimulatory effect on mitogenic response of Se-deficient sheep lymphocytes. The present study was designed to examine the anti-*Haemophilus somnus* antibody production in the cattle with marginally deficient Se and adequate Vit E status, and compared with that in the cattle supplemented with Se.

Ten breeding Japanese Black female cattle at mid- and term-pregnant stages, 3 to 15 years of age, were used in an 8-week trial. They were kept indoors and divided into two groups. The cattle with marginal blood Se status, having been vaccinated with *H. somnus* two years before the trial, were fed Se-deficient and Vit E-fortified diets (basal diets) twice a day. Basal diets consisted of corn silage, straw and Italian rye grass. Six out of 10 cattle were i.m. injected with 25 mg Se and 500 mg Vit E on week 0 and 2 of the present study. Although only Se should have been injected for accurate study on influence of Se deficiency on anti-*H. somnus* antibody production, we could not obtain commercial Se used for therapy. All cattle were vaccinated with formalin-inactivated *H. somnus*, strain M-1 Br, on weeks 2 and 5 of the present experiment.

Anti-*H. somnus* antibody titer was determined by the ELISA technique. The modified method of Purnell and Hendry [10] and Canto *et al.* [1] was used for ELISA procedure. In brief, round-bottom ELISA plates (Immulon; Gliner Inc.) were coated with 0.1 ml of outer membrane complex antigen diluted 1:200 in carbonate buffer (pH 9.6). After incubation, the plates were washed 4 times with 0.02% Tween-20 in PBS (Tween-PBS, pH 7.4), were then blocked with 0.25 ml of PBS containing 0.05% of ovalbumin type VII for 16 hr at 4°C. After

washing the plates, 0.1 ml of serum diluted 1:400 in Tris-buffer (pH 7.4) was added, and the plates were then incubated at 30°C for 1 hr. Washing procedure was repeated 4 times and 0.1 ml of rabbit peroxidase-conjugated anti-bovine IgG antibody diluted in Tween-PBS was added. The plates were incubated at 30°C for 30 min, and thereafter washed again. Then 0.1 ml of substrate solution (o-phenyldiamine in phosphate-citrate buffer, pH 5.2) was added and the plates were incubated at 30°C for 30 min. Finally, colorimetric reaction was terminated by adding 0.1 ml of 1 M sulfuric acid. The well contents were measured for the absorbance at 492 nm.

Serum Se concentration was determined by atomic absorption spectrometry [8]. Serum Vit E content was determined by a fluorimetric method [16]. Glutathione peroxidase (GSH-px) activity in whole blood was measured according to the method of Paglia and Valentine [9].

As shown in Figs. 1 and 2, the cattle supplemented with Se showed significantly high serum Se concentration ($p < 0.001$) and whole blood GSH-px activity ($p < 0.01$) after the 2nd week, whereas the cattle without Se supplementation exhibited marginally low serum Se concentration and whole blood GSH-px activity throughout the experimental period. Because Se works as a component of GSH-px [12], serum Se level was well correlated with the whole blood GSH-px activity. Serum Vit E level increased and remained far above the low end of the normal range of 150–200 $\mu\text{g/dl}$ [3] in both groups (Fig. 3), which may be due to the feeding of Italian rye grass. Regarding anti-*H. somnus* antibody production (Fig. 4), the cattle supplemented with Se manifested significantly higher anti-*H. somnus* antibody level on the 8th week, although until the 6th week the cattle without Se supplementation exhibited the upward trend of anti-*H. somnus* antibody production as did the cattle supplemented with Se.

As observed on the 8th week, Se supplementation for cattle with adequate Vit E and marginally deficient Se status produced significantly stronger anti-*H. somnus* antibody response. There was no significant difference in average age and pregnant period between the groups. Se status may therefore account for such a prominent

Fig. 1. Time course of serum selenium concentration of the cattle fed basal diets (selenium-deficient and vitamin E-fortified diets) with (●) and without (○) selenium and vitamin E injection. Selenium and vitamin E injection (SE) was carried out on week 0 and 2 of the experiment. Data represent the mean $\pm$ S.D. *: Significantly different from the cattle without selenium and vitamin E injection; $p < 0.001$.

Fig. 2. Time course of the whole blood glutathione peroxidase activity of the cattle fed basal diets (selenium-deficient and vitamin E-fortified diets) with (●) and without (○) selenium and vitamin E injection. Selenium and vitamin E injection (SE) was carried out on week 0 and 2 of the experiment. Data represent the mean $\pm$ S.D. *: Significantly different from the cattle without selenium and vitamin E injection; $p < 0.01$. e.u. = μ M NADPH oxidized/min/g Hb.

difference in serum anti-*H. somnus* antibody level between the groups. Important roles of Se in optimal humoral immune response were reported by many investigators [5, 6, 14, 15]. On the contrary Turner and Finch [17] noted that Se supplementation for marginally Se-deficient and Vit E-adequate lambs induced little enhanced antibody response to a *Salmonella dublin* vaccine. Thus the effects Se exerts on immune response vary with

Fig. 3. Time course of serum vitamin E level of the cattle fed basal diets (selenium-deficient and vitamin E-fortified diets) with (●) and without (○) selenium and vitamin E injection. Selenium and vitamin E injection (SE) was carried out on week 0 and 2 of the experiment. Data represent the mean $\pm$ S.D.

Fig. 4. Time course of anti-*Haemophilus somnus* antibody titer of the cattle fed basal diets (selenium-deficient and vitamin E-fortified diets) with (●) and without (○) selenium and vitamin E injection. Selenium and vitamin E injection (SE) was carried out on week 0 and 2 of the experiment. *H. somnus* (H.S.) vaccination was performed on weeks 2 and 5 of the experiment. Data represent the mean $\pm$ S.D. *: Significantly different from the cattle without selenium and vitamin E injection ; $p < 0.05$.

species and antigen.

It should be noted that even the Se-deficient cattle demonstrated the same magnitude of anti-*H. somnus* antibody response as did the cattle supplemented with Se until the 6th week. Considering a markedly increased serum Vit E level far above the low end of the normal range of 150–200 μ g/dl during the experiment, it is suggested that Vit E might make up for Se deficiency with its complementary antioxidant activity.

Se and Vit E may be involved in membrane fluidity of lymphoid cells, thus affecting immune response mechanism [13]. However, the mechanism where influencing

immune response is different, indicating coupled with our present results that Vit E, making amends for Se deficiency to a certain degree, cannot substitute for Se as modulator of immune response.

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