

## Effect of supplemental vitamin E on the peripheral blood leukocyte population in Japanese Black calves

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(Received 27 January 2015/Accepted 4 March 2015/Published online in J-STAGE 3 April 2015)

**ABSTRACT.** We investigated the effect of supplemental vitamin E on the peripheral blood leukocyte population in Japanese Black calves. Twenty-six calves kept at the same farm were studied. They were divided into two groups; thirteen calves received 300 IU/day of vitamin E orally from 1 to 3 months of age (VE group), and the other thirteen calves did not receive the vitamin E supplement (control group). The VE group showed a higher serum vitamin E concentration at 2 and 3 months of age compared with the control group ( $P<0.01$ ). The numbers of CD3<sup>+</sup> cells and CD4<sup>+</sup> cells were higher in the VE group than in the control group, and the difference was statistically significant at 3 months of age ( $P<0.05$ ). The numbers of CD21<sup>+</sup> cells were higher in the VE group than in the control group, and the difference was statistically significant at 2 months of age ( $P<0.05$ ). The numbers of CD335<sup>+</sup> cells tended to be higher in the VE group than in the control group. The numbers of CD8<sup>+</sup> cells and CD14<sup>+</sup> cells tended to be higher in the VE group than in the control group at 3 and 4 months of age. This study demonstrated that the supplementation of suckling Japanese Black calves with vitamin E might affect the numbers of some immune cell types in the peripheral blood.

**KEY WORDS:** calf, Japanese Black, leukocyte population, vitamin E

doi: 10.1292/jvms.15-0060; *J. Vet. Med. Sci.* 77(8): 985–988, 2015

Vitamin E plays an important role as antioxidant inside the body for maintaining the stability of biological membranes as well as the function of the immune system [13]. Overall, studies of supplementation of animals with vitamin E have reported improvements in immune function [1, 3, 8, 10–12]. Supplementation of Holstein calves with vitamin E has improved function of T and B cells, and increased production of antibodies after vaccination [20]. In addition, oral administration of vitamin E to feedlot cattle immediately after introducing them into a farm decreased the morbidity of infectious diseases [6].

Young calves have immature immune systems compared with adult cows, due to their lower numbers of peripheral blood T and B cells, which are responsible for cell-mediated and humoral immunity [9]. Furthermore, Japanese Black calves have lower numbers of peripheral blood T and B cells compared with Holstein calves, which makes Japanese Black calves more prone to infectious diseases [15]. Thus, supplementation of Japanese Black calves with vitamin E was expected to improve immune status. The blood leukocyte population in cattle has proved to be a good indicator of immune status in studies of bovine viral diarrhea-mucosal disease and infection with *Mannheimia haemolytica*, which showed a decrease in the numbers of CD4<sup>+</sup> cells and CD8<sup>+</sup>

cells in peripheral blood [7]. Also, malnutrition of cattle decreased the numbers of CD3<sup>+</sup> cells, CD4<sup>+</sup> cells and CD21<sup>+</sup> cells in peripheral blood [14]. However, there have been no reports about the changes in the blood leukocyte population of Japanese Black calves supplemented with vitamin E.

The main objective of this study was to assess the effect of vitamin E supplementation on the peripheral blood leukocyte population in clinically healthy Japanese Black calves.

Twenty-six Japanese Black calves kept at one farm in Aomori Prefecture were used in this study. The calves, born between spring and fall in 2011, were alternately assigned to two groups; 13 calves received 300 IU/day of vitamin E powder (this dose was determined based on the studies by Rajeesh *et al.* [18] and Reddy *et al.* [19]) mixed with milk replacer from 1 to 3 months of age (VE group), and the other thirteen calves received only milk replacer (control group). All calves were fed to meet their nutritional requirements according to the Japanese Feeding Standard for Beef Cattle [2] and managed in the same manner. Peripheral blood samples were collected from all calves at 1, 2, 3 and 4 months of age, once for each month, via the jugular vein using plain Vacutainer tubes and Vacutainer tubes containing dipotassium edetic acid (EDTA-2AK). Serum was separated from blood samples collected in plain tubes by centrifugation and stored at  $-20^{\circ}\text{C}$  until analysis. Blood collected in tubes containing EDTA-2AK was used for white blood cell (WBC) analysis within 4 hr after collection.

The serum vitamin E concentration was measured using a high performance liquid chromatograph (LC-2000, JASCO, Tokyo, Japan) as previously reported [5]. The serum total cholesterol (T-Cho) concentration was measured using a Labospect 7180 autoanalyzer (Hitachi High-Technologies

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Table 1. Primary antibodies used to identify peripheral blood leukocytes

Antigen	MAB clone	Isotype	Specificity	Source
CD3	MM1A	IgG1	Pan T cell	VMRD
CD4	CACT138A	IgG1	Helper T cell	VMRD
CD8	CACT80C	IgG1	Cytotoxic T cell	VMRD
CD14	MY4	IgG1	Monocyte	Coulter
CD21	CACT108A	IgG1	B cell	VMRD
CD335	MCA2365E	IgG1	Natural killer cell	AbD Serotec

VMRD: VMRD, Pullman, WA, U.S.A., Coulter: Beckman Coulter, Brea, CA, U.S.A., AbD Serotec: AbD Serotec, Ltd., Kidlington, U.K.

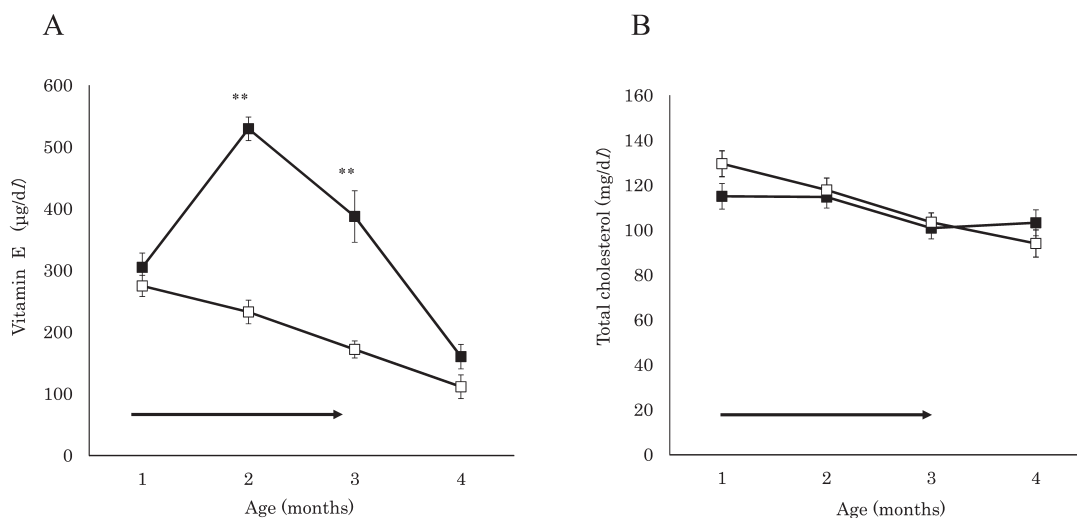


Fig. 1. Changes in serum vitamin E concentration (A) and serum total cholesterol concentration (B). Vitamin E group (dark squares) and control group (empty squares). Data are shown as the mean  $\pm$  SE. The arrows indicate the vitamin E supplementation period for the vitamin E group. Asterisks indicate significant differences between groups at the same age (\*\* $P < 0.01$ ).

Corp., Tokyo, Japan). The total WBC were determined with a blood cell counter. Peripheral blood mononuclear cells (PBMCs) and granulocytes (Grans) in WBCs were analyzed by WBC cytogram, and then, the numbers of PBMCs and Grans were calculated using their percentages and total WBC counts. The peripheral blood leukocyte population was assessed using a FACScan flow cytometer (Becton, Dickinson and Co., Mountain View, CA, U.S.A.) as previously reported [15]. The primary antibodies used and a description of the working solutions are listed in Table 1. Data for the serum vitamin E concentration and peripheral blood leukocyte population were expressed as the mean  $\pm$  standard error. Differences between groups were examined using the Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

The serum vitamin E concentration at 1 month of age did not show a significant difference between groups (Fig. 1A). The serum vitamin E levels of the VE group at 2 ( $P < 0.01$ ) and 3 ( $P < 0.01$ ) months of age were significantly higher than those of the control group. The serum T-Cho concentration did not show a significant difference between groups (Fig. 1B). The numbers of CD3<sup>+</sup> cells and CD4<sup>+</sup> cells were

higher in the VE group than in the control group, and the differences were statistically significant at 3 months of age ( $P < 0.05$ ) (Table 2). The numbers of CD21<sup>+</sup> cells were higher in the VE group than in the control group, and the difference was statistically significant at 2 months of age ( $P < 0.05$ ). The numbers of CD8<sup>+</sup> cells and CD14<sup>+</sup> cells at 1 and 2 months of age tended to be lower in the VE group than in the control group, and they tended to be higher in the VE group than in the control group at 3 and 4 months of age. The numbers of CD335<sup>+</sup> cells tended to be higher in the VE group than in the control group. The numbers of WBCs and granulocytes tended to be higher in the control group than in the VE group.

The results of the present study suggested that the increase in the serum vitamin E concentration caused by oral supplementation in the suckling Japanese Black calves changed the numbers of immune component cells in the peripheral blood. All T cells express the CD3 marker, and most CD4<sup>+</sup> cells are helper T cells. CD21 is expressed on B cells, which play an important role in antibody production [4]. Vitamin E has been reported to enhance interleukin-2 (IL-2) production [1, 13]. IL-2 is secreted from helper T cells, and it plays a role in activation and proliferation of NK cells, monocytes,

Table 2. Numbers of cells in each leukocyte subset

Cell type	1 month		2 months		3 months		4 months	
	VE group	Control group	VE group	Control group	VE group	Control group	VE group	Control group
WBC ( $\times 10^2/\mu\text{l}$ )	91.1 $\pm$ 5.2	98.3 $\pm$ 5.4	86.4 $\pm$ 5.1	101.4 $\pm$ 5.1	85.2 $\pm$ 3.7	93.4 $\pm$ 4.5	88.1 $\pm$ 3.0	93.2 $\pm$ 4.6
PBMC ( $\times 10^2/\mu\text{l}$ )	52.6 $\pm$ 2.9	51.8 $\pm$ 4.3	53.2 $\pm$ 3.3	52.9 $\pm$ 3.3	58.3 $\pm$ 3.1	50.5 $\pm$ 2.1	61.9 $\pm$ 2.9	57.8 $\pm$ 2.9
Gran ( $\times 10^2/\mu\text{l}$ )	38.5 $\pm$ 4.7	46.5 $\pm$ 5.5	33.2 $\pm$ 6.4	50.9 $\pm$ 6.4	26.9 $\pm$ 3.4	42.9 $\pm$ 3.8	26.1 $\pm$ 1.5	35.4 $\pm$ 3.2
CD3 <sup>+</sup> ( $\times 10^2/\mu\text{l}$ )	27.5 $\pm$ 1.8	25.4 $\pm$ 3.1	25.7 $\pm$ 2.3	24.2 $\pm$ 2.3	28.5 $\pm$ 1.3*	24.0 $\pm$ 1.2	32.4 $\pm$ 1.7	29.1 $\pm$ 1.5
CD4 <sup>+</sup> ( $\times 10^2/\mu\text{l}$ )	9.4 $\pm$ 0.7	7.9 $\pm$ 1.3	9.5 $\pm$ 0.9	7.7 $\pm$ 0.9	10.3 $\pm$ 0.7*	7.9 $\pm$ 0.5	12.1 $\pm$ 0.9	10.4 $\pm$ 0.7
CD8 <sup>+</sup> ( $\times 10^2/\mu\text{l}$ )	4.8 $\pm$ 0.5	5.3 $\pm$ 0.8	5.1 $\pm$ 0.7	5.5 $\pm$ 0.7	7.0 $\pm$ 0.6	5.5 $\pm$ 0.5	7.8 $\pm$ 0.4	6.4 $\pm$ 0.7
CD14 <sup>+</sup> ( $\times 10^2/\mu\text{l}$ )	8.1 $\pm$ 1.3	10.7 $\pm$ 0.8	8.9 $\pm$ 1.0	11.5 $\pm$ 1.0	8.1 $\pm$ 0.6	7.9 $\pm$ 0.6	9.2 $\pm$ 0.7	8.5 $\pm$ 0.9
CD21 <sup>+</sup> ( $\times 10^2/\mu\text{l}$ )	7.7 $\pm$ 1.0	6.3 $\pm$ 0.7	8.9 $\pm$ 0.7*	6.0 $\pm$ 0.7	9.4 $\pm$ 0.7	8.5 $\pm$ 0.6	11.0 $\pm$ 0.8	9.6 $\pm$ 1.2
CD335 <sup>+</sup> ( $\times 10^2/\mu\text{l}$ )	3.5 $\pm$ 0.5	3.4 $\pm$ 0.4	3.9 $\pm$ 0.3	2.9 $\pm$ 0.3	3.7 $\pm$ 0.9	2.7 $\pm$ 0.2	3.1 $\pm$ 0.3	2.8 $\pm$ 0.4

Data are expressed as the mean  $\pm$  SE. Asterisks indicate significant differences between two groups at the same age ( $P < 0.05$ ).

T cells and B cells [1, 12, 13, 21]. Vitamin E also alleviated the damage caused by free radicals and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which have a suppressive effect on several indices of immunity [3, 13, 22].

Previous studies of oral administration of vitamin E to calves revealed a stable increase in serum vitamin E [16, 18, 19] that was similar to that in the present study. Therefore, the changes in the numbers of immune cells in the VE group compared with the control group in the present study might have been associated with decreased free radical formation, reduced PGE<sub>2</sub> synthesis and increased IL-2 production.

We previously reported that the supplementation of Japanese Black calves with vitamin E could increase antibody production after vaccination with modified live bovine herpesvirus-1 [16]. In the present study, the VE group exhibited an increase in the number of helper T cells and B cells. Thus, the increased antibody production in the calves supplemented with vitamin E in our previous study might have been associated with increased numbers of helper T cells and B cells.

We previously reported that the serum vitamin E concentration was well correlated with the serum T-Cho concentration in Japanese Black fattening steers [17]. In the present study, the serum vitamin E concentration in the VE group was significantly higher than in the control group at 2 and 3 months of age, but the serum T-Cho concentration in the VE group was not higher than that in the control group at 2 and 3 months of age. These results suggested that the increased serum vitamin E concentration in the VE group might not be due to feed, such as milk replacer, but due to good absorption of supplemental vitamin E.

The results of our investigation confirmed that oral supplementation of suckling Japanese Black calves with vitamin E changed the numbers of immune cells in the peripheral blood. Supplementation of young Japanese Black calves with vitamin E seemed to enhance their immune statuses. In order to reduce the incidence of infection diseases in Japanese Black calves, further studies are needed to clarify how and when oral vitamin E administration improves the peripheral blood leukocyte population in Japanese Black calves.

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