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NOTE

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Effects of vitamin E supplementation on serum oxidative stress biomarkers, antibody titer after live bovine respiratory syncytial virus vaccination, as well as serum and fecal immunoglobulin A in weaned Japanese Black calves

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ABSTRACT. The purpose of this study was to determine the effects of vitamin E supplementation on blood oxidative stress biomarker in weaned calves. Thirty clinically healthy 12 weeks of age Japanese Black calves were randomly assigned to two groups: 15 calves received 300 IU of vitamin E daily from 12 to 18 weeks of age (VE group), and the other 15 calves did not receive the vitamin E (control group). Blood samples were taken at 12, 14, 16, 18, and 20 weeks of age. The concentration of serum reactive oxygen metabolites at 20 weeks of age were significantly lower in the VE group than those in the control group. Vitamin E supplementation to weaned calves might affect blood oxidative stress.

KEYWORDS: Japanese Black calf, oxidative stress, vitamin E, weaned calf

Weaning causes great stress for calves, due to physical changes and environmental changes, as well as switching from liquid feed to solid feed [4]. Therefore, the risk of disease in calves after weaning is high, which makes them particularly susceptible to respiratory diseases [32]. Bovine respiratory syncytial (RS) virus alone causes respiratory diseases in calves, which is further exacerbated by a bacterial infection [7]. Therefore, in Japan, vaccination is generally performed as a preventive measure against bovine RS virus infection.

Immunoglobulin A (IgA) serves as one of the first defenses to prevent pathogenic microorganisms from crossing the intestinal epithelial cell barrier and is considered an important regulator of the intestinal tract mucosa [5].

Vitamin E is one of the fat-soluble vitamins, and it plays an important role in improving disease resistance by stabilizing the biological membrane, removing active oxygen species in the body, and providing an antioxidative effect [9, 34]. Many studies on vitamin E supplementation in humans have reported the removal of oxidative stress and the improvement of immune function [9, 10, 13].

In cattle, it has been reported that vitamin E supplementation to newborn calves reduced oxidative stress [20], and thus vitamin E supplementation in suckling calves affects antibody production after vaccination [24]. However, to our knowledge, there have been no reports investigating the effects of vitamin E supplementation on blood oxidative stress, antibody production against vaccination, and IgA production in weaned calves.

The purpose of this study was to evaluate the effects of vitamin E supplementation on the serum biochemical values including oxidative stress, the antibody response to bovine RS virus vaccination, as well as the serum and fecal IgA, in Japanese Black weaned calves in order to keep calves healthier.

Thirty clinically healthy 12 weeks of age Japanese Black calves kept at one farm in Kagoshima Prefecture, Japan, were used in this study. All the calves stayed with their mothers for 4 days after birth and were housed indoors. Starting from 5 days after birth, they were fed with milk replacer and were raised individually in calf hutches. The amount and nutrient composition of feed are shown in Table 1. Including weaning at 12 weeks of age, all calves were managed in the same manner and fed to meet their nutritional requirements

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Table 1. The amount and nutrient composition of feed without supplement (per head per day)

		Weeks of age							
		1	4	8	12	14	16	18	20
Amount (dry matter; kg)									
Milk replacer		0.45	0.92	0.92					
Calf stater			0.10	0.50	1.36	1.02	0.68		
Concentrate						0.61	1.22	2.26	2.61
Hey (oats)			0.01	0.05	0.25	0.25	0.25	0.25	0.50
Hey (timothy)						0.26	0.43	0.95	0.95
Composition (dry basis)									
Total Digestible Nutrients	(%)	101.6	99.2	93.1	77.8	74.5	73.0	70.3	70.3
Crude Protein	(%)	28.2	27.3	25.0	18.5	16.8	16.1	14.7	14.3
Crude Fat	(%)	16.4	14.9	11.2	2.5	2.1	2.0	1.7	1.7
Calcium	g/kg	13.9	13.1	11.1	5.9	5.9	5.9	5.9	5.6
Phosphorous	g/kg	7.3	7.1	6.6	5.1	4.7	4.5	4.1	4.1
Magnesium	g/kg	1.0	1.1	1.4	2.1	2.5	2.6	2.9	2.8
Zinc	g/kg	0.10	0.10	0.09	0.06	0.06	0.06	0.06	0.06
Vitamin E	IU/kg	27.1	25.9	23.1	15.8	15.7	15.8	15.6	15.2

according to the Japanese beef cattle feeding standard [19]. The calves were randomly assigned to two groups: 15 calves were orally supplemented with 300 IU vitamin E (alpha-tocopherol, Rovimix vitamin E, DSM Nutrition Products, Basal, Switzerland) (the dose was based on the study by Rajeesh [27]) once daily from 12 to 18 weeks of age (VE group), and 15 calves were not supplemented with vitamin E (control group). All calves were vaccinated with commercially available live RS virus vaccine (No.52 strain, Kyoto Biken Laboratories Inc., Kyoto, Japan) at 14 weeks of age following the manufacturer's instruction. All the calves ate all the provided feed, and no calves developed diseases during the experimental period. Blood samples were taken at 12, 14, 16, 18, and 20 weeks of age from the jugular vein into plain Vacutainer tubes. Serum was isolated from the blood samples by centrifugation and stored at −30°C until analysis. For fecal samples, an arm covered with a plastic sleeve was inserted into the rectum of calves, and about 5 g of rectal stools were collected at 12, 14, 16, and 18 weeks of age. These fecal samples were stored at −30°C until analysis. The calves were raised according to guidelines of animal care of the Joint Faculty of Veterinary Medicine at Kagoshima University. The protocol was reviewed and approved by the Kagoshima University Laboratory Animal Committee, Japan (number VM19046).

The following serum biochemical parameters were determined using a Labospect 7020 autoanalyzer (Hitachi High-Technologies, Japan): aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), creatine kinase (CK), urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, triglyceride, beta-hydroxybutyric acid (BHB), calcium, inorganic phosphorus, magnesium, and zinc. The serum alpha-tocopherol (vitamin E) concentration was measured using high-performance liquid chromatography (Prominence, Shimazu Corp., Tokyo, Japan) as previously reported [3]. The serum oxidant status was determined using the diacron reactive oxygen metabolites (d-ROMs) test, which determines hydroperoxides [1, 28]. The serum concentrations of antioxidant capacity were measured using a biological antioxidant potential (BAP) test [1, 28]. Both d-ROMs and BAP were determined using a free radical analyzer (FREE Carrio Duo, Diacron International, Grosseto, Italy) [1]. The degree of oxidative stress was expressed as the oxidative stress index (OSI) calculated with the formula of (d-ROMs/BAP) × 100 =OSI [1, 28]. The serum antibody titers to bovine RS virus were determined by a neutralization test. The neutralization test was performed as previously described by Kubota *et al*. [12]. The serum and fecal IgA concentrations were measured by ELISA. ELISA was performed as previously described by Otomaru *et al.* [25].

Data of the serum biochemical values, vitamin E, d-ROM, BAP, OSI, as well as the serum and fecal IgA values were expressed as mean ± standard deviation. The serum antibody titers to bovine RS virus were expressed as geometric mean. Statistical analysis was conducted to determine the differences between the two groups at the same weeks of age using Student's *t*-test with SPSS statistics 26 software (IBM, Tokyo, Japan). *P* values less than 0.05 were considered statistically significant.

The concentrations of serum biochemical parameters were not significantly different between the groups (Table 2). The serum vitamin E concentration in the VE group remained above 100 IU/dL during the experimental period (Table 2). Whereas in the control group, they were less than 100 IU/dL after 14 weeks of age, and less than 60 IU/dL from 18 to 20 weeks of age. The difference between the groups was statistically significant at 14, 16, 18, and 20 weeks of age ($P<0.05$). The serum d-ROMs concentration in the control group was higher than that in the VE group from 14 to 20 weeks of age (Fig. 1A), and the difference between the groups was statistically significant at 20 weeks of age ($P<0.05$). The serum BAP was not significantly different between the groups (Fig. 1B). However, the OSI in the control group was significantly higher than that in the VE group at 20 weeks of age ($P<0.05$) (Fig. 1C). The antibody titers against RS virus gradually decreased in both groups, and they were not significantly different between the groups (Table 3). The serum and fecal IgA concentrations were not significantly different between the groups (Fig. 1D and 1E).

Vitamin E has an antioxidant effect of scavenging free radicals such as active oxygen species in the body [9], and also has a deoxidization effect of reducing hydroperoxides [34]. Vitamin E has also been shown to have a protective effect against infectious diseases by activating immunity, such as improvement of natural killer cell function [13], increase of interleukin 2 production from T

Table 2. Biochemical and vitamin parameters

Parameter		Weeks of age				
		12	14	16	18	20
AT[a] (IU/L)	Vitamin E group	105 ± 50	79 ± 23	95 ± 76	68 ± 18	80 ± 38
	Control group	108 ± 60	88 ± 28	72 ± 22	71 ± 14	74 ± 24
GGT[b] (IU/L)	Vitamin E group	31 ± 11	27 ± 9	22 ± 7	19 ± 4	19 ± 4
	Control group	34 ± 15	29 ± 11	23 ± 5	20 ± 4	20 ± 5
CK[c] (IU/L)	Vitamin E group	200 ± 160	180 ± 51	212 ± 104	190 ± 65	195 ± 71
	Control group	160 ± 47	207 ± 133	160 ± 41	216 ± 88	165 ± 43
Urea nitrogen (mg/dL)	Vitamin E group	10.7 ± 2.7	10.1 ± 2.0	10.5 ± 3.2	11.9 ± 3.8	11.0 ± 3.9
	Control group	10.8 ± 2.4	10.3 ± 2.4	10.8 ± 3.1	11.9 ± 2.7	10.6 ± 3.8
Creatinine (IU/L)	Vitamin E group	1.00 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
	Control group	1.00 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Total protein (g/dL)	Vitamin E group	6.2 ± 0.3	6.2 ± 0.2	6.2 ± 0.3	6.2 ± 0.4	6.4 ± 0.3
	Control group	6.3 ± 0.5	6.2 ± 0.4	6.1 ± 0.4	6.2 ± 0.6	6.2 ± 0.5
Albumin (g/dL)	Vitamin E group	3.3 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.2	3.2 ± 0.2
	Control group	3.3 ± 0.2	3.2 ± 0.2	3.2 ± 0.3	3.2 ± 0.3	3.2 ± 0.2
Globulin (g/dL)	Vitamin E group	2.9 ± 0.2	2.9 ± 0.1	2.9 ± 0.3	3.0 ± 0.4	3.2 ± 0.3
	Control group	3.0 ± 0.4	3.0 ± 0.3	2.9 ± 0.3	3.0 ± 0.4	3.1 ± 0.4
Albumin/globulin ratio	Vitamin E group	1.15 ± 0.09	1.13 ± 0.06	1.12 ± 0.17	1.06 ± 0.17	1.03 ± 0.16
	Control group	1.10 ± 0.12	1.07 ± 0.11	1.12 ± 0.13	1.06 ± 0.13	1.05 ± 0.15
Total cholesterol (mg/dL)	Vitamin E group	83 ± 28	67 ± 19	65 ± 14	58 ± 15	64 ± 20
	Control group	76 ± 22	55 ± 15	56 ± 7	48 ± 24	53 ± 23
Triglyceride (mg/dL)	Vitamin E group	11.1 ± 6.0	11.7 ± 5.4	11.7 ± 5.3	8.8 ± 3.3	13.6 ± 5.3
	Control group	13.3 ± 4.6	12.4 ± 5.7	9.9 ± 4.9	10.9 ± 6.0	11.9 ± 6.5
BHB[d] (μmol/L)	Vitamin E group	289 ± 76	356 ± 76	489 ± 187	450 ± 97	398 ± 74
	Control group	343 ± 115	423 ± 127	420 ± 96	479 ± 157	463 ± 119
Calcium (mg/dL)	Vitamin E group	10.6 ± 0.4	10.7 ± 0.3	10.5 ± 0.4	10.3 ± 0.5	10.4 ± 0.4
	Control group	10.5 ± 0.5	10.5 ± 0.4	11.1 ± 2.4	10.3 ± 0.7	10.5 ± 0.5
Inorganic phosphorus (mg/dL)	Vitamin E group	8.1 ± 0.7	8.5 ± 0.8	8.8 ± 0.7	8.7 ± 0.8	9.1 ± 1.1
	Control group	8.2 ± 1.1	8.0 ± 1.5	8.3 ± 1.1	8.5 ± 1.5	8.6 ± 0.9
Magnesium (mg/dL)	Vitamin E group	2.7 ± 0.2	2.8 ± 0.3	2.8 ± 0.2	2.7 ± 0.2	2.8 ± 0.2
	Control group	2.6 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	2.7 ± 0.2
Zinc (μg/dL)	Vitamin E group	100 ± 16	95 ± 17	92 ± 16	100 ± 19	96 ± 19
	Control group	90 ± 23	94 ± 15	93 ± 15	102 ± 23	108 ± 20
Vitamin E (IU/dL)	Vitamin E group	170 ± 55	160 ± 62*	152 ± 50*	128 ± 45*	129 ± 48*
	Control group	163 ± 49	88 ± 26	71 ± 20	52 ± 23	55 ± 25

[a] Aspartate aminotransferase, [b] Gamma-glutamyltransferase, [c] Creatine kinase, [d] Beta-hydroxybutyric acid. Data are shown as mean ± SD. The asterisk indicates a significant difference between groups at the same age ($P<0.05$).

lymphocytes [13], increase of B cell number in blood [10], and increase of antibody production after vaccination [27].

Recently, because of increased global antimicrobial resistance, careful use of antibacterial agents in humans and livestock animals is recommended [18]. Therefore, there is increasing interest in disease prevention through nutritional management, and one of them is the effective use of vitamin preparations [16].

The recommended amount of vitamin E for cattle is 15–60 IU/kg in feed dry matter according to the National Research Council (NRC) feed standard [21]. In calves, vitamin E is low when the blood concentration is less than 100 IU/dL [17, 23]. In this study, although the amount of vitamin E in the basic feed met the NRC standard value, the serum vitamin E concentration in the control group was less than 100 IU/dL from 14 to 20 weeks of age. Therefore, the amount of vitamin E in the feed in the control group might not be sufficient. Smith *et al.* [29] also suggested that 15 IU/kg of vitamin E in the feed dry matter for cattle may not be sufficient. On the other hand, the serum vitamin E concentration in the VE group was above 100 IU/dL during the experimental period. Additionally, there was no significant difference in biochemical values between the groups during the experimental period. The vitamin E supplementation was considered to have almost no effect other than serum vitamin E concentration.

Oxidative stress indicates the balance between reactive oxygen species (ROS) and antioxidants [14]. It develops when the production of reactive oxygen species increases and the amounts of antioxidants decreases [14]. The d-ROMs and BAP can be easily measured, and these tests can provide an understanding of the clinical status of oxidative stress [1, 28]. High d-ROMs values indicate increased production of ROS, and high BAP values indicate increased antioxidant capacity. Because these parameters are interdependent [14], it is also important to evaluate ROS and antioxidants jointly rather than separately. In addition, based on the d-ROMs and BAP values, the oxidative stress index (OSI) can be calculated to evaluate the degree of oxidative stress [1, 14]. In this study, the d-ROMs and OSI at 20 weeks of age were significantly higher in the control group than those in the VE group. The serum vitamin E concentration

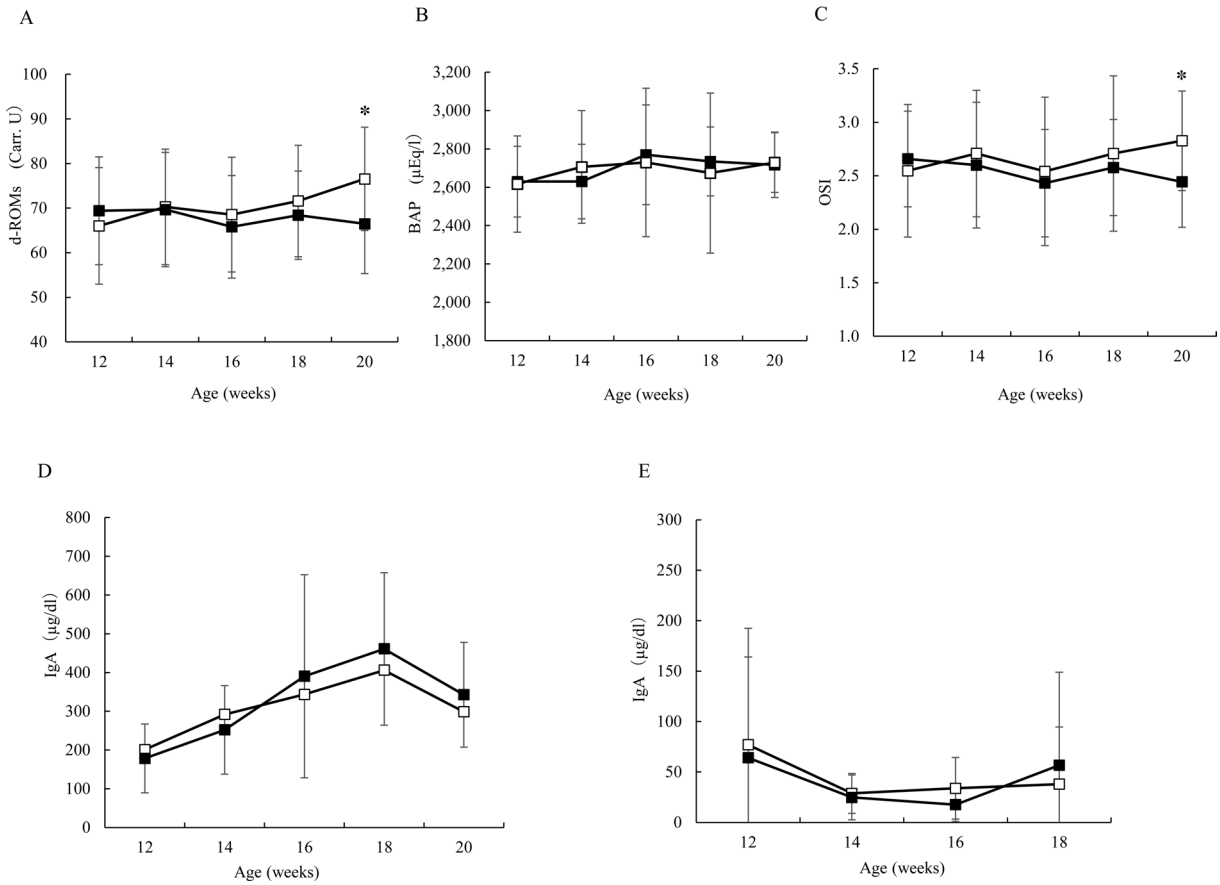

Fig. 1. (**A**) Changes in serum reactive oxygen metabolites (d-ROMs) in the vitamin E group (solid squares) and the control group (empty squares). The asterisk indicates a significant difference between groups at the same age ($P<0.05$). Data are shown as mean ± SD. (**B**) Changes in serum biological antioxidant potential (BAP) in the vitamin E group (solid squares) and the control group (empty squares). Data are shown as mean ± SD. (**C**) Changes in oxidative stress index (OSI) in the vitamin E group (solid squares) and the control group (empty squares). The asterisk indicates a significant difference between groups at the same age ($P<0.05$). Data are shown as mean ± SD. (**D**) Changes in the serum IgA concentration in the vitamin E group (solid squares) and the control group (empty squares). Data are shown as mean ± SD. (**E**) Changes in the fecal IgA concentration in the vitamin E group (solid squares) and the control group (empty squares). Data are shown as mean ± SD.

in the control group was significantly lower after 14 weeks of age compared to the VE group. Therefore, in the control group, the ability to remove oxidative stress might be reduced due to a decrease in vitamin E, and increases in the d-ROMs. There were no clear reasons why the increase in d-ROM levels in the control group was observed at 20 weeks of age, not immediately after weaning when vitamin E concentration was low. These changes in d-ROMs levels might be related to other antioxidants, and further research is necessary to clarify these factors.

On the other hand, in the VE group, the vitamin E concentration in the blood remained in the normal range, and thus the abilities to prevent the production of oxidants and to remove oxidative stress might have remained.

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 [2, 11]. It has also been reported that young calves had a lower ability of antibody production against vaccination [8]. 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 [22]. In general, the antibody titers in neonatal calves were affected by the maternal antibody titers in the colostrum [2], and vaccination to calves carrying the maternal antibody titers reduces the effectiveness of the vaccine. In this study, although the live bovine RS virus vaccine was administered to calves at 14 weeks of age, the antibody titer against bovine RS virus gradually decreased from 12 to 20 weeks of age in both groups. It was difficult to clarify the effects of vitamin E supplementation and live bovine RS virus vaccination on antibody production in this study. The factors for these results might include vaccine break due to the influence of the maternal antibody titers in calves. Or due to immature immunity of calves at younger age when antibody production after vaccination tends to be lower. Previous reports have also reported that live bovine RS virus vaccination to calves in the presence of antibody attenuates the antibody production [12, 31].

It has been reported that vitamin E improves immune function by increasing the number of B lymphocyte cells in the Peyer's patches of the intestinal tract [6]. In addition, it has been reported that vitamin E supplementation increases IgA concentration in the intestinal

Table 3. Antibody titers to bovine respiratory syncytial virus

	Animal number	Weeks of age				
		12	14	16	18	20
Vitamin E group	1	8	2	4	8	16
	2	256	512	128	64	32
	3	<2	<2	4	16	4
	4	256	256	128	128	32
	5	1,024	512	1,024	256	512
	6	256	128	64	32	16
	7	2	<2	2	2	2
	8	2	<2	2	2	4
	9	32	32	16	16	8
	10	64	64	16	8	8
	11	4	4	2	2	2
	12	1,024	256	128	128	32
	13	32	16	16	8	4
	14	64	32	32	8	8
	15	128	64	32	16	16
		40.3[a)]	25.4	21.1	16.0	11.6
	Animal number	Weeks of age				
		12	14	16	18	20
Control group	1	1,024	256	128	128	16
	2	64	16	16	8	16
	3	32	32	32	16	32
	4	32	64	128	64	64
	5	256	128	64	32	8
	6	512	256	64	32	32
	7	<2	<2	2	16	8
	8	32	8	8	8	4
	9	64	64	32	16	4
	10	32	8	8	4	4
	11	128	32	16	16	8
	12	32	8	64	64	64
	13	32	32	32	32	32
	14	128	128	32	64	32
	15	32	16	32	32	16
		58.4	30.6	27.9	24.3	15.3

[a)] Geometric mean.

tract in rats [33], and increases the serum IgA concentration in poultry [15]. Regarding IgA in calves, there have been reports on the changes in blood and feces over time in suckling calves [26, 30, 35]. However there have been no reports of weaned calves and the effects of vitamin E supplementation on IgA in calves. In this study, there was no significant difference in the serum and fecal IgA concentrations between the groups. Unlike in other animal species, in ruminants including calves, vitamin E supplementation might have little effect on IgA concentration in blood and feces. The relationship between vitamin E supplementation in calves and IgA production was not clear in this study, and thus further research in the future is warranted.

In conclusion, vitamin E supplementation to calves after weaning may reduce the blood cell oxidative stress. However, further studies are needed to clarify how it affects the blood oxidative metabolism, as well as an appropriate dose of vitamin E supplementation in the calves.

POTENTIAL CONFLICTS OF INTEREST. The authors declare that there is no conflict of interest regarding the publication of this article.

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