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Effects of vitamin C supplementation on the blood oxidative stress and antibody titre against *Histophilus somni* vaccination in calves

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Abstract

Introduction: The purpose of this study was to determine the effects of vitamin C supplementation on blood oxidative stress biomarkers and antibody response to vaccination in calves. **Material and methods:** Thirty-four clinically healthy 2 week old Japanese Black calves were randomly assigned to two groups. Seventeen calves formed the VC group which received 1,000 mg of vitamin C daily from 2 to 8 weeks of age, and the other 17 calves of the control group did not receive supplementation. All calves received an inactivated *Histophilus somni* vaccine at 4 and 8 weeks of age. Blood samples were taken at 2, 4, 8 and 12 weeks of age. **Results:** The concentration of the serum reactive oxygen metabolites (d-ROMs), and the oxidative stress index (OSI), which is calculated from the d-ROMs and biological antioxidant potential, were significantly lower at 8 weeks of age in the VC group than in the control group ($P < 0.05$). The antibody titres to *H. somni* in the VC group were significantly higher than those in the control group at 12 weeks of age after the second vaccination ($P < 0.05$). **Conclusion:** Vitamin C supplementation to calves may reduce oxidative stress and enhance the antibody production after vaccination with *H. somni*.

Keywords: calves, *Histophilus somni*, oxidative stress, vaccination, vitamin C.

Introduction

Young suckling calves have immature immune systems as evidenced by their lower numbers of lymphocytes in the peripheral blood, which are responsible for humoral and cellular immunity (9). This was demonstrated by a weak antibody response to vaccination (16). Additionally, calves of Japanese Black cattle have a lower number of immune cells than other breeds and thus tend to be more susceptible to diseases compared to other cattle breeds (17).

Histophilus somni is a common inhabitant bacterium of the upper respiratory tract of healthy cattle (2), and has been most commonly isolated in bovine respiratory diseases (2, 6). Antimicrobial agents are generally used to treat bacterial infections. However, in recent years, their use in livestock animals has been restricted. To prevent respiratory infections in cattle by bacteria such as *H. somni*, vaccination has precedence over antibiotic therapy. Thus, in Japan, almost all Japanese Black calves are vaccinated with an *H. somni* preparation.

Vitamin C is an important free radical scavenger and as an antioxidant, acts inside the body to stabilise biological membranes and maintain immune function (4, 15, 22). Many studies investigating vitamin C administration have reported more efficient quenching of singlet oxygen and strengthened immunity (4, 15, 20). Therefore, supplementation of vitamin C to Japanese Black calves was expected to reduce oxidative stress and improve immune function. The purpose of this study was to evaluate the beneficial effects of such supplementation on the serum oxidative stress biomarkers and the antibody response to inactivated *H. somni* vaccination in Japanese Black calves.

Material and Methods

Thirty-four clinically healthy 2-week-old Japanese Black calves kept on 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 at 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. The husbandry of all calves was unvaried, they were weaned at 12 weeks of age and fed to meet their nutritional requirements according to the Japanese beef cattle feeding standard (13). The calves were randomly assigned to two groups. Seventeen calves were orally supplemented with a dose based on the study by Hemingway (7) of 1000 mg of vitamin C (Rovimix vitamin C; DSM Nutrition Products, Basel, Switzerland) once daily from 2 to 8 weeks of age (VC group), and 17 calves were not supplemented with vitamin C (control group). All calves were vaccinated with a commercially available inactivated *H. somni* vaccine (M-1 Br strain; Kyoto Biken Laboratories Inc., Kyoto, Japan) at 4 and 8 weeks of age following the manufacturer's instructions. All the calves ate all the provided feed, and no calves developed disease during the experiment. Blood samples were taken at 2, 4, 8 and 12 weeks of age from the jugular vein using Vacutainer tubes containing dipotassium edetic acid (EDTA-2AK) and plain Vacutainer tubes. Serum was isolated from the blood samples in plain tubes by centrifugation and stored at –30℃ until analysis.

White blood cells (WBC) and red blood cells (RBC) were counted and haemoglobin (Hb) and haematocrit (Ht) were measured in the blood samples in the EDTA-2AK tubes using a 6550 automatic haemocytometer (Nihon Kohden, Tokyo, Japan) within 4 h of collection. The serum oxidant status was determined using a diacron reactive oxygen metabolites (d-ROMs) test, which determines hydroperoxides (1, 18, 21). The serum concentrations of antioxidant capacity were measured using a biological antioxidant potential (BAP) test (1, 18, 21), and the d-ROMs and BAP were measured using a free radical analyser (FREE Carrio Duo, Diacron International, Grosseto, Italy) (1, 18). The degree of oxidative stress was expressed as the oxidative stress index (OSI) calculated with the formula (d-ROMs/BAP) × 100 = OSI (1, 21). Serum antibody titres against *H. somni* were detected in ELISA, as described in a previous study (3, 14). *H. somni* of the M-1 Br strain was dispensed into wells of a microtitre plate, and serum samples at 400-fold dilution were added to the wells and incubated, at 30℃ for 1 h. After washing, peroxidase-conjugated anti-bovine IgG was added and the samples were incubated again, at 30℃ for 30 min. After a second washing, o-phenylenediamine was added in a citrate–phosphate buffer and the plates were incubated once again, at 30℃ for 30 min. The reaction was stopped and the optical density was read at 492 nm, using reference wavelength of 630 nm.

Data were expressed as the mean ± standard error. Statistical analysis was conducted to determine the differences between the two groups in the same weeks of age using Student's *t*-test with SPSS statistics 24 software (IBM, Tokyo, Japan). P values less than 0.05 were considered statistically significant.

Results

The values of WBC, RBC, Hb and Ht were not significantly different between the groups (Table 2). The serum d-ROMs concentration in the control group was higher than that in the VC group from 2 to 12 weeks of age (Fig. 1), and the difference between the groups was statistically significant at 8 weeks ($P < 0.05$). The serum BAP was not significantly different between the groups (Fig. 2). However, the OSI in the control group was significantly higher than that in the VC group at 8 weeks of age ($P < 0.05$) (Fig. 3). Fig. 4 shows the changes in the serum antibody titres against *H. somni*. In the VC group, the antibody titres against *H. somni* were significantly higher than those in the control group at 12 weeks of age after the second vaccination ($P < 0.05$).

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

	Weeks of age			
	2	4	8	12
Amount (dry matter; kg)				
Milk replacer	0.90	1.08	1.08	0.00
Concentrate	0.04	0.09	0.45	1.08
Oats	0.01	0.01	0.08	0.16
Composition (dry basis; %)				
Total digestible nutrients	103.4	102.4	95.8	77.5
Crude protein	27.4	27.1	24.9	18.8
Crude fat	15.5	15.1	11.8	2.2
Calcium	1.34	1.31	1.13	0.61
Phosphorous	0.72	0.71	0.66	0.52
Magnesium	0.11	0.11	0.14	0.21

Table 2. Haematological parameters

Parameter		Weeks of age			
		2	4	8	12
White blood cells (10^2 cell/μL)	VC group	86.0 ± 5.4	80.4 ± 3.6	81.6 ± 5.4	82.4 ± 5.9
	Control group	79.6 ± 5.3	88.4 ± 4.1	88.2 ± 5.5	83.8 ± 3.2
Red blood cells (10^4 cell/μL)	VC group	846 ± 23	948 ± 30	1022 ± 22	1057 ± 20
	Control group	893 ± 29	1007 ± 26	1102 ± 24	1099 ± 36
Haemoglobin (g/dL)	VC group	9.8 ± 0.2	10.5 ± 0.4	10.9 ± 0.2	11.2 ± 0.2
	Control group	9.9 ± 0.3	10.6 ± 0.3	11.4 ± 0.2	11.7 ± 0.2
Haematocrit (%)	VC group	31.9 ± 0.8	33.6 ± 1.1	34.4 ± 0.7	35.3 ± 0.7
	Control group	32.1 ± 1.0	33.6 ± 0.9	35.6 ± 0.7	35.3 ± 0.8

Data are shown as mean ± SE

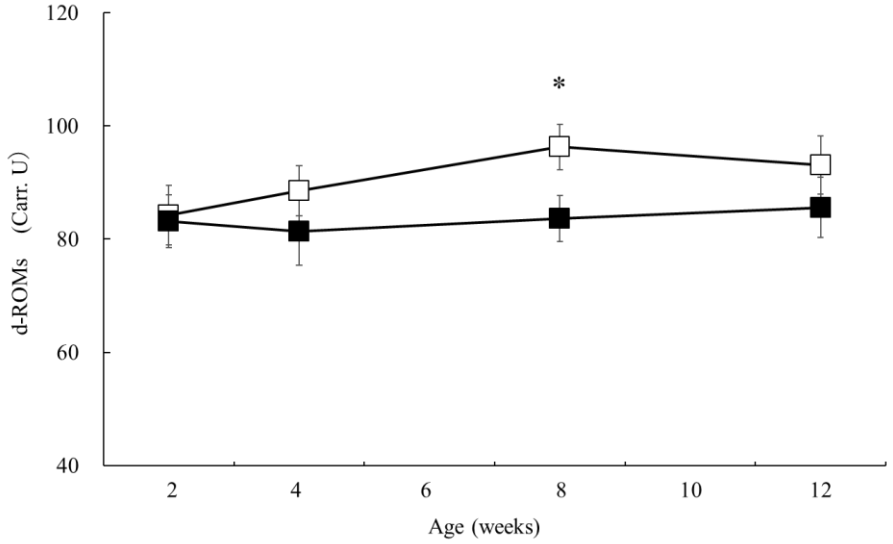

Fig. 1. Changes in serum derivatives of reactive oxygen metabolites (d-ROMs) in the VC group (solid squares) and the control group (hollow squares)
The amount of d-ROMs corresponds to the amount of hydrogen peroxide
The asterisk indicates a significant difference between groups at the same age ($P < 0.05$)
Data are shown as mean ± SE

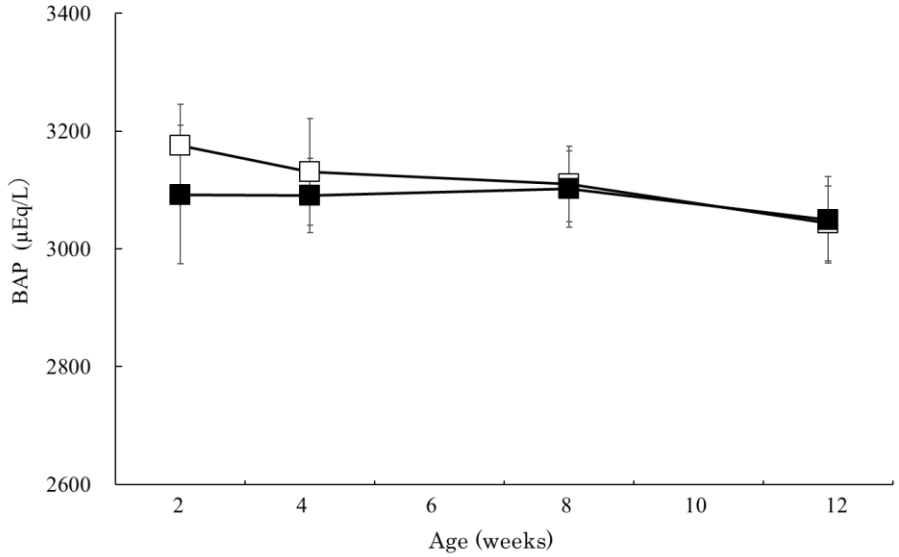

Fig. 2. Changes in serum biological antioxidant potential (BAP) in the VC group (solid squares) and the control group (hollow squares)
Data are shown as mean ± SE

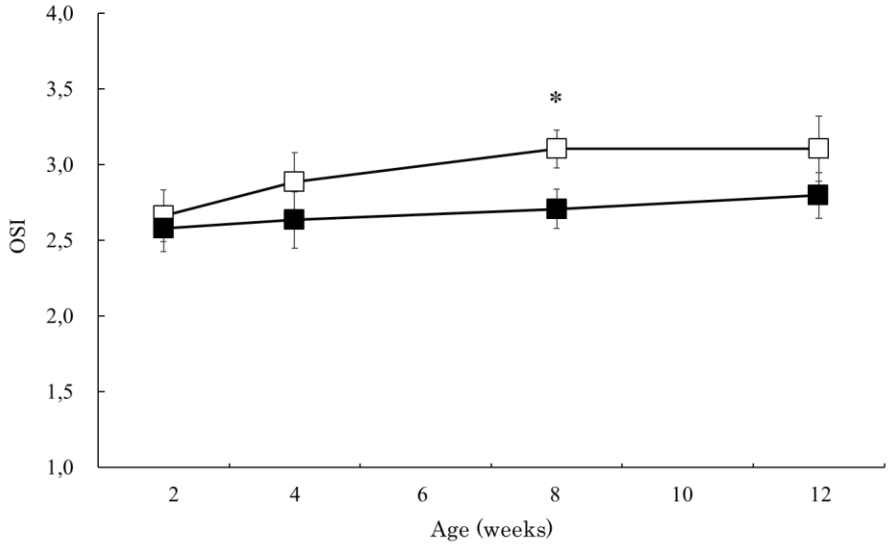

Fig. 3. Changes in oxidative stress index (OSI) in the VC group (solid squares) and the control group (hollow squares)
The asterisk indicates a significant difference between groups at the same age ($P < 0.05$)
Data are shown as mean ± SE

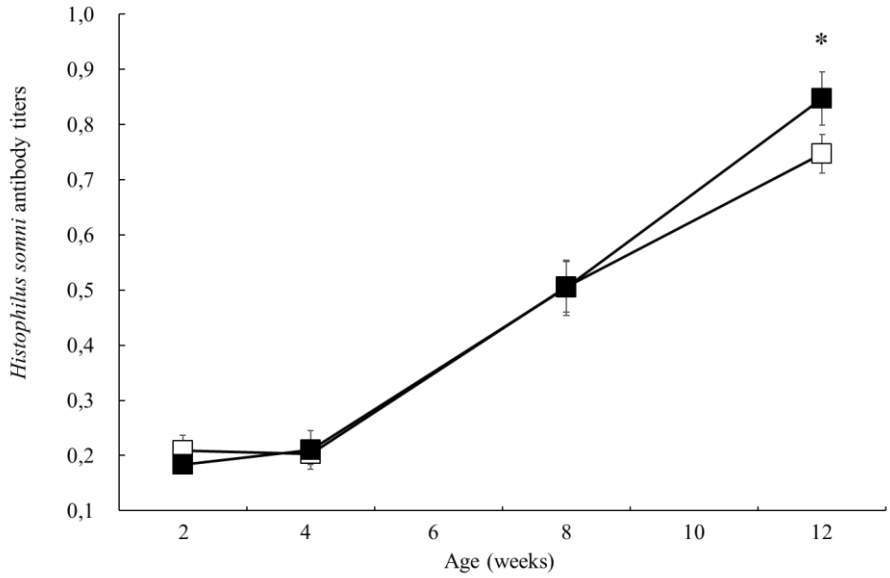

Fig. 4. Changes in antibody titres against *Histophilus somni* in the VC group (solid squares) and the control group (hollow squares)
The asterisk indicates a significant difference between groups at the same age ($P < 0.05$)
Data are shown as mean ± SE

Discussion

In this study, the values of WBC, RBC, Hb and Ht in calves of both groups were within the normal range and there was no significant difference between the groups. All calves were also clinically healthy during the experiment period. Therefore, it is considered that this study was conducted without negatively affected by vitamin C supplementation to calves.

Oxidative stress is indicated by the concentration balance of reactive oxygen species (ROS) and antioxidants (12). It develops when production of reactive oxygen species increases and amounts of antioxidants decrease (12). The d-ROMs and BAP can easily be measured, and tests for them can be applied to gain an understanding of the clinical status of oxidative stress (1, 18, 21). High d-ROMs values indicate increased production of ROS, and high BAP values indicate increased antioxidant capacity. Because these parameters are interdependent (12), it is also important to evaluate ROS and antioxidants jointly rather than separately, and from the d-ROMs and BAP values the grade of oxidative stress can be evaluated using the oxidative stress index (OSI) (1, 21). Vitamin C plays an important role as an antioxidant (15), efficiently contributes to defence against lipid peroxidation, and

scavenges peroxyl radicals (4, 15, 22). A previous study in humans has also shown that supplementation with vitamin C decreased several oxidative stress marker concentrations in blood (24). In this study, the d-ROMs and OSI at 8 weeks of age were significantly lower in the VC group than those in the control group. Therefore, vitamin C supplementation in the VC group might have contributed to oxidative stress reduction.

Several studies revealed that vitamin C has immunomodulatory functions (4, 8, 19, 22, 23). Vitamin C supplementation to animals enhanced phagocytosis of macrophages and proliferated helper T cells and B cells (4, 8). Ascorbic acid also activates dendritic cells which present antigens to helper T cells in an antigen-antibody reaction and those cells are responsible for antibody production (10). Clinical trials in mice and humans revealed that vitamin C supplementation was associated with a significant increase in serum IgG concentration (20, 23). Furthermore, guinea pigs which received vitamin C supplementation produced more antibodies to the inoculum (19). In general, the inactivated vaccine is administered at least twice in order to obtain a booster effect (5). In this study, administration of the inactivated vaccine to calves was performed twice, at 4 and 8 weeks of age. As a result, at 8 weeks of age before the second vaccination there was no difference in antibody titres between the two groups, but at 12 weeks of age after the second vaccination, the VC group had a significantly higher antibody titre than the control group. Therefore, vitamin C supplementation to Japanese Black calves may enhance the booster effect against vaccination. It has also been reported that in Brown Swiss calves, antibody production was promoted by administration of vitamin C in addition to vitamins A, D3 and E at the time of foot-and-mouth disease vaccination (11).

In conclusion, vitamin C supplementation to calves may reduce the blood cell oxidative stress and enhance antibody production after the second vaccination with inactivated *H. somni*. However, further studies are needed to clarify how it affects the blood oxidative metabolism and immune functions in the calves.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The present study was carried out in accordance with Kagoshima University animal care guidelines.

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