JARQ 56 (2), 171-176 (2022) https://www.jircas.go.jp

Effect of Ascorbic Acid Supplementation on Fecal Immunoglobulin A in Japanese Black Calves

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Abstract

This study aimed to assess the effect of ascorbic acid supplementation on fecal immunoglobulin A (IgA) in calves. A total of 30 Japanese Black calves were assigned to the supplementation and control groups. The supplementation group received 1,000 mg/day of ascorbic acid orally from 2 to 4 weeks of age (n = 15), whereas the control group did not (n = 15). Blood and fecal samples were collected at 2 and 4 weeks of age. There is no significant difference in the values of hematological and biochemical parameters between the two groups. The fecal IgA concentration in the control group decreased from 2 to 4 weeks of age, whereas it increased in the supplementation group from 2 to 4 weeks of age. The difference between the groups was statistically significant at 4 weeks of age ($P < 0.05$). These results suggested that ascorbic acid supplementation to calves may increase IgA production in the intestinal tract.

Discipline: Animal Science
Additional key words: calf, immunity, vitamin

Introduction

Young calves have an immature immune system (Kampen et al. 2006, Rajala & Castrén 1995). Despite advances in veterinary medicine and livestock and animal welfare, the incidence of diseases, such as respiratory diseases and diarrhea, from birth to weaning remains high in calves (Gorden & Plummer 2010, Cho & Yoon 2014).

The Japanese Black is a breed of Wagyu (Japanese beef cattle). The Japanese Black, known for its capacity to produce highly marbled beef, is raised throughout Japan and constitutes more than 90% of Wagyu raised and fattened in Japan (Gotoh et al. 2009, Matsuzaki 1997). Because of the increasing markets for highly marbled meat in Japan and overseas, the Japanese Black cattle became one of the major breeds in beef cattle. However, the calves of the 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 (Ohtsuka et al. 2011).

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 (Fagarasan & Honjo 2003, Stelwagen et al. 2009).

Ascorbic acid plays an important role in maintaining the stability of biological membranes, synthesizing collagen, and immune function in the body (Mousavi et al. 2019). In cattle, it has been reported that dietary ascorbic acid supplementation in dairy suckling calves reduced the incidence of diarrheal diseases (Hemingway 1991, Seifi et al. 1996). The reduction of diarrheal diseases in calves might be associated with increased IgA production, which protects the intestinal tract. This study aimed to clarify the effect of ascorbic acid supplementation on IgA production in the intestinal tract in Japanese Black calves.

Materials and methods

1. Animals and study site

A total of 30 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 dams for 4 d after birth and were housed indoors.

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Received 25 February 2021; accepted 30 June 2021.

Starting at 5 d after birth, they were fed with milk replacer and individually raised in calf hutches. The amount and nutrient composition of feeds are shown in Table 1. All calves were managed in the same manner, and the amount and nutrient composition of feed met their nutritional requirements in accordance with the Japanese beef cattle feeding standard (Ministry of Agriculture, Forestry and Fisheries Research Council 2008).

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

	2 weeks of age	4 weeks of age
Amount (dry matter; kg)		
Milk replacer	0.90	1.08
Concentrate	0.04	0.09
Oats	0.01	0.01
Composition (dry basis; %)		
Total Digestible Nutrients	103.40	102.40
Crude Protein	27.40	27.10
Crude Fat	15.50	15.10
Calcium	1.34	1.31
Phosphorus	0.72	0.71
Magnesium	0.11	0.11

The calves were randomly assigned to the supplementation group (n = 15; 16.8 ± 0.5 d of age) and the control group (n = 15; 16.4 ± 0.4 d of age). The supplementation group received 1,000 mg of ascorbic acid (DSM Nutritional Products, Basel, Switzerland) orally once daily for 14 d from 2 to 4 weeks of age. This dose was based on the study by Hemingway (1991) and Seifi et al. (1996). Blood samples were collected from the jugular vein at 2 and 4 weeks of age and placed into ethylenediaminetetraacetic acid-containing and plain vacutainer tubes. Serum was isolated from the blood samples in plain tubes 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 2 and 4 weeks of age. The fecal samples were taken at 10-11 a.m. (2 h-3 h after feeding). These fecal samples were stored at −30°C until analysis. Body weight was measured at 2 and 4 weeks of age. All the calves ate all the provided feed, and no calves developed disease during the study period.

2. Sample analysis

White blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), and hematocrit (Ht) were measured using the 6550 Automatic Hemocytometer (Nihon Kohden, Japan) within 4 h after collection. The following serum biochemical parameters were determined using a Labospect 7020 autoanalyzer (Hitachi High-Technologies, Japan): aspartate aminotransferase, gamma-glutamyl transferase, creatine kinase, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, triglyceride, nonesterified fatty acids, calcium, inorganic phosphorus, and magnesium. The fecal IgA concentrations were measured using enzyme-linked immunosorbent assay (ELISA). ELISA was performed as previously described with some modifications (Niimi et al. 2017). The fecal samples (400 mg each) were suspended using a bead crusher after the addition of 1,000 µL of PBS/sample and centrifuged at 15,000 rpm for 10 min. The supernatant was used for measurement. Affinity Purified Sheep Anti-Bovine IgA (Bethyl, USA) was diluted 500-fold using carbonate bicarbonate buffer (Sigma, Japan). The diluted IgA antibody was placed in a 96-well plate at 100 µL per well and immobilized overnight at 4℃. After removing the solution, the plate was washed with 200 µL of wash buffer per well five times. After blocking with 0.05% Tween-20 solution, the plate was allowed to stand at room temperature for 1 h. The stool supernatant was serially diluted using wash buffer and dispensed into the wells of a plate. Reaction was conducted at room temperature for 2 h. After the well contents were removed, the plate was washed using a wash buffer. Properly diluted horseradish peroxidase-conjugated sheep anti-bovine IgA (Bethyl, USA) was added to each well, and reaction was conducted at room temperature for 1 h. After removing the supernatant, the plate was washed using a wash buffer; then, 100 µL/well of color-developing solution was added. After 5 min, the reaction was stopped using diluted sulfuric acid. The absorbance was read at 450 nm using a spectrophotometer. The detection range of the assay was 1.37-1,000 ng/mL.

3. Statistical analyses

Daily gain was calculated from body weight at 2 and 4 weeks of age. Data were expressed as the mean ± standard error. Statistical analysis was conducted to determine the differences between the two groups at the same weeks of age using Student's *t*-test with the IBM SPSS Statistics version 26 software (IBM, Japan). *P*-values of 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). Additionally, there was also no significant difference in the serum biochemical parameters between the groups (Table 3). Body weight and daily gain were not significantly different between the two groups (Table 4). The fecal IgA concentration was not significantly different between the groups at 2 weeks of age (Fig. 1). The fecal IgA concentration decreased in the control group from 2 to 4 weeks of age, whereas it increased in the supplementation group from 2 to 4 weeks of age. The difference between the groups was statistically significant at 4 weeks of age ($P < 0.05$).

Discussion

Previous reports have shown that oral feeding of Holstein calves with 1,000 mg of ascorbic acid once daily had reduced the incidence of diarrhea and mortality (Hemingway 1991, Seifi et al. 1996). Therefore, in this study, the calves in the supplementation group received 1,000 mg of ascorbic acid orally once daily. All the calves were clinically healthy, suggesting that they were at low risk of infection diseases during the experimental period.

Generally, the nutritional status of young calves is influenced by the nutritional status of mother cows (Ogata et al. 1999, Zanker et al. 2001). In this study, the hematological parameters did not show significant differences between the groups. The serum biochemical parameters were also within the normal range (Otomaru et al. 2016), without significant differences between the groups. Additionally, all calves consumed all feed provided, and body weight and daily gain did not show significant differences between the groups, suggesting that ascorbic acid supplementation to calves did not have any negative effects in this study.

IgA is one of the first lines of defenses against pathogenic microorganisms in the intestinal tract. IgA can bind to pathogenic microorganisms and prevent them from invading the intestinal tract. It plays an important role in the intestinal health of calves (Fagarasan & Honjo 2003, Stelwagen et al. 2009). Most IgA in newborn calves is derived from the colostrum. Newborn calves acquire immune components such as IgA from the colostrum to maintain their health (Stelwagen et al. 2009, Tsuruta et al. 2009).

Generally, immune function is reduced by stress-associated substances, such as corticoids or oxidants (Rook 1999, Staley et al. 2018). Ascorbic acid has an antioxidative effect, scavenging free radicals from superoxide and preventing the production of inducible NO synthase. Additionally, ascorbic acid can suppress the production of cortisol (Park et al. 2020, Smitha & Kannan 2014). Ascorbic acid can also improve the immune function by increasing the number of B lymphocyte cells and activating dendritic cells (Tanaka et al. 1994, Kim et al. 2012). This in turn could induce the differentiation of the cells into IgA-producing plasma cells in the Peyer's patches of the intestinal tract (Tezuka & Ohteki 2019).

In this study, the fecal IgA concentration in the control group decreased over the course of time, most likely because of the decrease in IgA derived from colostrum. Moreover, the change in IgA was similar to the previous reports in calves (Tsuruta et al. 2009). Contrarily, the fecal IgA concentration in the supplementation group increased from 2 to 4 weeks of age, showing significantly higher value than that in the control group at 4 weeks of age. In this study, the supplementation group might have decreased stressors or

Table 2. Hematological parameters

		2 weeks of age	4 weeks of age
WBC (10^2 cells/μL)	Supplementation group	83.7 ± 5.4	79.8 ± 4.0
	Control group	80.2 ± 6.0	85.4 ± 3.9
RBC (10^4 cells/μL)	Supplementation group	832 ± 24	947 ± 34
	Control group	898 ± 32	1,010 ± 28
Hb (g/dL)	Supplementation group	9.7 ± 0.2	10.5 ± 0.4
	Control group	9.9 ± 0.3	10.6 ± 0.3
Ht (%)	Supplementation group	31.5 ± 0.8	33.8 ± 1.3
	Control group	32.1 ± 1.1	33.5 ± 1.0

Data are shown as mean ± standard error.

Table 3. Biochemical parameters

		2 weeks of age	4 weeks of age
AST (IU/L)	Supplementation group	40.3 ± 1.3	51.1 ± 1.7
	Control group	40.4 ± 1.8	48.1 ± 2.2
GGT (IU/L)	Supplementation group	99.3 ± 9.5	49.3 ± 7.0
	Control group	98.9 ± 13.0	38.3 ± 3.6
CK (IU/L)	Supplementation group	169 ± 31	172 ± 26
	Control group	150 ± 15	139 ± 13
Urea nitrogen (mg/dL)	Supplementation group	12.8 ± 0.7	14.1 ± 0.4
	Control group	12.0 ± 0.6	13.2 ± 0.5
Creatinine (IU/L)	Supplementation group	1.00 ± 0.05	0.92 ± 0.05
	Control group	0.92 ± 0.03	0.93 ± 0.03
Total protein (g/dL)	Supplementation group	6.3 ± 0.2	6.2 ± 0.1
	Control group	6.2 ± 0.1	6.0 ± 0.1
Albumin (g/dL)	Supplementation group	2.9 ± 0.1	3.3 ± 0.1
	Control group	3.0 ± 0.1	3.2 ± 0.0
Globulin (g/dL)	Supplementation group	3.4 ± 0.2	2.9 ± 0.1
	Control group	3.2 ± 0.1	2.8 ± 0.1
Albumin/globulin ratio	Supplementation group	0.93 ± 0.07	1.12 ± 0.04
	Control group	0.96 ± 0.05	1.11 ± 0.04
Total cholesterol (mg/dL)	Supplementation group	108 ± 10	176 ± 8
	Control group	109 ± 8	157 ± 16
Triglyceride (mg/dL)	Supplementation group	23.4 ± 2.4	18.6 ± 2.4
	Control group	22.3 ± 3.5	19.5 ± 3.9
NEFA (μEq/L)	Supplementation group	247 ± 17	297 ± 21
	Control group	250 ± 25	254 ± 21
Calcium (mg/dL)	Supplementation group	10.6 ± 0.2	10.7 ± 0.2
	Control group	10.4 ± 0.2	10.6 ± 0.3
Inorganic phosphorus (mg/dL)	Supplementation group	9.3 ± 0.2	9.6 ± 0.1
	Control group	9.0 ± 0.3	9.3 ± 0.2
Magnesium (mg/dL)	Supplementation group	2.1 ± 0.1	2.0 ± 0.1
	Control group	2.1 ± 0.1	2.0 ± 0.1

Data are shown as mean ± standard error.

Table 4. Changes in body weight

	Supplementation group	Control group
2 weeks of age (kg)	48.1 ± 1.5	51.9 ± 1.6
4 weeks of age (kg)	62.8 ± 1.7	66.0 ± 1.5
Daily gain (kg)	1.09 ± 0.07	1.01 ± 0.06

Data are shown as mean ± standard error.

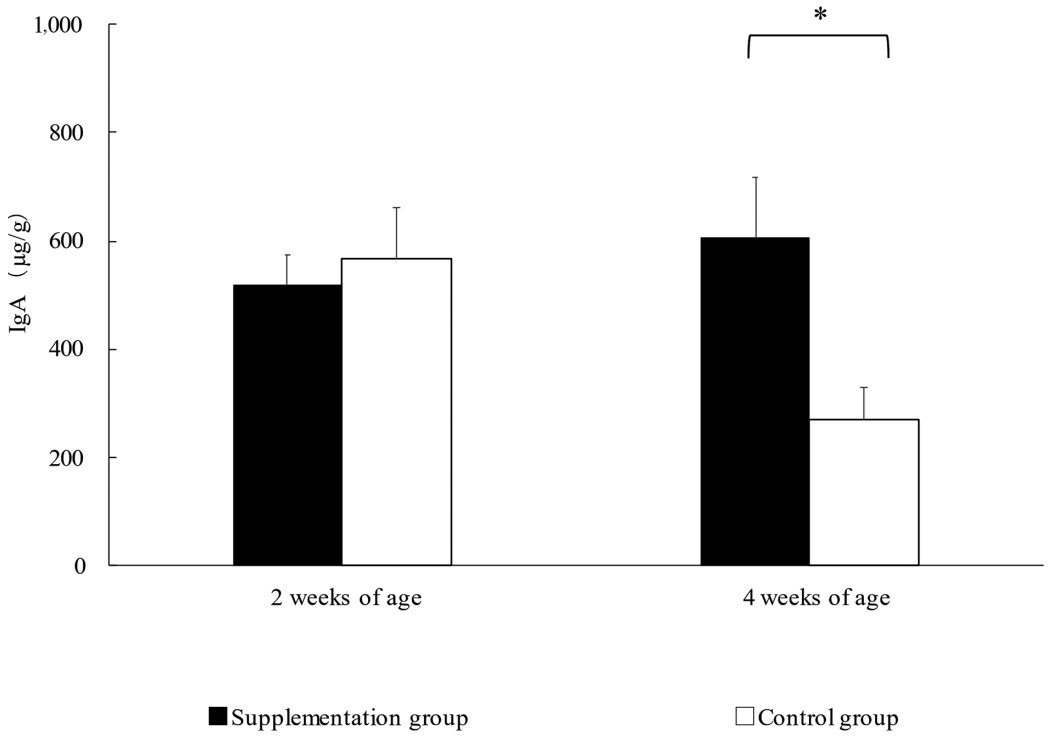

Fig. 1. Changes in fecal immunoglobulin A (IgA) concentration in the ascorbic acid supplementation and control groups
The asterisk indicates a significant difference between the two groups at the same age (*: $P < 0.05$). Data are shown as mean ± standard error.

more activated immune cells, resulting in improved IgA production in the intestinal mucosa.

In conclusion, although this study has limitations in the number of calves and the duration of the experiment, ascorbic acid supplementation to calves might have promoted IgA production in the intestinal tract. Further studies are needed to clarify how ascorbic acid supplementation promotes IgA production in calves.

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