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NOTE

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Single oral β-cryptoxanthin administration increases its serum concentration and enhances peripheral blood neutrophil function in Holstein cattle

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ABSTRACT. We investigated the effect of oral administration of β-cryptoxanthin (β-CRX) on its serum concentration and peripheral neutrophil functions by the chemiluminescence (CL) response in Holstein cattle. A single oral administration of β-CRX was performed for serum β-CRX concentration (0, 0.05, 0.1, or 0.2 mg/kg body weight [BW]) and for peak CL response of peripheral neutrophils (0.2 mg/kg BW). The serum β-CRX concentration was peaked on 2 days after, similar to peak CL response on 3 days after β-CRX administration. Therefore, a single oral administration of β-CRX (0.2 mg/kg BW) induces higher serum concentration and concurrently enhances bactericidal ability of peripheral neutrophils in Holstein cattle.

KEY WORDS: β-cryptoxanthin, chemiluminescence response, Holstein cattle, peripheral neutrophils

Carotenoids, the most important of which are α-carotene, β-carotene, β-cryptoxanthin (β-CRX), lycopene, lutein, and zeaxanthin, are plant pigments and precursors for vitamin A synthesis. They modulate the immune response by functioning as an antioxidant but also stimulating blastogenesis by lymphocytes and phagocytosis by neutrophils in humans [5], dairy cattle [6], and mice [9]. Specifically, in cattle, dietary β-carotene increased the blood β-carotene concentration and enhances host defenses by promoting the functions of lymphocytes and phagocytes [4, 6]. However, the effect of oral administration of β-CRX on its serum concentration and on the physiological responses of the host is unclear in cattle despite previous results suggested a possible benefit of β-CRX in the improvement in peripheral antioxidant capacity. Therefore, the objectives of this study were to evaluate the effect of oral administration of β-CRX on its serum concentration and peripheral neutrophils function. We examined longitudinal changes in the serum β-CRX concentration in response to a single oral administration of β-CRX, and its effects on peripheral blood phagocytosis by neutrophils. We hypothesized that oral administration of β-CRX would increase its serum concentration and enhance the chemiluminescence (CL) responses of peripheral neutrophils (antioxidant capacity) related to the serum concentration.

Animal care and experimental procedures were performed according to protocols approved by the Iwate University Laboratory Animal Care and Use Committee (A201201; Morioka, Japan). Eight Holstein heifers (non-pregnant; 20.1 ± 1.2 months of age; 506 ± 32 kg, mean ± SE) were used for determination of the serum β-CRX concentration. A single oral administration of 0 (n=4) and 0.05 (n=4) mg/kg body weight (BW) β-CRX (water-diluted) (Unitika, Kyoto, Japan) was performed using an oral tube on day 0, and blood samples were collected at right before administration (day 0) and 1, 2, 3, 4, 8, 12, and 16 days after administration. After 2-wk wash-out period, single oral administration of β-CRX (0.2 and 0.1 mg/kg BW) and blood collection were repeated in the same manner. The serum concentration of β-CRX was determined by high-performance liquid chromatography with absorbance detection, as described by Nantz *et al*. [11]. Briefly, 150 µl of serum was extracted by hexane, and dried and re-dissolved in ethyl acetate extracts were measured by absorbance at 452 nm. The coefficient of variation for β-CRX was <11%. The effect of β-CRX on the functions of peripheral neutrophils was evaluated by CL assay in four Holstein bulls (6.0 months of age; 144 ± 5 kg). A single

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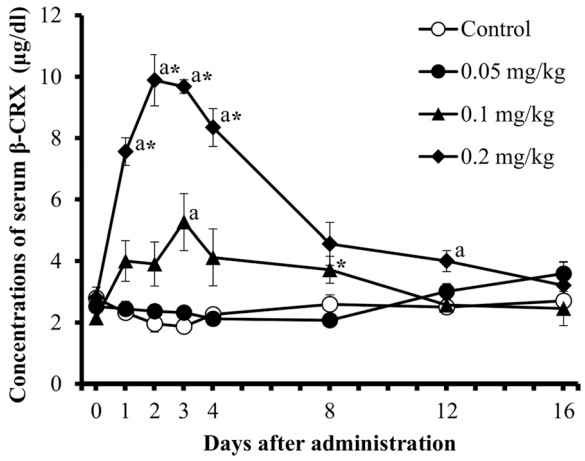

Fig. 1. Serum β-cryptoxanthin (β-CRX) concentration at 0, 1, 2, 3, 4, 8, 12, and 16 days after oral administration of 0 (control), 0.05, 0.1, or 0.2 mg/kg body weight β-CRX. [a]Significant difference (P<0.05) compared with the control. *Significant difference (P<0.05) compared with day 0. Values are mean ± SE (n=4).

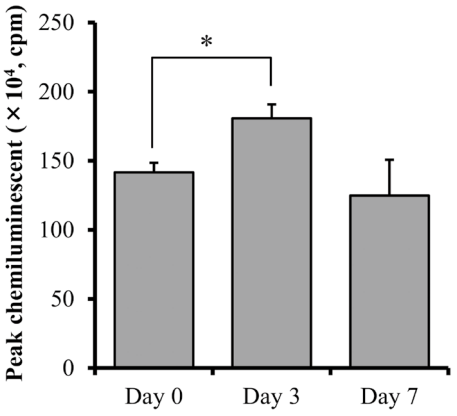

Fig. 2. Peak chemiluminescence (CL) values at 0, 3, and 7 days after oral administration of β-cryptoxanthin (β-CRX) (0.2 mg/kg body weight). *Significant difference (P<0.05) compared with day 0. Bars represent mean ± SE (n=4).

oral administration of 0.2 mg/kg BW β-CRX was performed on day 0. For CL assays, blood samples were collected at 0, 3, and 7 days after administration, and neutrophils were isolated by Ficoll-Conray (GE Healthcare, Uppsala, Sweden) density gradient centrifugation followed by hypotonic hemolysis [8]. All procedures were performed within 2 hr of collection, and the purity and viability of neutrophils thus obtained manually were 95% and 97%, respectively. A luminol-dependent CL assay was performed as described by Nagahata *et al*. [10]. The normality of the distributions of variables was assessed using the Shapiro–Wilk test. Significant differences among groups were evaluated using the Mann–Whitney *U*-test for non-normal variables (Prism ver. 8.10; GraphPad Software, La Jolla, CA, USA). One-way repeated-measures ANOVA, followed by Dunnett's multiple comparison method, was used to determine within-group differences compared with day 0 in each group. Significant differences were considered significant at P<0.05.

The mean serum β-CRX concentration before administration was 2.14–2.80 μg/dl (Fig. 1). In the 0.2 mg/kg BW group, the β-CRX concentration peaked at 2 days (9.88 μg/dl) and increased significantly (P<0.05) on days 1, 2, 3, and 4 compared with day 0 after administration. The 0.2 mg/kg BW group showed a significantly (P<0.05) higher serum β-CRX concentration than the control and 0.1 mg/kg BW groups on days 1, 2, 3, 4, and 12. In the 0.1 mg/kg BW group, the serum β-CRX concentration was significantly (P<0.05) higher only on day 3 compared with the control group, and increased significantly on day 8 compared with day 0. In contrast, the 0.05 mg/kg BW group showed no significant changes in the serum β-CRX concentration. The peak CL value of neutrophils in the 0.2 mg/kg BW group was 142 ± 7 × 10^3 counts per minute (cpm) on day 0, and increased significantly (P=0.038) on day 3 (181 ± 10 × 10^3 cpm) compared with day 0 (Fig. 2).

Until recently, the investigation of β-cryptoxanthin feeding as an anti-oxidant to cattle are limited. Therefore, the present study increases understanding of the response of peripheral blood neutrophil function to single oral β-cryptoxanthin administration in Holstein cattle. The antioxidant activity of β-CRX prevents free radical-mediated damage to lipids, proteins, and nucleic acids [9], and dietary β-CRX has greater bioavailability than dietary α-carotene or β-carotene [1, 2]. Although it is still unclear that β-CRX is degraded or absorbed in the rumen, these may explain the slower response of the serum β-CRX concentration to oral administration in cattle compared to that in the domestic cat [3] and human [12], which lack a rumen. In the present study, the CL response on day 3 corresponded to significant increase in serum β-CRX concentration on days 1 to 4 than the control group. Because a reduced CL value indicates reduced ingestion of *Staphylococcus aureus* by bovine neutrophil and consequently host defenses during the periparturient period [7], the CL response in the present study suggested that the enhancement of neutrophil phagocytosis on day 3 in the two independent experiments (serum concentration of β-CRX and luminol-dependent CL assay experiments) despite the β-CRX administration and the CL experiments were performed independently. Furthermore, a gradual decrease in the serum β-CRX concentration after peak response may not consistently preserve a higher CL value even if the serum β-CRX concentrations were still greater than the control group heifers. Therefore, the β-CRX administration in Holstein heifers and bulls of different age (21 *vs.* 6 months of age) induces serum β-CRX peak in the similar day point, and a single oral administration of β-CRX temporally modulated the functions of neutrophils in the present study. Collectively, these results suggest that an oral administration of β-CRX (0.2 mg/kg BW) resulted in an increase in the serum concentration of β-CRX and concurrently enhanced phagocytosis by peripheral neutrophils.

CONFLICT OF INTEREST. The authors have nothing to disclose.

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