FULL PAPER

Internal Medicine

Effects of dietary feed supplementation of heat-treated *Lactobacillus sakei* HS-1 on the health status, blood parameters, and fecal microbes of Japanese Black calves

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ABSTRACT. This study investigated the effect of heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) on the health and fecal bacteriological change of suckling Japanese Black calves as a supplement in milk replacers. Twelve calves were separated from dams to calf-hatch after calving for milk replacers feeding. They were randomly assigned to an HK-LS HS-1 supplement or a control without HK-LS HS-1 group in milk replacers. HK-LS HS-1 was administered from separation day to 3 weeks. Blood and fecal samples were examined. Two calves with a haptoglobin concentration of >500 μg/ml on day 0 were excluded from the experiment, and 10 calves were finally included. Glucose and vitamin A levels on day 7 were significantly higher ($P<0.05$) in the supplement group than in the control group. No significant differences were observed in haptoglobin or serum amyloid A between the groups. The number of *Escherichia coli* in feces was lower in the control group than in the supplement group on day 21 ($P=0.06$). No difference was observed in the number of bifidobacteria, but that of lactic acid bacteria was significantly higher ($P<0.05$) in the supplement group on day 21. The number of medications administered was significantly lower ($P<0.05$) in the supplement group (5.2 ± 3.9) than in the control group (10.6 ± 5.9) during the experimental period. The results indicated that HK-LS HS-1 is potentially beneficial for improving intestinal microbes and reducing the number of medical treatments.

KEY WORDS: cattle, feed supplementation, intestinal microbes, lactic acid bacteria, probiotic

The intestine of a newborn calf is sterile and colonization of the gastrointestinal (GI) tract begins immediately after birth [38]. Therefore, appropriate development of the GI tract microbiota in the early weeks of life is crucial for a functional immune system [26]. The intestinal microbiota is affected several factors. These include but are not limited to: diet, antibiotic treatment, environments for as growth and feeding, and stress. The period from birth to weaning is stressful to young calves, causes decreased immunity, and reduces calf herd productivity [29, 34].

Japanese Black (JB) is the most popular breed of beef cattle in Japan. However, compared with other breeds, these cattle are immunologically weak. Thus, they are more prone to disease during the early postnatal period, even despite adequate passive immunity. During this period, the high risk of infections in JB calves may be ascribed to reduced lymphocyte proliferation [11, 22, 23]. Although progress has been made in developing vaccines and improving herd management practice and treatment protocols, diseases in calves such as diarrhea and pneumonia, during the lactating period cause considerable economic loss to

the JB cattle herds. To prevent such adverse cases, instead of antibiotic use in animal production that may contribute to human pathogen resistance, alternatives such as probiotics and prebiotics have been proposed worldwide [8, 13, 17, 29, 36, 38]. Probiotics have been defined as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host". Several lactic acid bacteria (LAB) strains with species belonging to the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*, are considered beneficial to the host and have, thus, been widely used as probiotics in cattle production [38]. A recent study with newborn calves indicated that intestinal colonization of newborn calves by *Lactobacillus* spp. takes place during the first 7 days of life [34]. Early colonization by LAB in the intestinal ecosystem may decrease pathogen adherence to the intestinal mucosa. Additionally, a stable microbial load of *Lactobacillus* species has been shown to improve weight gain and immunocompetence in young calves [2, 38].

Most studies involving human or animal supplementation with LAB refer to immunomodulatory effects of viable bacteria. However, heat-killed LABs, such as *Lactobacillus acidophilus* or *Lactobacillus plantarum*, are more effective than viable LABs in immunomodulation and also stimulate phagocytic activity in macrophages [5, 9, 15]. They have longer shelf life and are easy to store and transport [12]. A recent study on pigs and broilers also revealed that dietary supplementation with heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) improves their growth performance [14, 31]. However, the effects of heat-killed LAB for cattle, especially for JB calf, are unknown. We hypothesized that supplementation of HK-LS HS-1 at age 7 days will enable LABs to establish a community in the intestinal tract and increase in concentration without harmful effects on the calves. The aim of this study was to investigate the effect of HK-LS HS-1 as a supplement in milk replacers (MR) on the clinical health (frequency of diarrhea and/or fever) as well as fecal bacteriological change of suckling JB calves from the early stage after birth. Moreover, measurements of hematology and blood chemistry were conducted to monitor and compare the states of hepatic, renal, nutritional, and mineral intake, immunoglobulins, and inflammation in calves with and without HK-LS HS-1 supplementation.

MATERIALS AND METHODS

The experiments were conducted according to the regulations concerning the protection of experimental animals and the guidelines of Yamaguchi University, Japan (No. 40, 1995; approval date: March 27, 2017) and we obtained informed consent from the farmer.

Twelve JB calves born between April and July 2019 on a private farm in Kagoshima Prefecture, Japan were studied. In this experiment, calves born to mothers who had problems during labor such as dystocia were excluded from the experiment. Calving occurred naturally in the stall in all cases, and the calves were fed fresh colostrum from their dam within 2 hr after birth. After the first feeding, the calves were orally administered a colostrum replacer including totally 60 g of IgG (Headstart; Bayer Co., Ltd., Tokyo, Japan) mixed into 1 liter warm water by feeding bottle within 6 hr of the calving. Separation of calves from dams to calf-hatch was conducted 2–12 days (mean: 6.7 ± 3.6 days) after calving for MR feeding based on the calf's health condition and willingness to feed. Thus, all the calves were considered to have similar levels of stress due to infection. The calves were randomly assigned to the HK-LS HS-1 supplement group (n=6) or control without HK-LS HS-1 group (n=6) in MR. HK-LS HS-1 (Lactobacillus-KDP®; Daiwa Pharmaceutical Co., Ltd., Tokyo, Japan) was administered (0.2% HK-LS HS-1 based on a preliminary trial) orally twice daily at 9:00 am and 4:00 pm from the day of separation to 3 weeks. The volume of the MR provided was initially 3 l (600 g MR)/day, but this was gradually increased to a maximum of 6 l (1,000 g MR)/day by the end of the sampling on day 21, regardless of the body weight and sex of the calves. The intake of calf starter (total digestible nutrients >76.0%, crude protein >23.0%; Banana Calf, Nippon Agricultural Industry Co., Ltd., Yokohama, Japan) was also monitored daily. Fresh water and a calf starter supplemented with minerals and vitamins were provided ad libitum during the experimental period.

General health, including appetite and fecal consistency, was monitored daily during the experimental periods by experienced farm staff. Additionally, the veterinarians not only visited the farm at the time of sampling from the test calves, but also during the week when there was no sampling. They visited the farm once a week to observe the health of the calves and check the progress of the experiment. Enteritis, bronchitis, and pneumonia were diagnosed based on previously reported clinical criteria such as diarrhea (gruel-like or watery feces), fever (rectal temperature >39.5°C), and signs of respiratory disease (severely increased respiratory sounds accompanied by fever and coughing or a grayish to yellowish nasal discharge) [17, 33]. In this experiment, the farm staff observed the stool properties of the calf at the time of AM and PM feedings; and in cases of mild diarrhea with good appetite, an oral antidiarrheal was administered after the milking that included berberine tannate, phenyl salicylate, acacia yak powder, and torula yeast (Geritomin; Kyoritsu Seiyaku Corp., Tokyo, Japan). In cases of a calf showing severe diarrhea or fever with no appetite at feeding time, injection of antibiotics was given under the direction of a veterinarian, these included penicillin, kanamycin, or oxytetracycline was injected by the farm staff. In this study, recovery from fecal characteristics and normal body temperature was judged to be curative, and medical treatment was discontinued. All treatment data were recorded for each calf.

Blood samples from the jugular vein were collected on the day of separation (before HK-LS HS-1 supplementation; day 0), and 7 (day 7) and 21 (day 21) days after the supplementation to determine the following: complete blood count (assessed on F-820; Sysmex, Japan) and blood urea nitrogen (BUN), serum aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), total protein (TP), total cholesterol (T-Cho), glucose (Glu), free fatty acid (FFA), 3-hydroxybutyric acid (3HB), albumin (Alb), calcium (Ca), magnesium (Mg), and inorganic phosphorus (iP) (measured on a Labospect 7080 autoanalyzer; Hitachi, Japan). Serum vitamin A (VA) and vitamin E (VE) levels were measured using a HPLC (Shimadzu, Kyoto, Japan) to evaluate the changes in the depletion of both vitamins during the experimental period. Serum haptoglobin (HG) concentration was measured using an enzyme-linked solvent assay kit (Life Diagnostics, Inc., West Chester, PA, USA). Serum amyloid A (SAA) concentration was also measured

using an automated biochemical analyzer (Pentra C200; HORIBA ABX SAS, Montpellier, France) with an SAA reagent special for animal serum or plasma (VET-SAA 'Eiken' reagent; Eiken Chemical Co., Ltd., Tokyo, Japan). The SAA concentration was calculated based on a standard curve made by a calibrator (VET SAA calibrator set; Eiken Chemical Co., Ltd.). Concentrations of serum immunoglobulin A (IgA) were measured using an enzyme-linked solvent assay kit (Bethyl Laboratories, Inc., Montgomery, TX, USA). The tests were performed to monitor hepatic (AST and GGT) and renal functions (BUN), nutritional status (T-Cho, glucose, FFA, 3HB, and Alb), mineral intake (Ca, Mg, and iP), inflammation (HG and SAA) and intake of immunoglobulin (GGT, TP, Glb, A/G ratio and IgA) of the calves in the two groups.

A bacteriological analysis was conducted to evaluate the effect of HK-LS HS-1 supplementation for monitoring fecal microbes, especially for the number of aerobic (Coliform group) and anaerobic bacteria (LAB, bifidobacteria) in the fecal sample according to a previous method [40]. Fecal samples were collected from all calves upon rectal stimulation on days 0, 7, and 21. The fecal samples (3 g) were immediately placed on ice in a 50 m*l* conical tube containing 27 m*l* of brain heart infusion broth (Difco; Tokyo, Japan) medium, stirred and then transported to the laboratory while refrigerated, and processed within 24 hr of the sampling. Dilutions of the fecal homogenized samples were made in modified phosphate-buffered saline (PBS) including 0.5 g of L-cysteine·HCl·H$_2$O, 0.5 g of sodium thioglycolic acid, and 1 g of agar. The relevant dilutions were plated in de Man, Rogosa, and Sharpe agar media (Kanto Chemical; Tokyo, Japan) for *Lactobacillaceae* spp., brinated horse blood agar media (Nissui Seiyaku, Tokyo, Japan) for *Bifidobacterium* spp., and in deoxycholate hydrogen sulfide lactose agar media (Nissui Seiyaku) for the coliform group. The plates were incubated in each anaerobic and aerobic conditions at 37°C for 48 hr. Subsequently, the agar plates were assessed for growth, and colonies were counted. Using the relevant calculations for the spiral plater, total cell counts of *Enterobacteriaceae* per gram of fecal sample were calculated and transformed into log10 values.

The results obtained for each group are expressed as the mean ± SD. The number of calves that did not receive any medical treatment was compared between the groups by using the Fisher's exact test. Values for blood parameters and mean duration (in days) of medical treatment were compared between the groups using the Student *t* test to determine the effects of HK-LS HS-1 on the calves. Additionally, the CFU within each group on day 0, 7, and 21 were compared using a one-way analysis of variance, followed by a *post-hoc* test, with Ekuseru-Toukei 2012 for Windows (version 1.11; Society Survey Research Information Co., Ltd., Tokyo, Japan). *P* values less than 0.05 were considered to indicate a statistically significant difference, whereas *P* values less than 0.1 were considered to indicate a significant tendency.

RESULTS

Two calves were excluded from the experiment because they showed an HG concentration higher than 500 μg/m*l* (SAA concentrations of these 2 calves [150.8 and 175.0 μg/m*l*] were also higher than the others). Finally, 5 calves (one male and four females) were assigned to the HK-LS HS-1 supplement group and 5 calves (three males and two females) to the control without HK-LS HS-1 group in MR.

Blood analysis

The results of the hematological and serum biochemical analyses are shown in Figs. 1 and 2. No significant differences were observed between the groups at any of the sampling times with regard to red and white blood cell counts, hemoglobin (Hb) levels, hematocrit values, and total protein concentrations.

No significant differences were observed between the groups with regard to AST, T-Cho, BUN, Ca, IP, Mg, VE, 3HB, TP, AG ratio, and Alb levels. The FFA at the first sampling (day 0) was significantly higher ($P<0.05$) in the HK-LS HS-1 group (355.7 ± 121.9 mmol/*l* versus control: 211.1 ± 43.8 mmol/*l*). The Glu and serum VA concentrations were significantly higher in the HK-LS HS-1 group on day 7 (98.6 ± 7.3 mg/d*l* versus control: 82.9 ± 14.9 mg/d*l*, and 91.6 ± 15.0 IU/d*l* versus control: 75.2 ± 5.3 IU/d*l*, respectively), and GGT was higher on day 21 (47.5 ± 9.8 U/*l* versus 29.0 ± 13.0 U/*l*) in the HK-LS HS-1 group.

The results of acute-phase protein (APP) in blood are shown in Fig. 3. No clear difference was found between the HK-LS HS-1 and control groups in serum HG and SAA. As for HG, the average value increased because of one calf in the HK-LS HS-1 group (more than 500 μg/m*l*) on day 7. Two calves in the control group (171 and 306 μg/m*l*) on days 7 and 21 gave a sudden high value, whereas the others showed an average value of <5 μg/m*l*. On the other hand, SAA tended to be higher at 39.0 ± 12.4 μg/m*l* in the LS HS-1 group and 39.9 ± 11.8 μg/m*l* in the control group on day 0, and gradually tended to decrease the mean SAA concentrations in both groups.

Health and medical treatments

Table 1 shows the incidence of illness (diarrhea or fever) and the number of treatments during the test period, which was divided according to the second sampling time (Day 7). Totally, four of the five calves required medical treatment in the HK-LS HS-1 supplement group and all five calves in the control group. The number of calves requiring medical treatment for diarrhea with oral antidiarrheal during the experimental periods was significantly lower in the HK-LS HS-1 group (5.2 ± 3.9) than in the control group (10.8 ± 5.5) ($P=0.07$). The mean (± SD) duration of medical treatment per calf was 2.6 ± 1.9 day (HK-LS HS-1 group) and 5.3 ± 2.6 day (control group) for diarrhea during the experimental period. Additionally, the cost of medical treatments significantly tended to be lower in the HK-LS HS-1 group (3,619.6 yen) ($P=0.07$) than in the control group (7,234.2 yen).
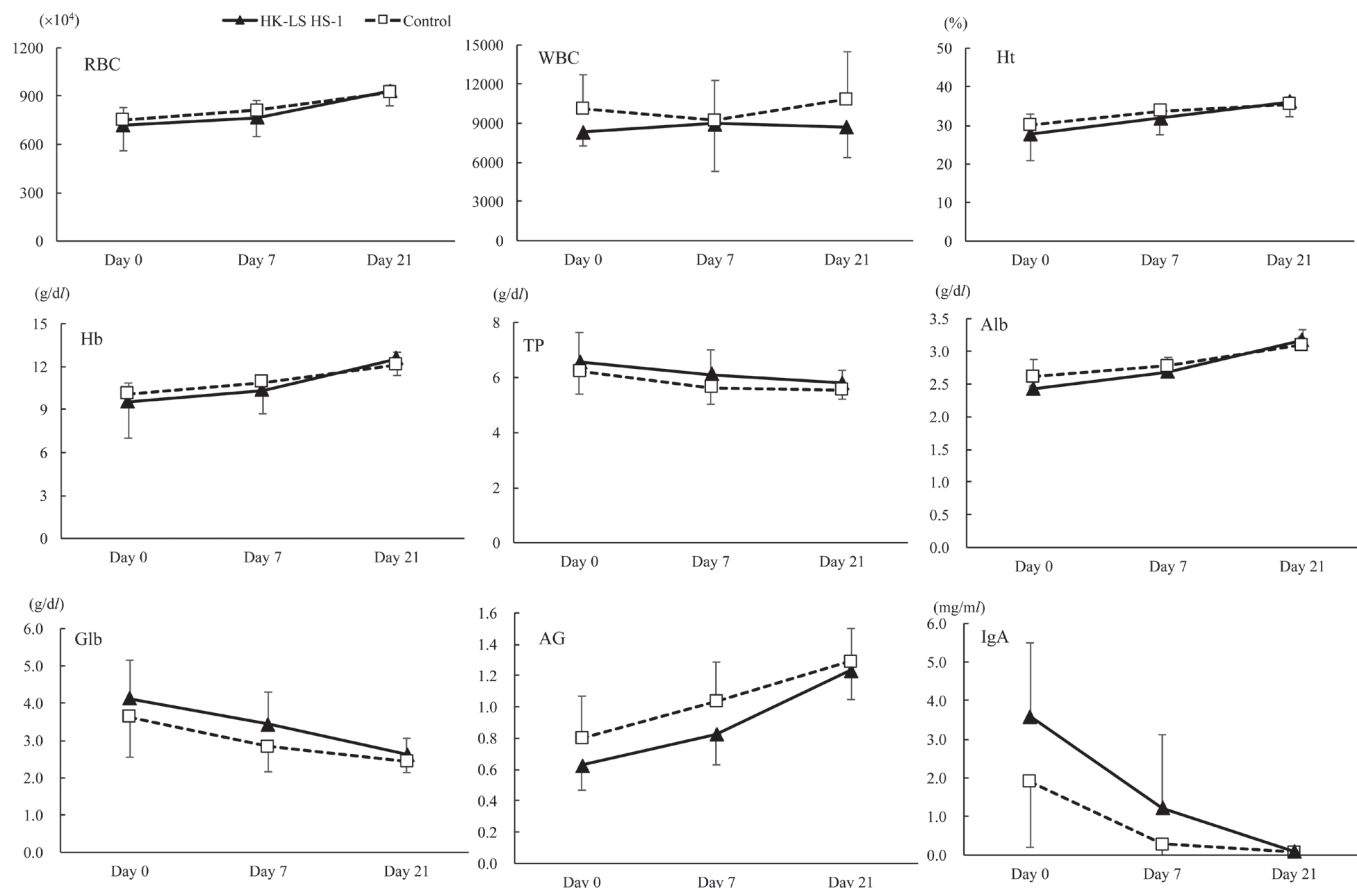
Fig. 1. Effects of heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) supplementation on periodic changes in hematology and serum total protein (mean ± SD) of calves.

Fecal coliform, LAB, and Bifidobacterium counts in fecal samples

The bacteria count (CFU/g) in feces from the 2 groups on days 0, 7 and 21 is shown in Fig. 4. The number of *E. coli* colonies tended to be smaller on day 21 in the control group than in the HK-LS HS-1 group (P=0.06). Although the number of Bifidobacterium colonies showed no difference between treatment groups, that of LAB colonies was significantly increased (P<0.05) in the HK-LS HS-1 group than in the control group on day 21.

DISCUSSION

The beneficial effects of probiotics include preventing the growth of pathogenic bacteria, increasing digestive capacity, lowering the pH of the intestinal tract, and improving mucosal immunity to improve production and health of animals have been widely studied in livestock animals [16, 38]. Additionally, because the live *Lactobacillus* are difficult to preserve and the effect is not constant, attempts have been made to produce "heat-killed" *Lactobacillus*. Several effects of the oral administration of heat-killed LAB as a supplement, such as induction of IL-12 which leads to a T helper 1 type immune response, suppression of IgE production against naturally fed food allergy, and improvement in the health-related quality of life, have also been reported previously in mice [12, 15], humans [3, 5, 9, 30] and farm animals such as pig [3], chicken [14, 28], and fishery [6, 37]. Additionally, regarding HK-LS HS-1 applications for farm animals, previous report revealed that daily gain in body weight and feed conversion were improved after supplementation for pigs [31]. Khonyoung and Yamauchi [14] also examined the effects of oral HK-LS HS-1 supplementation on the growth performance of broiler chickens and found that body weight and feed efficiency increased in the HK-LS HS-1 group than without supplementation, which might be due to the morphological change in the hypertrophied intestinal absorptive epithelial cells on the villus apical surface. However, information on the application of "heat killed" LAB for cattle is still not available. Thus, the present study was conducted to confirm the effects of the daily oral administration of HK-LS HS-1 on the incidence of diseases and fecal counts of both LAB and coliforms in the early stage of newborn calves. Results of our study showed the effects of HK-LS HS-1 supplementation for the first time in calves. We found a significant increase in LAB CFU/g at each successive sampling point and a low number of calves not requiring medical treatment for diarrhea during the HK-LS HS-1 administration period in the treatment group were found. Based on these observations, it was therefore suggested that the beneficial effects of HK-LS HS-1 reflected by their fecal conditions, particularly on the digestive health of newborn calves.
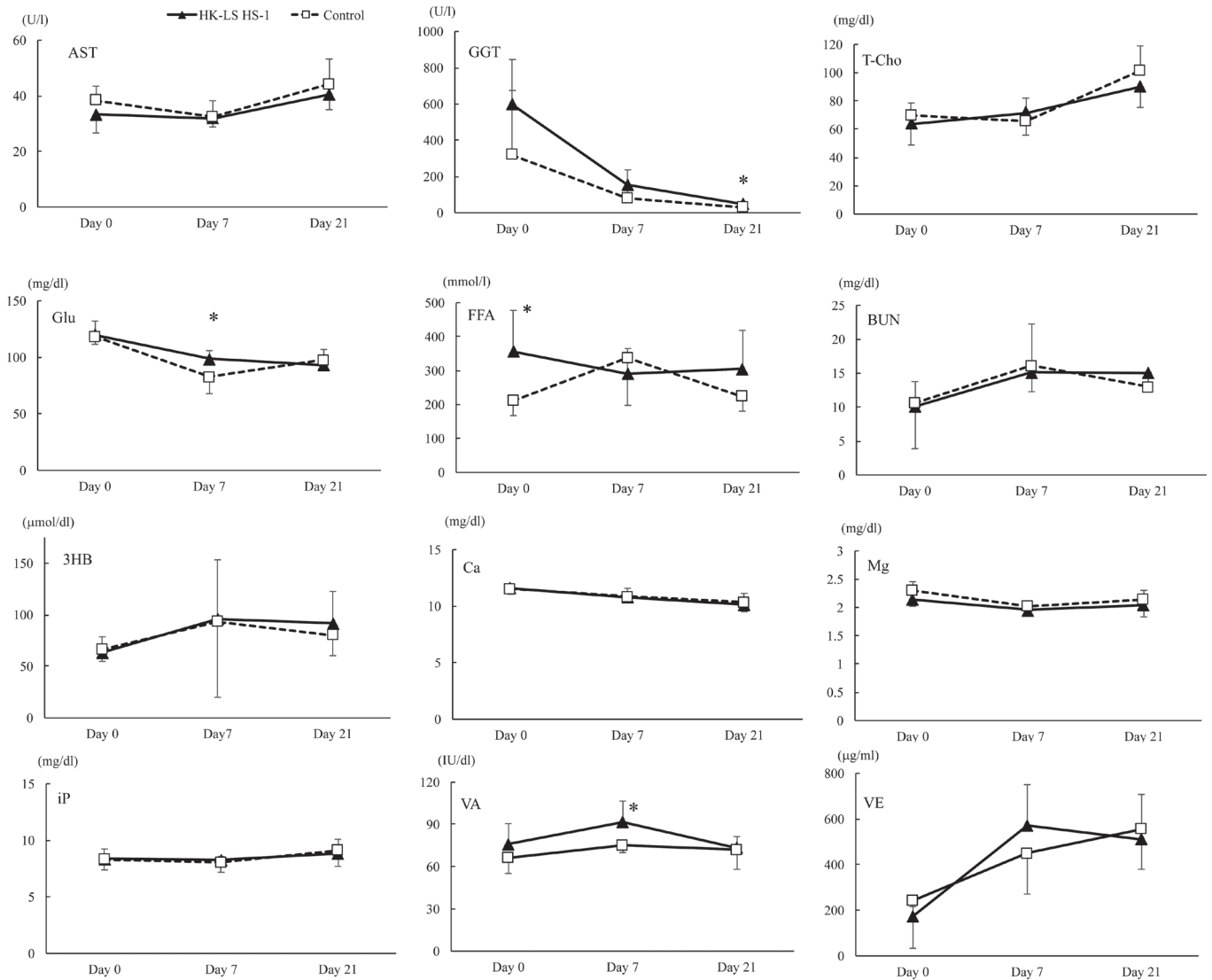
Fig. 2. Periodic alterations in serum biochemical parameters (mean ± SD) of calf supplemented with or without heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) (significant difference between the supplement and control groups **P*<0.05).
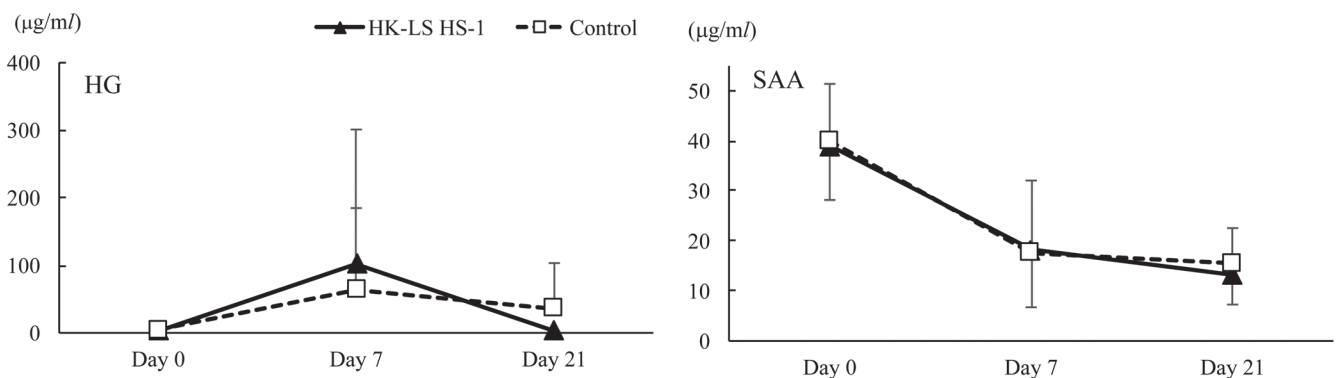

Fig. 3. Periodic alterations in serum haptoglobin (HG) and serum amyloid A (SAA) (mean ± SD) with heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) and control groups.

Table 1. Efficacy of heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) supplementation on calf number requiring medical treatment and frequencies of medical administrations

	HK-LS HS-1 (n=5)		Control (n=5)	
	Day 0–Day 7	Day 8–Day 21	Day 0–Day 7	Day 8–Day 21
No. of calf with treatment	2	4	2	4
Total	4		5	
Mean No. of treatments	0.6 ± 0.8	4.6 ± 3.7	1.8 ± 3.1	9.0 ± 7.2
Oral antidiarrheal	2	20	6	43
Antibiotics injection	1	3	3	1
Total (Day 0–Day 21)	5.2 ± 3.9[a]		10.6 ± 5.3[a]	
Cost of treatment (Yen)	3,619.6 ± 2,616.1[b]		7,234.2 ± 3,577.2[b]	

a) Significant difference of tendency between the HK-LS HS-1 supplementation group and control group (P=0.07).
b) Significant difference of tendency between the HK-LS HS-1 supplementation group and control group (P=0.07).

In the present study, measurements of hematology and blood chemistry were conducted to monitor the states of nutritional intake, immunoglobulins, and some harmful (side) effects of HK-LS HS-1 supplemented calves, as compared with the control calves. It was indicated that serum TP greater 5.5 g/dl of calves between 24 hr and 7 days of age is considered an accurate indicator of the serum IgG concentration of the animal; in case of Ht is also measured as a proxy for hydration status [10]. In the present study, the TP concentrations at Day 0 (starting day of the experiment of each calf) were more than 6.0 g/dl; the concentrations of Alb, Glb, and the A/G ratio were not significantly different between the two groups during the experimental period. In the colostrum, Alb functions as a transport protein; it is absorbed through the intestine of calves, and the concentrations of Alb and TP reflect the immunity of calves and can be used diagnostically [27]. Additionally, serum IgA concentrations, which also reflect the colostrum intake after birth, were not different between the two groups, with both having similar concentrations and decreasing patterns, as previously report [19]. Therefore, it was assumed that the immunity of the examined calves in the two groups were at the same levels as in the present study. In the present study, metabolic evaluation revealed significant differences in some serum biochemical parameters between the calves receiving HK-LS HS-1 and those not within the normal reference ranges, for example, in levels of FFA on day 0, Glu and vitamin A on day 7, and GGT on day 21, although the reasons for these significant differences are unknown. The differences might reflect the milk intake status between birth and day 0 (the day of start of the experiment) in FFA; the health status between day 0 and day 7 of the calves as shown in Table 1 (although the number of treatments was not significantly different). The GGT concentrations of both groups decreased in a time-dependent manner, and a significant difference was observed between the groups on day 21 sampling. As previously indicated [25, 35, 39], the GGT concentration of newborn calves that have fed on the colostrum is usually extremely high (300 <U/l) and decreases in a time-dependent manner. In the present study, the mean first sampling day (Day 0) after calving was 5.8 days in the HK-LS HS-1 group and 7.6 days in the control group, in which the GGT concentrations were more than 300 U/l. Therefore, we assume that the GGT concentrations on Day 0 from both groups also reflect a sufficient intake of colostrum after birth, and these differences might reflect the different day interval from birth to start of the experiment of the calves or the health and nutritional status of the calves during the experimental period.

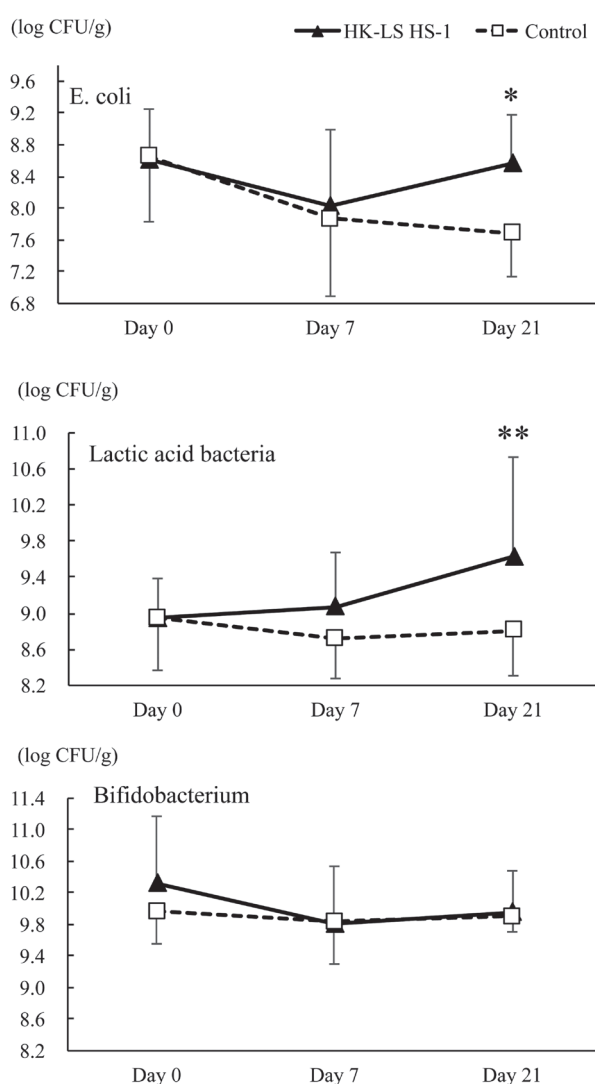APPs are secreted during the acute-phase response, as part

Fig. 4. Population of fecal aerobic bacteria colony (*Escherichia coli*) and anaerobic bacteria colonies (lactic acid bacteria, bifidobacteria) (mean ± SD) in the heat-killed *Lactobacillus sakei* HS-1 (HK-LS HS-1) group and control group (*P=0.06, **P<0.05).

of the innate immune response to different stimuli such as infection and inflammation [7, 20]. Haptoglobin and SAA are the most prominent APPs in cows [4], and HG is a major APP in ruminants in which the serum HG concentration of healthy cattle is less than 20 mg/*l* but can increase to 2 g/*l* within 2 days of infection [7]. Based on previous reports regarding HG, the 2 calves that showed high HG concentrations (more than 500 μg/m*l*) were excluded from the experiment. Interestingly, the SAA concentrations of the excluded calves were also extremely higher (151 and 175 μg/m*l*) than the other examined 10 healthy calves that showed less than 20 mg/*l* in HG but higher SAA concentrations in both groups (HK-LS HS-1 group, 39.0 μg/m*l*; control group, 39.9 μg/m*l*) on day 0. In cattle, the SAA3 isoform has been detected in high concentrations in the colostrum [4, 24]; thus, the high levels of SAA in the blood of calves may be reasonable after ingesting the colostrum, as observed in the 2 excluded calves that showed extremely higher concentrations. Daily HK-LS HS-1 administration for 21 days after calving had etiotropic effects on the animal's health, particularly diarrhea in the present study, and significantly reduced the number of calves diagnosed with and treated for diarrhea. These results imply that the enteric inflammation is not as severe in the treatment group (also as shown in APP results) compared with the control group that received medical treatments. Therefore, the SAA may also be a possible marker for acute inflammation especially for newborn JB calves. Further clinical research is necessary to obscure the correlation of both HG and SAA in newborn calves.

Recently, the first week of life has been reported to be an important period for the intestinal colonization of calves by LAB spp. [34]. Moreover, the administration of LAB spp. and Bifidobacterium to newborn calves during the first week of life increases weight gain and feed conversion ratio, and decreases diarrhea incidences [1, 16]. These effects are most pronounced in pre-weaned calves, suggesting that probiotic supplements are more effective when the gut microbiota is being established and less effective when the microbiome has stabilized [1, 16]. Our hypothesis was that supplementation of HK-LS HS-1 at age 7 days will enable LAB to establish a community in the intestinal tract and increase in numbers. Although the number of *E. coli* colonies tended to be smaller in the control group than in the HK-LS HS-1 group on day 21, possibly due to more frequent administrations of antidiarrheal in the control group as shown in Table 1, our results indicated a significant increase in log CFU/g of LAB in the feces collected on Day 21 from HK-LS HS-1 group. Therefore, it was suggested from the results that the HK-LS HS-1 supplementation to calves from approximately at age 7 days would enable LAB to establish a community in the intestinal tract and increase in concentration similar to live probiotics. Previously, the concept of "biogenics" was defined as "food ingredients which beneficially affect the host by directly immunostimulating or suppressing mutagenesis, tumorigenesis, peroxidation, hypercholesterolemia, or intestinal putrefaction" and suggested the administration of non-viable probiotic bacteria to obtain some "probiotic" effects [18, 21]. It was also proposed a concept "paraprobiotic" defined as "non-viable microbial cells (intact or broken) or crude cell extracts, which, when administered in adequate amounts, confer a benefit on the human or animal consumer" [32]. On the other hand, prebiotics are defined as "non-digestible food ingredients that, when consumed in sufficient amounts, selectively stimulate the growth and/or activity of one or a limited number of microbes in the gut" [38]. Our findings suggest that HK-LS HS-1 may have etiotropic effects (based on the clinical observations) on the health of calves with affecting the number of intestinal LABs in the early stage of pre-weaning. Hence, HK-LS HS-1 is also expected to function as a biogenic/paraprobiotic in JB calves. Further research is necessary to clarify the functional mechanisms of HK-LS HS-1 for calves.

In conclusion, the present study revealed the potential benefit of HK-LS HS-1 to improve the intestinal LAB, improving the etiotropic effects of the calf, and reducing the number of medical treatments compared with control calves. Further research is required to elucidate the mechanism of action of HK-LS HS-1 as a biogenic/paraprobiotic that improves immunological functions to newborn calves, which may include a change in the number of fecal anaerobic bacteria such as *Lactobacilli*.

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