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Physiological Responses of Japanese Black Calves to Supplementation with Sodium Butyrate in Milk Replacer

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Abstract

Genetic factors are important for meat quality in cattle and gene expression may be determined by epigenetic control. This may involve Histone Deacetylases (HDACs), which suppress or enhance gene expression, and beta-hydroxybutyric acid (BHBA, produced from butyric acid), which inhibits HDAC activity and increases gene expression. This study aimed to determine how supplementing Japanese Black calves with sodium butyrate in milk replacer affects the relationship between plasma BHBA, HDAC activity in tissues, and blood metabolites. Eight Japanese Black female calves were randomly assigned to two groups of four animals each (control group, and supplementation with sodium butyrate group; NaB). Calves in both groups were fed milk replacer containing 26% Crude Protein (CP), 25.5% Crude Fat (CF), and 116% Total Digestible Nutrients (TDNs), and all calves received calf starter and hay *ad libitum*. Sodium butyrate was administered to the calves in the NaB group at daily doses of 3 g (from 3 to 30 days of age), 5 g (from 31 to 60 days of age), and 7 g (from 61 to 90 days of age). The supplementation of Japanese Black calves with sodium butyrate in milk replacer decreased plasma concentrations of BHBA.

Keywords: Histone Deacetylases (HDACs); Japanese Black Calves; Plasma Beta-Hydroxybutyric Acid (BHBA); Sodium Butyrate

Introduction

Genetic factors derived from sires and dams are considered important for meat quality and meat production. Japanese Black cattle are known for their great potential to produce marbled beef and, in recent years, studies have focused on providing a high-fat milk replacer to Japanese Black calves during pre-weaning to enhance lipid metabolism [1]. To support the bias in lipid development for marbling to occur, it is now known that changes in gene expression can be regulated by histone acetylation as well as the nucleotide sequences of genes [2]. Gene expression without changes in DNA base sequence or changes in cell phenotype is known as epigenetic control. Epigenetic control is regulated by the nutritional environment in the early growth phase and occurs even after cell division. It is well known that histone acetylation caused by histone deacetylases (HDACs) inhibitors is associated with increased or suppressed gene expression. Butyric acid suppresses the activity of HDACs involved in the epigenetic process [3]. Beta-hydroxybutyric Acid (BHBA) also inhibits HDAC activity and increases gene expression by causing histone hyperacetylation in the promoter region [4]. BHBA is produced through the decomposition of butyric acid in the liver, and the serum BHBA concentration is increased by daily butyrate intake in milk-fed Holstein calves [5]. Unlike other breeds, it is possible that Japanese Black calves may metabolize fatty acids, including butyrate, differently, to produce marbled fat. However, there is a lack of information about the effect of butyrate intake on BHBA concentration and HDAC activity in pre-weaned Japanese Black cattle. Therefore, in this study, we sought to assess how butyrate intake affects the relationship among BHBA, HDAC activity, and blood metabolites in Japanese Black calves. Based on previous findings, we hypothesized that inhibition of HDAC activity through butyrate supplementation involves BHBA, which is increased by

the decomposition of butyric acid.

Materials and Methods

Experimental Animals

All experimental procedures, including animal care and handling, were performed in accordance with guidelines from the Committee for Animal Welfare of Kyushu University. Eight, three-day old, Japanese Black female calves were randomly assigned to two groups of four animals each (control group, and supplementation with sodium butylate group; NaB). Calves in both groups were fed milk replacer containing 26% Crude Protein (CP), 25.5% Crude Fat (CF), and 116% Total Digestible Nutrients (TDNs). The quantity of milk replacer and sodium butyrate were determined based on previous studies [6,7]. Calves in both groups were provided with 600 g/day of milk replacer from 4 to 90 days of age. Milk replacer was offered to the calves at 08:00, 11:00, 14:00, and 17:00 each day. Sodium butyrate was given to the calves in the supplementation with sodium butyrate group at daily doses of 3 g (from 3 to 30 days of age), 5 g (from 31 to 60 days of age) and 7 g (from 61 to 90 days of age). The calves in both groups were fed calf starter (TDN, 72%; CP, 18%; ether extract, 2%) and hay (CP, 13.4%; CF, 3.6%; TDN, 59.3%) *ad libitum* from 4 to 90 days of age. We measured body weight and collected tissue and blood samples at 90 days of age.

Collection and Analysis of Tissue and Blood Samples

When calves were 90 days of age, blood samples were collected from the jugular vein into heparinized tubes at 10:00. After blood samples were collected, calves were anesthetized with an intramuscular injection of 0.3 mg/100 kg general anesthesia (Intervet, Osaka, Japan) and 5 mL of a local anesthetic (AstraZeneca KK, Osaka, Japan). Liver tissues (4 cm) were obtained from between 11th and 12th rib, and longissimus thoracis tissues (8 cm) were obtained from between the 12th and 13th thoracic vertebrae using needle biopsy (Baxter, Valencia, CA, USA). The liver and muscle samples were immediately frozen in liquid nitrogen and stored at -80˚C until analysis. Nuclear DNA was extracted from liver and muscle tissues using an EpiQuik Nuclear Extraction Kit I (Epigentek, Brooklyn, NY). Total HDACs activities were measured using the EpiQuik HDAC Activity/Inhibition Assay Kit (Epigentek). All blood samples were centrifuged at 2330 × *g* for 30 min at 4˚C, and plasma was stored at -80˚C until analysis. Concentrations of BHBA, insulin, and Non-Esterified Fatty Acid (NEFA) were measured using BHBA enzyme-linked immunosorbent assay kits (Cusabio Biotech, Wuhan, China), bovine insulin enzyme-linked immunosorbent assay kits (Mercodia, Uppsala, Sweden) and the acyl-CoA synthetase-acyl-CoA oxidase enzymatic method (FFAC; Wako Pure Chemical, Japan), respectively, according to manufacturers' instructions.

Statistical Analysis

The data on body weight, milk replacer intake, CP intake, CF intake, total HDAC activity, and plasma concentrations of BHBA, insulin and NEFA are expressed as mean ± standard error. Statistical significance was tested with the Mann-Whitney test. Stat View 5 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. Treatment differences were considered significant at $P \leq 0.05$.

Results and Discussion

Supplementing Japanese Black calves with sodium butyrate in milk replacer had no effect on feed intake or body weight (Table 1). The effects of sodium butyrate on feed intake and body weight in calves were described in a previous study [8]. Gorka et al. [8] demonstrated that supplementation of starter and milk replacer with sodium butyrate increased feed intake and body weight in calves. Furthermore, it has been suggested that supplementation of solid feed with butyric acid results in more effective feeding and improved rumen development [8,9]. Therefore, supplementation of liquids, such as milk replacer, with sodium butyrate may not result in increased feed intake and body weight in calves.

Item	Group		P-value
	Control	NaB	
Body weight (kg)			
Initial	35.38 ± 5.28	36.38 ± 4.72	NS
90 days	100.98 ± 6.29	103.14 ± 2.72	NS
Mean body weight gain (kg)	0.73 ± 0.07	0.74 ± 0.08	NS
Feed intake of DM (kg)			
Milk replacer	336.74 ± 11.26	343.10 ± 6.57	
CP	13.13 ± 0.44	13.38 ± 0.26	NS
CF	12.88 ± 0.43	13.12 ± 0.25	NS
TDN	58.59 ± 1.96	59.70 ± 1.14	NS

Starter	57.40 ± 9.36	70.31 ± 20.59	
CP	10.33 ± 1.68	12.66 ± 3.71	NS
CF	1.15 ± 0.19	1.41 ± 0.41	NS
TDN	42.28 ± 6.92	52.03 ± 15.24	NS
Hay	9.20 ± 3.84	16.74 ± 5.58	
CP	0.89 ± 0.37	0.91 ± 0.30	NS
CF	0.23 ± 0.10	0.24 ± 0.08	NS
TDN	5.75 ± 2.40	5.85 ± 1.95	NS

Table 1: Body weight, and consumption of milk replacer, calf starter and hay intake-crude protein, crude fat, and total digestible nutrients-from the feed in calves in the control and supplementation sodium butyrate (NaB) groups.

We found that plasma concentrations of BHBA were significantly lower in the Japanese Black calves supplemented with sodium butyrate in milk replacer than in Japanese Black calves in the control group (Table 2). Although butyric acid and BHBA are reported to suppress HDAC activity in mammals [3,4], in our study, lower BHBA concentrations in the NaB group resulted in higher total HDAC activity in the liver (approximately 1.5 times higher) and the longissimus thoracis muscle (approximately 2 times higher), although these results were not statistically significant (Table 2). As such, sodium butyrate may have little effect on total HDAC activity, and BHBA may affect HDAC activity in Japanese Black calves.

Item	Group		P-value
	Control	NaB	
HDAC activity (OD/min/mg)			
Longissimus thoracis muscle	1.20 ± 0.39	2.27 ± 1.12	NS
Liver	3.57 ±.1.01	4.97 ± 1.37	NS
Plasma concentrations			
BHBA (nmol/ml)	218.59 ± 34.41	165.90 ± 7.19	0.04
Insulin (ng/ml)	0.15 ± 0.06	0.82 ± 0.90	NS
NEFA (mEq/ml)	0.10 ± 0.02	0.09 ± 0.02	NS

Table 2: Total HDAC activity (longissimus thoracis muscle and liver), and plasma beta-hydroxybutyric acid (BHBA), insulin, and non-esterified fatty acid (NEFA) in calves in the control and NaB groups at 90 days of age.

Supplementing calves with coated calcium butyrate increased the concentrations of serum BHBA in calves and increased the calves' starter intake [10]. Conversely, starter intake did not change when supplementing calves with sodium butyric acid in milk replacer. The results in that experiment indicated that the dietary levels of butyrate do not result in changes in serum BHBA concentrations in calves [11] but do suggest that an increase in serum BHBA concentrations due to butyrate supplements contributes to an increase in starter intake in calves. Interestingly, Japanese Black calves had low plasma concentrations of BHBA, despite the fact that starter intake did not change due to supplementation with sodium butyrate. The mechanism underlying the decreased plasma BHBA concentrations in Japanese Black calves supplemented with sodium butyrate is unclear. It is thought that metabolism and/or utilization of BHBA in the liver was accelerated. Some reports support the hypothesis that ingested butyric acid disappears from the gastrointestinal and is metabolized in the liver [7]. Vazquez-Anon et al. [12] suggested that metabolic changes in gluconeogenesis in the liver decreased plasma BHBA concentrations. Nutrition during the pre-weaning stage has been found to induce metabolic change in Japanese Black calves [1]. Thus, the reduced plasma concentrations of BHBA in Japanese Black calves supplemented with sodium butyrate may be due to metabolic changes in the liver.

Plasma concentrations of insulin and NEFA were not affected by supplementation with sodium butyrate (Table 2). Based on previous studies, blood samples were taken 120 min after calves were fed the milk replacer, targeting the time that we expected BHBA and insulin concentrations to increase [10]. Kato et al. [7] demonstrated that, 75 min after feeding calves milk replacer with or without sodium butyrate, plasma insulin levels were lower in calves supplemented with sodium butyrate than in calves not supplemented with sodium butyrate, although there were no differences at other sampling points between 0 min to 120 min, and concentrations of NEFA did not differ at any of the sampling points. In order to examine the changes in plasma concentrations of insulin and NEFA in detail, the number of blood sampling points should be increased.

The current findings show that supplementing Japanese Black calves with sodium butyric acid decreased plasma BHBA concentrations. Further studies are needed to determine the mechanisms by which BHBA secretion is altered in ruminants supplemented with sodium butyrate in milk replacer, and whether such changes affect HDAC activity and gene expression. The data from this study provides evidence that different species or breeds may have different responses to sodium butyrate.

Conclusions

The supplementing Japanese Black calves with sodium butyrate did not affect their weight, HDAC activity, plasma concentrations of insulin or non-esterified fatty acids, but did decrease plasma

BHBA concentrations.

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