Effects of lactose content in milk replacer on apparent digestibility, growth, liver mRNA expression, and blood parameters related to metabolism of dairy calves

R. Fukumori,1 M. Hirose,1 I. Norimura,2 T. Nakayama,1 K. Shimada,35 H. Mineo,4 M. A. Steele,5 S. Gondaira,1 H. Higuchi,1 K. Chisato,1 S. Oikawa,1 and K. Izumi2*

Abstract: Even with the same energy supply, differences in energy sources may affect calf growth and metabolism. In this study, we evaluated the effects of feeding 3 different milk replacers (MRs) with different lactose supplies under isoenergetic conditions on calf digestibility, growth, and metabolism-related markers. Fifteen Holstein bull calves were randomly assigned to one of 3 MR-feeding treatments: low-lactose (L:38%), medium-lactose (M:41%), or high-lactose (H:46%). After birth, calves were provided colostrum and treatment MRs were fed from 1 d of age and gradually increased to a maximum feeding rate at 20 d of age (L:1.16 kg/d, M:1.21 kg/d, H:1.26 kg/d DM) without feeding solid feeds during the experimental period. Blood samples were temporarily collected weekly to assess blood concentrations of metabolites and hormones. From 30 to 36 d of age, the calves were euthanized and liver samples were collected to determine growth-related mRNA expression. L calves showed a greater body length than H calves and the highest GHR mRNA expression. Plasma concentrations of total cholesterol, urea nitrogen, total protein, albumin, insulin, and insulin-growth factor-1 were not different, but plasma concentrations of triglycerides were higher in order H, M, and L. These results showed that the difference in lactose content in the MR affected calf metabolism, and the L-MR was suggested to be more likely to enhance growth into the peripheral tissues.

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Recently, researchers and the dairy industry have been increasingly pressed to improve pre-ruminant calf nutritional management because higher pre-weaning growth is associated with calf health and future performance (Soberon et al., 2012). The energy sources of milk replacer (MR) are lactose and fat, and their ratios vary based with the product, which may affect calf growth and metabolism. In a previous study where high-fat MR and high-lactose MR were fed ad libitum (Berends et al., 2020), high-lactose MR showed greater ME intake but no impact on developmental outcomes was observed. However, no conclusions have been reached regarding the optimal balance of fat and lactose as energy sources in terms of metabolic traits as well as body weight gain. Lactose, a disaccharide synthesized from glucose and galactose, accounts for more than 80% of the sugar fraction in milk (Urashima and Saito, 2005) and provides the same amount of nutrients to the intestinal tract at half the osmolality of monosaccharides, reducing the risk of diarrhea due to increased osmolality. Galactose has been reported to contribute to neonatal and early life brain development (Cederlund et al., 2013), and various benefits are expected for calf health and growth. In addition, ruminants have lower activity of ATP-citrate lyase and NADP-malate dehydrogenase (Hanson and Ballard, 1967), indicating that extra glucose might not be easily converted to body fat, supposing high lactose MR may prevent fat accumulation compared with high fat MR.

Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) play important roles in body growth and mammary gland development (Schaff et al., 2016). Blood circulating IGF-1 is mainly produced in the liver and its synthesis depends on hepatic GH receptor (GHR) abundance (Butler et al., 2003). The mRNA expression of these factors has been reported to be associated with energy and protein intake (Haisan et al., 2018, Firmenich et al., 2020), but the effect of energy sources, such as lactose content in the MR, on the GH-IGF-1 axis has not been determined. In hepatocyte studies, stimulation with glucose or insulin increases GHR expression (Dehkhoda et al., 2018). It was therefore hypothesized that for the same energy content, high lactose MR would be more effective for development via increased GH and IGF-1 synthesis. This study aimed to determine the effects of replacement of lactose by fat in MR under conditions of equal ME supply on growth performance, blood metabolites and hormones, and growth-related mRNA expression in the liver of dairy calves.

The calves used in this study were housed at the Rakuno Gakuen University Large Animal Experiment Station (Ebetsu, Hokkaido, Japan) and all procedures were approved by the Animal Experiment Committee of Rakuno Gakuen University (approval # VH21C6).

1Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan 069-8501, 2Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu, Japan 069-8501, 3The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Shinjuku, Tokyo, Japan 969-0223, 4Department of Health and Nutrition, Hokkaido Bunkyo University, Eniwa, Japan 060-1449, 5Department of Animal and Bioscience, University of Guelph, Guelph, Ontario, Canada, N1G2W1. *Corresponding Author: izmken@rakuno.ac.jp. © 2024, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association*. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). Received December 03, 2023. Accepted April 10, 2024.

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-24. Nonstandard abbreviations are available in the Notes.
Fifteen Holstein bull calves born on 2 farms (4 and 11 calves were provided, respectively) were used in this study and assigned to one of the 3 dietary treatments with consideration given to avoid farm and dam’s parity bias. The treatments consisted of MR lactose content (L: low lactose MR, M: medium lactose MR, and H: high lactose MR) for 30-d periods. Calves were fed colostrum within 3 h of birth and then transferred to the experimental location within 1 d of birth. All calves had serum immunoglobulin G concentrations, measured using a bovine ELISA kit (Betyl laboratories, TN, USA), above 10 mg/mL 24 h after ingestion of colostrum, with no differences between treatments (L: 24.5 mg/mL, M: 21.1 mg/mL, and H: 21.5 mg/mL, $P = 0.84$). Calves were kept in individual calf pens (2.42 m x 1 m, 2.42 m²/head) and fed the MR treatment 3 times a day at 0700h, 1300h, and 1900h. Calves were allowed to drink water ad libitum; however, solid feed was not provided during the experiment. The lactose contents were 38%, 41%, and 46%, the CP contents were 25.2%, 25.4%, and 24.4%, and the fat contents were 17.8%, 15.8%, and 13.6% for L, M, and H, respectively. The calves were fed MR at 0.6 kg/d in L, 0.625 kg/d in M, 0.65 kg/d in H (1 to 9 d of age), at 0.9 kg/d in L, 0.938 kg/d in M, 0.975 kg/d in H (10 to 19 d of age), and at 1.2 kg/d in L, 1.25 kg/d in M, 1.3 kg/d in H (20 d of age to end of experiment) on powder basis. All calves were not found to have leftover drink. Amounts of MR were adjusted to achieve equivalent ME, and the dilution rate of each MR was adjusted to 451 mOsm/kg. The concentrations of the prepared MR were 16.7%, 15.6% and 14.9% (wt/wt) in L, M, and H, respectively. Blood samples were collected weekly (at 1, 7, 14, 21, and 28 d of age) before morning feeding to determine the plasma concentrations of total cholesterol (T-Chol), albumin, total protein (TP), urea nitrogen (UN), insulin, and insulin-like growth factor-1 (IGF-1). Blood samples (5 mL) were collected in heparinized evacuated tubes (Terumo, Tokyo, Japan). The tubes were then immediately placed on ice. The tubes were then centrifuged at 1,950 × g at 4°C for 30 min at 4°C. The harvested plasma was stored at −30°C until analysis.

At 30–36 d of age, the calves were euthanized and liver samples were collected. For euthanasia, xylazine (10 μL/kg BW) was used as a sedative, isosol (20 mg/kg BW) as an anesthetic and suxamethonium (0.2 mg/kg BW) as muscle relaxant, all of which were intravenously injected. After confirmation of cardiac arrest by auscultation, the liver was excised, and samples were cut into 5 mm square approximately 1 cm from the surface of the liver in the center of the right lobe. The samples were promptly frozen in liquid nitrogen and stored at −80°C until mRNA expression analysis. The MR samples were sent to the Japan Food Research Laboratory (Chitose, Japan) for high-performance liquid chromatography and analyzed for lactose content, and DM, CP, and ether extracts were analyzed (AOAC International, 2000).

All analyses of plasma metabolite concentrations were performed by Sapporo Clinical Laboratory Co. (Kushiro, Hokkaido, Japan) using an automatic analyzer (OLYMPUS AU680, Olympus Corporation, Tokyo, Japan). Sicari Kit GLU (Kanto Chemical Co., Ltd., Tokyo, Japan) was used for glucose, Quick Auto Neo BUN (Shino-Test Cooperation, Tokyo, Japan) for BUN, Albumin HRII (Fujifilm Wako Pure Chemicals Corporation, Osaka, Japan) for Albumin, Exdia XL Eiken TP (Eiken Chemical Co., Ltd., Tokyo, Japan) for TP, Deteminerr C TG S (Kyowa Medex, Tokyo, Japan) for TG and Deteminerr C-TC S (Kyowa Medex) for T-Chol were used for measurements. Plasma concentrations of insulin and IGF-1 were measured using a time-resolved fluorescence immunoassay (competitive solid-phase immunoassay). Insulin was measured as described by Masuda et al. (2020) and IGF-1, as described by Laarman et al. (2012), respectively. Total RNA extraction, quantification, and real-time PCR were conducted as described by Nishi et al. (2020). Total RNA extraction was conducted using TRI reagent (Sigma-Aldrich, St. Louis, MO, USA), and DNase digestion was performed using TURBO DNA-free DNase (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was quantified via spectrophotometry using a BioSpec-nano (Shimadzu, Kyoto, Japan). For each reaction, a parallel negative control reaction was performed in the absence of reverse transcriptase (Toyobo, Osaka, Japan) using 1 μg RNA and analyzed by the β-actin band using PCR and 1.5% agarose gel stained with ethidium bromide, visualized on a UV transilluminator. We used the melting curve analysis to evaluate each primer pair for specificity to ascertain that only one product was amplified. The reaction was performed using a Thunderbird SYBR qPCR mix (Toyobo) and a CFX Connect TM Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA), as per the manufacturer’s instructions. Thermal cycling consisted of initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Growth hormone receptor (GHR), GHR1A, and IGF-1 as growth-related markers and GAPDH as the candidate or internal control were measured. The sequences of the primers used is same as those described in Witte et al. (2019) for GHR, GHR1A, and IGF-1, and GAPDH for Nishi et al. (2020).

All data are presented as LSM ± SEM. For mRNA expression in the liver, the mean of the 2−ΔΔCt values of 5 calves in the L group was set to 1, after which the values for individual were represented as relative values. All data were analyzed using the fit model procedure of JMP (version 13.2.1; SAS Institute Inc., Cary, NC, USA). The present study has sufficient power ($α = 0.05$, $β = 0.8$) to detect a 15% difference in blood parameters and 10% in mRNA expression. For variables not measured over time, such as growth performance and mRNA expression, the model included fixed effects of treatment and farm of origin, and random effects of the calf. For variables measured over time, such as plasma parameters, the model included the fixed effects of treatment, farm of origin, collection d of age as a repeated measure, their interaction, and the random effect of the calf. Farm had no effect on all variables ($P > 0.247$). Differences were considered significant at $P < 0.05$, and a tendency was declared at $P < 0.10$.

BW, body size, and growth during the 4 wk period are presented in Table 1. BW and ADG were not affected by the dietary treatments ($P = 0.333$ and $P = 0.678$, respectively). Wither height at 27 d of age in L calves was higher than H calves (L: 90.1 cm vs. H: 87.0 cm, $P < 0.05$), but this was also the case at the start (2 d of age; L: 84.2 cm vs. H: 80.9 cm, $P < 0.05$) and there was no difference in growth range. Body length did not differ at the start (L: 73.1 cm vs. H: 70.1 cm, $P = 0.216$), but that in L calves was longer than H calves (L: 69.8 cm vs. H: 74.8 cm, $P < 0.05$). In the present study, lactose content did not influence ADG (L: 0.719 kg/d, M: 0.693 kg/d, and H: 0.758 kg/d, respectively, $P = 0.678$), which is consistent with the results of previous studies (Berends et al., 2020, Echeverry-Munera et al., 2021, Yohe et al., 2020). Body length of H calves was smaller than that of L calves. In contrast, a
companion study by Fukumori et al. (submitted) showed that the gastrointestinal weight to BW ratio tended to increase with increasing lactose feeding. Therefore, it was suggested that high-lactose feeding increases the available nutrient to the gastrointestinal tissues rather than the peripheral tissues.

Weekly changes in the basal plasma concentrations of glucose (A), TG (B), T-Cho (C), UN (D), TP (E), albumin (F), insulin (G), and IGF-1 (H) are shown in Figure 1. Except for insulin, those profiles were changed by weekly: Plasma T-Cho and albumin concentrations increased weekly (Figure 1C, 1F, \( P < 0.001 \)), but plasma UN and TP decreased [Figure 1D (\( P = 0.003 \)), 1E (\( P < 0.001 \))]. Plasma IGF-1 concentrations decreased at 7 d of age and increased again at 14 d of age (Figure 1H, \( P < 0.001 \)). No dietary effects were observed in plasma parameters other than TG. Plasma TG concentrations were affected by dietary treatment (\( P = 0.02 \), Figure 1B) were higher in order H, M, and L (\( P < 0.05 \)). Unexpectedly, plasma glucose concentrations were not different between treatment (\( P = 0.353 \)) and plasma TG concentrations were higher when calves fed higher amount of lactose in MR. The reason for unchanged plasma glucose may be that the H calves had increased glucose consumption in the gastrointestinal tract due to an increased gastrointestinal tract weight ratio, which is the tissue consumes a large amount of glucose (Britton and Krehbiel, 1993). The response in plasma TG concentration to feeding MRs differing in lactose content was not changed and increased again at 14 d of age (Figure 1H, \( P < 0.001 \)). No significant differences were found in IGF-1 and GHR1A gene expression or plasma IGF-1 concentrations among the dietary treatments. The GHR1A, a specific GHR in the liver and its mRNA expression is positively correlated to IGF-1 mRNA expression (Butler et al., 2003, Kim et al., 2004), and in a previous study in young goats, IGF-1 and GHR1A mRNA expression levels were reduced when fed a protein-restricted diet (Firmenich et al., 2020). When MR (20% fat and 30% CP) intake was increased to achieve ADG of 0.5, 0.95, and 1.4 kg/d, calf hepatic GHR1A and IGF-1 mRNA expression and plasma IGF-1 concentration increased with greater nutrient intake (Smith et al., 2002). Since the present study was adjusted MR feeding amount for isoenergetic and iso-protein intake, there was probably no difference in these items. In addition, previous studies in rats have shown that plasma IGF-1 levels are mainly dependent on the protein content of the diet and are less influenced by carbohydrate content (Gehring et al., 2021). This suggests that the increased lactose content in the MR had little effect on IGF-1 levels in the liver and blood.

While previous studies have examined the ratio of lactose to fat in MR-fed solid feeds together (Berends et al., 2020, Echeverry-Munera et al., 2021, Yohe et al., 2021), the present study fed only MR to eliminate other factors. The results showed that high-lactose MR had a slightly negative effect on growth and length development. Taken together with the companion report, it was suggested that high-lactose MR may reduce the nutrient supply to peripheral tissues instead of promoting gastrointestinal development.

### Table 1. Effects of lactose content in MR on growth performance (LSM ± SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L (38% lactose)</th>
<th>M (41% lactose)</th>
<th>H (46% lactose)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>48.7</td>
<td>48.3</td>
<td>44.9</td>
<td>1.89</td>
<td>0.333</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>84.2a</td>
<td>82.4ab</td>
<td>80.9b</td>
<td>0.90</td>
<td>0.074</td>
</tr>
<tr>
<td>Hip height (cm)</td>
<td>86.9</td>
<td>85.9</td>
<td>85.5</td>
<td>1.13</td>
<td>0.070</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>73.1</td>
<td>71.5</td>
<td>70.1</td>
<td>1.15</td>
<td>0.216</td>
</tr>
<tr>
<td>Heart girth (cm)</td>
<td>86.8</td>
<td>87.4</td>
<td>84.0</td>
<td>1.78</td>
<td>0.383</td>
</tr>
<tr>
<td>At 27 d of age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td>67.4</td>
<td>66.3</td>
<td>64.6</td>
<td>1.55</td>
<td>0.473</td>
</tr>
<tr>
<td>Wither height (cm)</td>
<td>90.1a</td>
<td>89.4ab</td>
<td>87.0b</td>
<td>0.85</td>
<td>0.057</td>
</tr>
<tr>
<td>Hip height (cm)</td>
<td>94.0</td>
<td>92.6</td>
<td>91.2</td>
<td>0.98</td>
<td>0.183</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>84.8a</td>
<td>82.8ab</td>
<td>80.8b</td>
<td>1.14</td>
<td>0.083</td>
</tr>
<tr>
<td>Heart girth (cm)</td>
<td>95.8</td>
<td>97.2</td>
<td>93.6</td>
<td>1.40</td>
<td>0.225</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.719</td>
<td>0.693</td>
<td>0.758</td>
<td>0.052</td>
<td>0.678</td>
</tr>
<tr>
<td>Body height growth (cm)</td>
<td>18.7</td>
<td>18.0</td>
<td>19.7</td>
<td>1.34</td>
<td>0.678</td>
</tr>
<tr>
<td>Body length growth (cm)</td>
<td>7.10</td>
<td>6.70</td>
<td>5.68</td>
<td>0.541</td>
<td>0.202</td>
</tr>
<tr>
<td>Heart girth growth (cm)</td>
<td>11.7</td>
<td>11.3</td>
<td>10.7</td>
<td>1.40</td>
<td>0.883</td>
</tr>
</tbody>
</table>

\( ^1 \) L = low-lactose MR (38% lactose), M = medium-lactose MR (41% lactose), H = high-lactose MR (46% lactose).

\( ^a,b \) Means within a row with different superscripts differ significantly (\( P < 0.05 \)).
Figure 1. Least squares means and SEM (n = 5) for weekly changes in plasma concentrations of glucose (A), triglycerol (TG, B), total-cholesterol (T-Cho: C), urea nitrogen (UN, D), total protein (TP, E), albumin (F), insulin (G), and insulin-like growth factor-1 (IGF-1, H) by calves fed milk replacer in different lactose content: low lactose (L:38%), medium lactose (M:41%), and high lactose (H:46%). Plasma TG concentrations were higher in the order H, M, and L (P < 0.05). Means with different superscripts are significantly different among treatments (P < 0.05).
Other studies have reported that a high lactose MR increases starter intake during the pre-weaning period, likely to compensate for energy intake (Berends et al., 2020), which may be beneficial in terms of gastrointestinal tract development for weaning. In the present study, although solid feed intake was not measured, the gut developmental effect of high lactose MR may contribute to higher solid feed intake during the weaning period. In contrast, high lactose been reported to have more therapeutic events than low-fat intake for a few weeks may be avoided from a calf health perspective. The changes in growth factors and blood parameters in this study also support the idea that some fat content is required in the first few weeks of life for body development and health. Further functional evaluation of the fat-to-lactose ratio with respect to metabolism, such as heat production and the immune system, is necessary. In conclusion, high-lactose (46%) MR showed smaller body length lower hepatic GHR mRNA expression than low-lactose (38%) MR, suggesting that too high amount of lactose may be undesirable for peripheral tissue development.

**References**


NOTES

R. Fukumori  https://orcid.org/0000-0001-6047-8709
M. A. Steele  https://orcid.org/0000-0001-6941-6205
S. Gondaira  https://orcid.org/0000-0001-9745-7792
K. Chisato  https://orcid.org/0009-0001-5610-3629
K. Izumi  https://orcid.org/0000-0002-9179-957X

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Conflict of Interest Mr. Kensuke Shimada is an employee of the National Federation of Dairy Co-operative Associations (ZEN-RAKUREN)