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Research article

Glucagon-like peptide 2 (GLP-2) in bovine colostrum and transition milk

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ABSTRACT

Bovine colostrum contains growth factors, cytokines, hormones, and enzymes, which have important roles in stimulating gastrointestinal development of neonatal calves. In the present study, we measured the concentration of glucagon-like peptide 2 (GLP-2), one of the gut-derived peptides secreted from intestinal L-cells, in colostrum and transition milk of Japanese black cattle. All colostrum samples were collected within 24 h after calving (d 0) and transition milk was collected at 24, 48 and 72 h relative to the time at colostrum sampling (d 1, d 2 and d 3, respectively). Concentrations of GLP-2 in colostrum were 5.53 ± 1.07 ng/mL on average (range = 0.94–9.60 ng/mL) and decreased from d 0 to 3 ($P < 0.01$). Furthermore, concentrations of GLP-2 in colostrum and transition milk were quadratically decreased with the elapsed time from parturition until colostrum sampling ($R^2 = 0.48$, $P < 0.01$). Our results show for the first time that GLP-2 is present in bovine colostrum and transition milk and that concentrations decreased with elapsed time from parturition.

1. Introduction

Glucagon-like peptide 2 (GLP-2) is one of the gut-derived peptides co-secreted with GLP-1 from intestinal L-cells in response to nutrient absorption [1, 2]. Treatment with GLP-2 has been shown to stimulate proliferation of intestinal crypt cells, reduce apoptosis and inflammation in intestinal mucosal epithelium, and enhance nutrient absorption and gut integrity after injury in non-ruminants [1, 2, 3, 4] and ruminants [5]. In particular, intravenous administration of GLP-2 was shown to stimulate the development of gut epithelium in dairy cows [6]. Therefore, GLP-2 has become a target of research that addresses improved health and productivity of dairy and beef cattle.

In ruminants, intake of colostrum is critical for the transfer of passive immunity from dam to calf and, in addition, affects metabolism, endocrine function and nutritional state, as reviewed by Guilloteau et al. [7] and Blum and Hammon [8, 9]. As well as immunoglobulin, bovine colostrum contains various bioactive agents such as growth factors, cytokines, hormones and enzymes, which are thought to impart numerous functions to aid the developing calf, among which is development of the gastrointestinal tract (GIT) [10, 11, 12, 13, 14]. Previous research has reported that insulin orally administered to calves was absorbed from the intestine and caused an increase in serum concentrations of insulin [15]. Therefore, other hormones present in colostrum might also be absorbed from the intestine of neonatal calves. We have previously reported that an extended feeding duration of colostrum or a 50:50 mixture of colostrum and whole milk (to mimic transition milk) increased plasma GLP-1 and GLP-2 concentrations in neonatal calves [16, 17]. Therefore, circulating concentrations of GLP-2 in calves may be derived from the dam via ingestion of colostrum and transition milk. If bovine colostrum contains GLP-2, there is a possibility that the stimulatory effects of colostrum on calf's GIT development is partly attributed to an action of colostral GLP-2.

It is not currently known what the concentrations of GLP-2 are in colostrum and transition milk of Japanese black cattle. Therefore, our aim was to collect and measure GLP-2 in colostrum within 24 h after parturition and transition milk over the first 3 d after parturition to establish a basal understanding for use as a reference in future studies.

2. Materials and methods

2.1. Animals and diets

The procedures used in the present study were performed according to the Guidelines for the Animal Experiments by the Faculty of Agriculture in Kyushu University and with the approval of the Kyushu University Laboratory Animal Care and Use Committee. Twelve, pregnant Japanese black cattle cows were used in the present study from 60 d prior to expected parturition date until 3 d after parturition. Throughout the experimental period, all cows were fed a commercial concentrate feed

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(Yamato, Japan Agricultural Cooperatives, Tokyo, Japan) and hay daily at 16:00 h to meet crude protein and total digestible nutrients requirements of breeding cows at late gestation and lactation periods according to Japanese Feeding Standard for Beef cattle [18]. The chemical composition of concentrate feed and hay are shown in Table 1. During the periparturient period, cows were monitored with monitoring device fitted on the cattle barn to allow confirmation of the exact time of parturition.

2.2. Sample collection and analysis

Immediately before collecting a milk sample, teats were washed with water and wiped dry with a paper towel. Colostrum samples were collected at 15:00 h (1 h before daily feeding) within 24 h after parturition (d 0). Transition milk was collected 24, 48 and 72 h relative to the time of colostrum sampling (d 1, d 2 and d 3, respectively). In addition, first colostrum samples were collected before calves drank the colostrum (immediately after the parturition) from additional three cattle. A total of 20 mL of colostrum and transition milk were collected per sampling from four teats. Collected milk was filtered through a piece of gauze cloth overlying the collection container to remove impurities. Blood samples were collected 1 h before daily feeding (at 15:00 h) at 7 d relative to expected parturition data (d -7) and right before the colostrum and transition milk sampling at d 0, 1, 2 and 3 using vacutainers for the collection of plasma (Venoject II VP-H100K with heparin sodium; Terumo Corporation, Tokyo, Japan). Immediately after collecting blood and milk samples (within 5 min after milk sampling), aprotinin (Sigma-Aldrich, Oakville, ON, Canada) was added to the colostrum and transition milk (500 kallikrein inhibitor units/mL of milk). Blood samples were centrifuged at 2,330 × g at 4 °C for 20 min, and plasma was collected. Colostrum, transition milk and plasma samples were stored at −80 °C until analyses. Calves were separated from their dams on all sampling days 4 h before collecting colostrum and transition milk (11:00 h) and returned to their dams until 4 h prior to the next sampling to obtain enough volume of sample and to remove the confounding effect of suckling by calves.

Concentrations of GLP-2 in plasma, colostrum and transition milk were measured by solid phase competition immunoassay using europium (PerkinElmer Japan, Kanagawa, Japan)-labeled bioactive human GLP-2 (1–33) (Peptide Institute Inc., Osaka, Japan), anti-bioactive GLP-2 (Rat) serum (1–33) (Yanaihara Institute Inc., Shizuoka, Japan) and polystyrene microtiter strips (Nalgene Nunc International, Tokyo, Japan) coated with anti-rabbit γ-globulin [19], according to the technique of time resolved fluoroimmunoassay (TR-FIA) previously described [20, 21].

2.3. Statistical analysis

Three cattle were removed from the statistical analyses for d 0 because insufficient colostrum was collected. We have previously noted that only a limited volume of colostrum is able to be collected from Japanese black cattle within 24 h after birth [21]. Data for concentrations of GLP-2 in plasma and the remaining samples of colostrum and transition milk were analyzed using the fit model procedure of JMP 14 (SAS Institute Inc., Cary, NC, USA) to evaluate the fixed effect of time (days after calving) as per the following model (eq. (1)):

$$Y_{ij} = \mu + Time_i + Cow_j + e_{ij}, \quad (1)$$

where Y_{ij} is the dependent variable, μ is the overall mean, $Time_i$ is the fixed effect of time (days relative to parturition or expected parturition data), Cow_j is the random effect of the cows, and e_{ij} is the error term.

Data for colostral- and plasma GLP-2 concentrations were further analyzed as per the following model (eq. (2)):

$$Y_{ij} = \mu + Sample_i + Cow_j + e_{ij}, \quad (2)$$

where Y_{ij} is the dependent variable, μ is the overall mean, $Sample_i$ is the fixed effect of sample type (colostrum vs plasma at d -7 vs plasma at d 0), Cow_j is the random effect of the cows, and e_{ij} is the error term.

Quadratic regression analysis was performed between colostral- and transition milk concentrations of GLP-2 and elapsed time from parturition to colostrum collection using fit Y by X procedure in JMP 14.

3. Results and discussion

3.1. GLP-2 assay

The calibration curve of the competitive TR-FIA assay for GLP-2 is shown in Figure 1: concentrations of the GLP-2 standard ranged from 0.1 to100 ng/mL. Intra-assay and inter-assay CVs were 7.3 % and 7.1 %, respectively. The minimum detectable level and 50 % inhibitory concentration were 0.02 ng/mL and 1.13 ng/mL, respectively. The GLP-2 tracer was displaced by bovine colostrum in a dose-response manner (Figure 1). Bovine GLP-2 shares 88 % sequence identity with human GLP-2 [1]. The quality control criteria in our TR-FIA protocol were satisfactory; therefore, the assay was suitable for determining bovine colostral GLP-2.

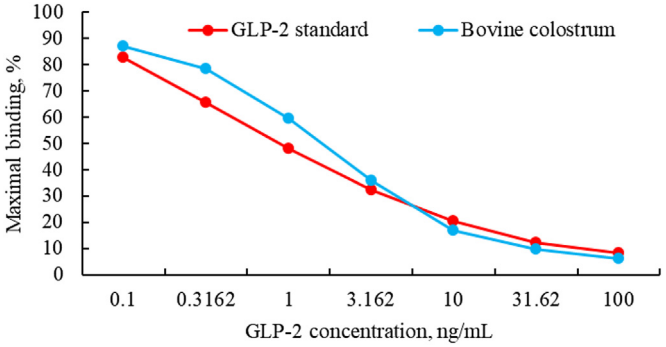

Figure 1. The competitive TR-FIA calibration curve for human glucagon-like peptide 2 (GLP-2) standard and bovine colostrum. Calibration curve was linearized using a Logit-log model. Each point means the average of triplicate measurements. TR-FIA = time-resolved fluoroimmunoassay.

Table 1. Nutrient composition of concentrate feed and hay.

Item[1]	Concentrate feed	Hay
DM, %	88.4	71.7
Nutrient composition, % DM		
TDN	45.6	60.0
CP	16.9	17.2
Crude fat	4.0	3.1
NDF	25.8	63.1
NFC	45.6	13.4

[1] DM = dry matter, TDN = total digestible nutrients, CP = crude protein, NDF = neutral detergent fiber, NFC = non fiber carbohydrate.

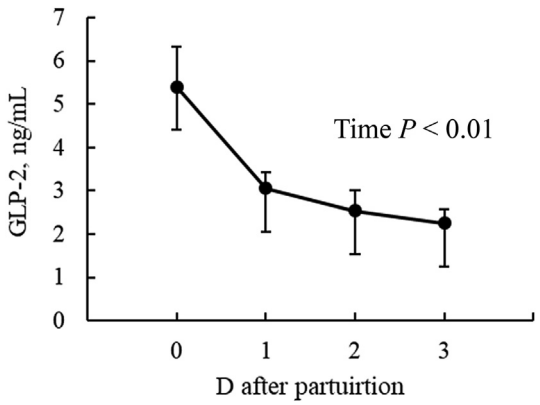

Figure 2. Glucagon-like peptide 2 (GLP-2) concentration in colostrum (d 0) and transition milk (d 1 to d 3). Results are expressed as mean ± SE.

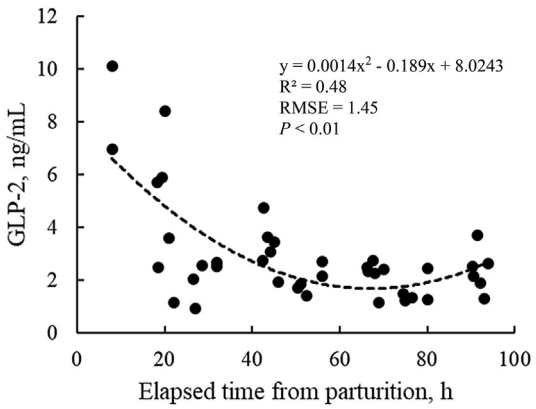

Figure 3. Relationship between colostral- and transition milk concentrations of GLP-2 and elapsed time from parturition to collecting colostrum estimated by regression analysis. GLP-2 = glucagon-like peptide 2. RMSE = Root mean square error.

3.2. GLP-2 concentration in colostrum and transition milk

We show for the first time that GLP-2 is present in bovine colostrum and transition milk (Figure 2). Concentrations of GLP-2 in colostrum were 5.53 ± 1.07 ng/mL (mean ± SE) on average, which was significantly higher ($P < 0.01$) than concentrations in plasma of cows at 7 d before the expected parturition date (0.87 ± 0.15 ng/mL on average and ranged from 0.22 to 1.79 ng/mL) and at parturition (d 0) (0.90 ± 0.15 ng/mL on average and ranged from 0.45 to 1.82 ng/mL) in the current study (Table 2). Previous studies have reported that plasma concentrations of GLP-2 were no higher than 1.0 ng/mL in mature sheep [22] and in lactating dairy cows [23]. Higher concentrations of hormones in colostrum compared with maternal blood are not unique to GLP-2 because concentrations of insulin and IGF-1 were reported to be higher in colostrum than in maternal blood [21, 24].

It remains unclear how concentrations of GLP-2 are increased in colostrum of periparturient cows. Glucagon-like peptides are inactivated and removed from circulation in proportion to the amount of dipeptidyl peptidase-IV secreted into circulation and by renal clearance once secreted from the intestinal L-cells [25, 26], resulting in short half-life of 7 min in circulation [27]. Therefore, it is possible that higher concentrations of GLP-2 in colostrum, than in plasma, is attributed to the difference between clearance of GLP-2 in colostrum and circulation. Kierson et al. reported that ghrelin, a peptide hormone secreted from stomach, is present in human colostrum at higher concentration than maternal blood [28]. Another study reported that the source of ghrelin in breast milk may arise from plasma [29]. Therefore, multiple circulating hormones could be transferred to and accumulate in colostrum, which is consistent with our finding.

Ingestion of bioactive substances in colostrum, such as hormones, have been shown to stimulate GIT development in neonatal calves [10, 11, 12, 13, 14]. Although the possibility and the degree of systemic uptake of colostral hormones by the neonate is still questioned, a previous study has shown that insulin administered orally to neonatal calves within 24 h after birth was absorbed and confirmed to be active because a marked hypoglycemia in calves was observed [30]. Similarly, Kirovski et al. [15] confirmed that orally administered insulin was absorbed within 1 h after birth in calves. In contrast, it was also reported that the stimulatory effects of colostral growth hormone, IGF and insulin on the GIT are exerted directly on the intestinal lumen in autocrine or paracrine manner in calves [31]. These findings extend our understanding of maternal investment in offspring, which continues post-partum with the delivery of bioactives in colostrum that, in addition to immunoglobulins, impart important roles in calf growth and health. As described above, a major action of GLP-2 is to stimulate GIT development [1, 2]. Thus, our results suggest the possibility that orally ingested GLP-2 via colostrum has beneficial impacts on GIT development of newborn calves, and further studies are warranted to confirm this conjecture.

In the present study, concentrations of GLP-2 in colostrum ranged from 0.94 to 9.60 ng/mL. This large variation in concentrations of GLP-2 is likely due to the difference in the timing of sampling, where the elapsed time from parturition to sampling of colostrum ranged from 9 to 21 h. This indicates that the number of suckling by calves until colostrum sampling also varied between individuals. Concentrations of GLP-2 were reduced ($P < 0.01$) in colostrum and transition milk from d 0 to 3 as shown in Figure 2. Moreover, concentrations of GLP-2 in colostrum and transition milk were quadratically decreased with elapsed time from parturition to sampling ($R^2 = 0.48$, $P < 0.01$; Figure 3). These results indicate that GLP-2 concentration in breast milk is reduced by elapsing time post-calving and/or suckling by calves. To test this theory, we collected first colostrum samples immediately after the parturition (before calves started drinking) from additional three cattle to measure colostral GLP-2 concentration with no confounding effect of difference in the elapsed time from parturition and number of suckling by calves. Concentrations of GLP-2 in the first colostrum samples of three cattle were 9.28, 8.80 and 11.8 ng/mL (9.72 ± 1.09 ng/mL on average), which was approximately twice as high as those in colostrum collected within 24 h after the parturition (5.41 ± 0.92 ng/mL), supporting above theory. Decreasing concentrations in colostrum with time post-calving is not unique to GLP-2. Others have reported that concentrations of IGF-1, insulin and IgG decrease in milk with time post-calving [32]. Overall, the increased concentrations of GLP-2 in colostrum that decrease over time in subsequent milkings are consistent with the dam providing ongoing investment in their growing calves via imparting factors that promote development of the GIT, which, in turn, is crucial for post-natal health and growth.

Table 2. Comparison of glucagon-like peptide 2 (GLP-2) concentrations between colostrum and plasma at 7 d before the expected parturition data (d -7) and at parturition (d 0).

		Plasma		
	Colostrum	d -7	d 0	*P*-value
GLP-2, ng/mL	5.53 ± 1.07[a]	0.87 ± 0.15[b]	0.90 ± 0.15[b]	<0.01

[a,b] Values within a row with different superscripts differ ($P < 0.05$).

In conclusion, we have shown that GLP-2 is present in bovine colostrum and transition milk at higher concentrations than in blood of periparturient Japanese black cattle. Furthermore, we have shown that concentrations of GLP-2 in colostrum and transition milk decreased with elapsed time from parturition, which is similar to the decrease in concentrations of IGF-1 and insulin in colostrum and transitional milk as previously reported [32]. The findings of the current study extend our basal knowledge and understanding of GLP-2 and provide an important reference for concentrations in colostrum and transition milk of Japanese black cattle.

Declarations

Author contribution statement

Yudai Inabu, Hideyuki Takahashi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hiroshi Yamamoto: Performed the experiments; Analyzed and interpreted the data.

Haruki Yamano, Yutaka Taguchi, Shunnosuke Okada, Tetsuji Etoh, Yuji Shiotsuka, Ryoichi Fujino: Performed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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