J. Dairy Sci. 101:1–5
https://doi.org/10.3168/jds.2018-14412

Short communication: The effect of delayed colostrum feeding on plasma concentrations of glucagon-like peptide 1 and 2 in newborn calves

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ABSTRACT

Glucagon-like peptide (GLP)-1 is involved in glucose homeostasis via its role in stimulating insulin secretion, whereas GLP-2 increases mucosal growth of the small intestine. To our knowledge, the effect of delayed colostrum feeding on plasma GLP-1 and GLP-2 in neonatal calves has not been evaluated. To investigate the effect of delayed colostrum feeding on plasma concentrations of GLP-1 and GLP-2 in newborn calves, we randomly assigned 27 Holstein bull calves to 1 of 3 treatment groups: those fed colostrum within 1 h after birth (control), 6 h after birth (6H), and 12 h after birth (12H; n = 9 for each treatment). Blood samples were obtained before the colostrum feeding and every 3 h after each colostrum feeding for a 36-h period, and plasma concentrations of GLP-1, GLP-2, insulin, and glucose were measured. Plasma GLP-1 concentration at 12 h after colostrum feeding was lower in 12H than in control calves. In addition, plasma insulin concentration was lower in the 6H and 12H calves than in the controls. Plasma glucose and GLP-2 concentrations were, however, not affected by treatment. These results indicate that delayed colostrum feeding can decrease plasma GLP-1 and insulin concentrations without affecting glucose or GLP-2 concentration.
Key words: gut-derived peptide, glucagon-like peptide 1, glucagon-like peptide 2, colostrum

Short Communication

It is well known that timing of the first colostrum feeding is critical in the transfer of passive immunity in newborn calves, because the efficiency of IgG absorption decreases over the first 24 to 30 h of life (Bush and Staley, 1980; Weaver et al., 2000). More specifically, Osaka et al. (2014) reported that the apparent efficiency of IgG absorption declined by less than 0.3%/h from calving to 12 h after birth, and then declined more rapidly at 2.5%/h to at least 18 h after birth. Similar to blood IgG concentrations, plasma concentration of hormones, such as IGF-1 and insulin, were also reported to decrease with a delay in colostrum feeding (Hammon et al., 2000). These findings indicate that the timing of colostrum feeding could affect blood hormone concentrations, as well as the transfer of passive immunity.

Gut-derived peptides are known to play important roles in nutrient utilization and in the physical, morphologic, and metabolic transformation of growing animals. Among such peptides are glucagon-like peptide (**GLP**)-1 and GLP-2, which are co-secreted from enteroendocrine L-cells (mainly located in the distal intestine) in response to nutrient intake (Burrin et al., 2003). It has been reported that GLP-1 plays a role in glucose homeostasis via the stimulation of insulin secretion in both nonruminants (O'Halloran et al., 1990; Holz et al., 1993) and ruminants (Faulkner, 1991; Edwards et al., 1997; Fukumori et al., 2012), as well as through its own direct action without mediating insulin action (Luque et al., 2002; Acitores et al., 2005; Sancho et al., 2005, 2006). It has been reported that insulin, which is present at high concentrations in colostrum (Hammon et al., 2013), could be absorbed from intestine (Kirovski et al., 2008), indicating that exogenous insulin provided via colostrum feeding may contribute to circulating insulin concentration. On the other hand, Hammon et al. (2000) suggested that colostrum feeding increases endogenous insulin production in newborn calves. Therefore, given that plasma insulin concentration decreases with a delay in first colostrum feeding (Hammon et al., 2000), we speculate that delayed colostrum feeding may affect GLP-1 secretion as well as insulin in neonatal calves. On the other hand, GLP-2 plays a role in stimulating gastrointestinal tract (**GIT**) growth in ruminants (Taylor-Edwards et al., 2011), as well as nonruminants (Drucker et al., 1996; Kato et al., 1999; Hartmann et al., 2000). Major morphological and functional changes in the calf's GIT are initiated by colostrum feeding (Ontsouka et al., 2016), suggest-

Received January 8, 2018.
Accepted March 6, 2018.
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ing that GLP-2 can exert its action after feeding the calf with colostrum. However, the effects of colostrum feeding and delayed colostrum feeding on plasma GLP-1 and GLP-2 concentrations have not been evaluated in newborn calves. Our objective in this study was to evaluate the effect of different delaying colostrum feeding on plasma concentrations of GLP-1 and GLP-2, as well as those of insulin and glucose.

All experimental procedures used in this study were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00001595) and conducted in accordance with the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada). A total of 27 Holstein bull calves were used for the present study. Calves were born as singlets, separated from their dams at birth, and then managed in individual pens. The pens in which calves were maintained were bedded with shavings on the bottom layer and fresh straw on the top. Calves were randomly assigned to 1 of 3 treatments: those fed pooled colostrum containing 62 g of IgG/L (Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada) at 7.5% of birth BW using an esophageal tube within 1 h after birth (control), 6 h after birth (**6H**), or 12 h after birth (**12H**; n = 9 for each treatment). Birth BW was not different across treatment groups (means ± SD; 43.5 ± 1.51 kg, 42.0 ± 1.74 kg, and 42.2 ± 0.96 kg for control, 6H, and 12H, respectively). At 12 h after being fed colostrum and every 6 h thereafter, calves were fed milk replacer (**MR**; Excel Pro-Gro Calf Milk Replacer; Grober Nutrition, Cambridge, ON, Canada) at 2.5% of birth BW. Calves were fed MR by bottle initially, but if the calves did not consume all the MR within 30 min, they were tube-fed the remainder of the MR volume. Colostrum was kept frozen at −20°C until thawing and, before calving, the colostrum was thawed in a water bath at a constant temperature of 50°C until it reached a temperature of 39°C. Similarly, MR was warmed to 39°C before feeding. Total protein, total fat, and lactose contents for colostrum were 14.2, 5.0, and 2.7%, respectively, and CP, crude fat, and lactose contents for MR were 26, 18, and 42%, respectively. For all treatments, blood samples were taken within 1 h after birth (before colostrum feeding) using evacuated tubes (Fisher Scientific Co., Nepean, ON, Canada) as baseline samples and every 3 h after each colostrum feeding through a jugular catheter (Thermo Fisher Scientific, Carlsbad, CA), which was fitted at 2 h after birth, for a 36-h period. The blood samples collected at 12, 18, 24, 30, and 36 h after colostrum feeding were taken immediately before MR feeding. Immediately after sample collection, aprotinin (Sigma Aldrich, Oakville, ON, Canada) was added to the blood samples for plasma preparation (in the ratio of 0.5 μL of aprotinin to 1 mL of blood). Blood samples were centrifuged at 3,000 × *g* and 4°C for 20 min, and plasma was collected and stored at −20°C until analysis.

Plasma samples were analyzed for hormone and glucose concentrations. Plasma hormone concentrations were measured following the time-resolved fluoroimmunoassay (TR-FIA) technique previously described by Sugino et al. (2004). Plasma GLP-1 concentration was measured using a solid-phase competition immunoassay with europium (Eu)-labeled human GLP-1 and polystyrene microtiter strips (Nalgene Nunc Int., Tokyo, Japan) coated with anti-rabbit γ-globulin (Inabu et al., 2017). Interassay coefficient of variation (**CV**) was 5.3%, and the least detectable level was 0.007 ng/mL. Plasma GLP-2 concentration was measured using a solid-phase competition immunoassay with Eu-labeled human GLP-2 (Peptide Institute Inc., Osaka, Japan), polyclonal anti-rat GLP-2 (Yanaihara Institute Inc., Shizuoka, Japan), and polystyrene microtiter strips coated with goat-anti-rabbit γ-globulin (Inabu et al., 2017). Interassay CV was 5.8% and the least detectable level was 0.038 ng/mL. The plasma concentration of insulin was measured using a solid-phase competition immunoassay with Eu-labeled bovine insulin and polystyrene microtiter strips coated with anti-guinea pig γ-globulin (Inabu et al., 2017). Interassay CV was 3.1%, and the least detectable level was 0.14 ng/mL. Plasma glucose concentration was analyzed using an enzymatic method with peroxidase glucose oxidase (Sigma, St. Louis, MO).

Data for plasma glucose, GLP-1, GLP-2, and insulin concentrations were analyzed using the fit model procedure of JMP 13 (SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijk} = \mu + T_i + H_j + C_k + TH_{ij} + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the fixed effect of treatment, H_j is the fixed effect of time after colostrum feeding used as a repeated measure, C_k is the random effect of calf, TH_{ij} is the interaction of treatment and time, and e_{ijk} is the error term. All values were expressed as least squares means ± standard errors of the means (LSM ± SEM). The effects of treatment, time, and treatment by time interaction were considered significant at $P < 0.05$ and tendencies were assumed at $0.05 \leq P < 0.10$.

Although we observed a time effect on plasma glucose concentration ($P < 0.01$; Figure 1A), this was mainly a result of the increased concentration after MR feeding, whereas we observed no increase up to 12 h after colostrum feeding. The small response of glucose to colostrum feeding was probably due to the lower lactose content in colostrum (only 2.7% in this study)