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Technical note: Establishment of an ileal cannulation technique in preweaning calves and use of a piecewise regression approach to evaluate effects on growth and pH fluctuation of ileal digesta

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ABSTRACT

Digestibilities of nutrients, especially protein, are crucial characteristics of milk replacers in a calf-rearing program. Endogenous synthesis of proteins and microbial fermentation in the large intestine alter apparent total-tract digestibility of AA. Therefore, collection of digesta samples at the end of the ileum is the only method to estimate true small intestinal digestibility of AA. The aim of this work was to evaluate the effectiveness of inserting a T-cannula into the distal ileum of preweaning calves for use in digestibility studies. A second objective was to evaluate the use of a "broken-line" statistical model to compare treatment effects on calf growth and digesta pH. A T-cannula was surgically installed in the terminal ileum of 2 calves approximately 5 cm anterior to the ileocecal junction at 15 d of age, and 2 paired noncannulated calves were used as controls. Cannulation did not affect mean body weight (BW), average daily gain, milk and water intakes, and body frame dimensions. However, final BW (89.2 vs. 94.6 kg) was lower and starter intake (0.06 vs. 0.21 kg/d) tended to be decreased in cannulated calves compared with control calves. No effects on health scores, rectal temperature, or the odds of incurring diarrhea or being medicated were observed. Flow of digesta (46.4 ± 0.04 g/h) increased linearly after feeding, whereas there was a quadratic effect of time on digesta pH, with the nadir at approximately 8.5 h postfeeding. The broken-line model successfully fitted daily fluctuations of pH and allowed us to detect differences in growth slopes between cannulated and control calves. Despite the expected negative effect on BW, we conclude that this technique permitted sampling of representative ileal digesta while allowing satisfactory growth and health of the calves.

Key words: calf, ileal cannulation

Technical Note

Calves are born with a reticular groove that enables milk to reach the abomasum, thereby bypassing the rumen (Meale et al., 2017). At birth, the rumen is not completely developed (Baldwin et al., 2004). Although the size (Warner et al., 1956) and metabolic activity of the rumen increase quickly (Rey et al., 2012), the rumen itself is not fully functional until at least 60 d of age (Eckert et al., 2015). Because of the lack of microbial fermentation in the rumen, preruminant calves rely solely on AA from feed to support growth (Hill et al., 2008). As long as energy is not limiting, body deposition of protein increases linearly as a function of dietary AA absorption (Drackley, 2008).

The use of plant-based protein has increased in the last decades at the expense of milk proteins (Gro Intelligence, 2017). The apparent digestibility of plant protein in milk replacers, however, is generally lower compared with milk proteins (Guilloteau et al., 1986). This difference is caused by several factors, including the effect of protein source on abomasal emptying, digestive secretions, rate of absorption, intestinal morphology and permeability, and flow of digesta (Lallès, 1993; Drackley, 2008). Plant proteins also can decrease apparent digestibility of AA due to a greater synthesis of endogenous protein (Montagne et al., 2000) or because of the presence of antinutritional factors that alter digestive and absorptive capacity of the intestinal epithelium (Lallès et al., 1999). Moreover, microbial fermentation in the large intestine alters the AA profile in feces independent of the dietary AA supply (Khorasani et al., 1989). Consequently, as recommended in pigs (Stein et al., 2007), collecting ileal digesta samples in young preweaning dairy calves is necessary to accurately determine true ileal digestibility of feed ingredients. Research using this technique was abundant in the past (e.g., Smith, 1962; Gorrill and Nicholson, 1972), but to our knowledge only 1 experiment using this technique has been reported in the last 2 decades (Montagne et al., 2003). The considerably greater milk allowance recommended today (Schäff et al., 2018) and

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the availability of nutritive supplements (Górka et al., 2018) and advanced plant-based alternative proteins (Cervantes-Pahm and Stein, 2010; Senevirathne et al., 2017) make it necessary to revisit the digestive physiology of young preruminant calves.

Piecewise or "broken-line" regression models are used with nonlinear data. Broken-line models are characterized by lines or curves connected by joint points called knots (Schwarz, 2015). Each line or curve is a continuous function that represents 1 piece of the polynomial function (Marsh and Cormier, 2002). The knots allow the function to better fit the data (Hurley et al., 2006). Piecewise regression models comprise plateaus (a_0) and 1 or several slopes (b_1, b_2) delimited by knots (x') plus the error term (e_i). The model predicting a dependent variable (y_i) from an independent variable (x_i) can be written as

$$y_i = a_0 + b_1 \times \text{minimum }(x_i, x') + b_2 \times \text{maximum }(0, x_i - x') + e_i.$$

The best location for a knot can be estimated by parametrically varying x' to find the value that results in the lowest residual sum of squares (Schwarz, 2015).

Our first objective was to adapt and validate a cannulation and sampling technique widely used in pigs (Stein et al., 1998) to 15-d-old calves and to evaluate the effect of the cannulation procedure on growth and health of calves. Our second objective was to characterize the flow of ileal digesta pH by applying a broken-line regression model to the data. To our knowledge, the opportunities provided by this model have not been explored extensively in calves or in other biological models (Shafii et al., 1990; Robbins et al., 2006).

The Institutional Animal Care and Use Committee at the University of Illinois at Urbana–Champaign approved all animal procedures (protocol no. 15167). Six male Holstein calves were acquired from a commercial dairy farm and enrolled at approximately 2 d of age. Calves were blocked by BW and assigned randomly to either treatment (cannulation) or control (no cannulation). One of the calves in the control group died at 8 d due to a clostridial infection and was not replaced. Calves were housed individually outdoors in polyvinyl chloride hutches that were bedded with straw. All calves were fed 2× daily a commercial milk replacer (28.5% CP, 15% fat) reconstituted to 15% solids, at a rate of 2% (DM) of BW daily, adjusted weekly. A texturized calf starter (20% CP) was offered for ad libitum intake only from wk 6 until the end of the trial to verify that the amount of digesta collected during the milk-only phase was sufficient for nutrient analysis. Weaning started on d 49 by progressive dilution of milk replacer until the end of the trial (d 56). Body weight and body frame measurements were recorded weekly, and water, milk, and starter intakes were measured daily.

Surgery was performed on d 15 after arrival. Calves were not fed the morning of the surgery to avoid seepage of gastrointestinal contents into the abdominal cavity. However, a solution (2 L/calf) containing electrolytes was offered to keep calves properly hydrated. Antibiotic injections of penicillin (22,000 IU/kg) were administered at 24 h, 12 h, and immediately before the surgery. Immediately before surgery, an analgesic (flunixin meglumine) was injected intravenously at a dose of 1 mL/45 kg of BW. Calves were anesthetized with xylazine (0.15 mg/kg of BW) and ketamine (3 mg/kg of BW) combined in a single intravenous injection, followed by immediate endotracheal intubation. Anesthesia was maintained with isoflurane (2–5% isoflurane in oxygen) using mechanical ventilation. The incision area was shaved with an electric razor and then cleansed with repeated applications of alcohol and povidone-iodine. Sterile drapes were used to protect the surgery site. A sterile scalpel was used to make an incision through the skin layer approximately 4 cm posterior to the last rib on the right side of the animal. Muscle layers were mechanically separated by hand, exposing the peritoneum. A scalpel was used to carefully cut the peritoneum and locate the intestinal area approximately 5 cm anterior to the ileocecal junction, where the incision for the T-cannula was to be made. A purse string suture was then inserted into the submucosa of the ileum using 2/0 absorbable chromic gut suture, and a 4-cm incision in the intestine was made inside the purse string. A titanium T-cannula, designed as described by Stein et al. (1998), was inserted into the opening and the suture was pulled tight to secure the cannula. A second reinforcing suture using 2/0 absorbable chromic gut was used to further secure the cannula. The barrel of the cannula was exteriorized through a stab wound anterior to the original incision. The cannula was pulled through this incision with the aid of tissue forceps. A plastic washer and a metal lock washer were screwed onto the threaded barrel to secure the exteriorized cannula, and a cap was placed on the top of the cannula to prevent digesta seepage. Penicillin was dripped into the body cavity (approximately 1 mL/10 kg of BW) through the initial incision to reduce the risk of tissue infection. The peritoneum layer was closed with 2/0 absorbable chromic gut suture in a continual pattern. Muscle, fat, and skin layers were sewn in an interrupted pattern using 0/0 synthetic absorbable suture. Each surgery took approximately 20 min. Once calves had recovered from the anesthesia, they were moved back to their hutches. Analgesics were administered intravenously for 3 d and penicillin for 7 d after the surgery, every

12 h. An ointment with 1% chlorhexidine (Nolvasan, Zoetis Inc., Parsippany, NJ) was applied to the incision site, which was sprayed with an aluminum-based aerosol bandage (AluShield, Neogen, Lexington, KY) immediately following surgery. In addition, the area of the incision site and the skin around the cannula were washed with povidone-iodine solution, and a zinc oxide-lanolin–based cream (Desitin, Pfizer Inc., New York, NY) was applied twice daily or more often if needed to minimize irritation and maintain skin integrity during the experiment. Animals returned to full feeding the evening of the day of the surgery. Calves were offered an electrolyte solution (~2 L/d per calf) between meals (at 1200 h) during the initial 15 d postsurgery. Body temperature was monitored 3 times daily for the initial 7 d postsurgery and daily thereafter.

After the 2-wk recovery period, digesta samples were collected in weekly periods for 4 wk. During each sampling period, a 250-mL plastic bag (Playtex, North Bergen, NJ) was attached to the cannula with an autolocking cable tie after removing the cap. Bags were removed when full or every 30 min continuously for 12 h (between the a.m. and p.m. meals) for 2 consecutive days. The contents of each bag were weighed, the digesta pH was recorded immediately with a portable pH meter (Accumet AP110, Fisher Scientific, Atlanta, GA), and the digesta sample was frozen at −20°C. Samples were pooled within calf by week. Cannulated calves were killed at 56 d of age.

Variables for BW, body frame measurements, and intakes recorded during the 6 wk after the surgery (starting at d 15) were analyzed using PROC MIXED in SAS (version 9.4; SAS Institute Inc., Cary, NC) including the REPEATED statement. The covariance structure that resulted in the smallest Bayesian and Akaike information criterion was chosen. Treatment, week, and their interaction were included as fixed effects, and calf was included as a random effect. Initial values (at wk 2) were included as covariates. Weekly BW and pH values were fit to the best broken-line model based on the adjusted coefficient of determination values, significance of the parameter estimates, and visual appraisal of the residuals using NLREG (version 6.5; http://www.nlreg.com/). Weekly BW increase was fitted in a broken-line model according to the equation

$$\mathrm{BW} = [b_1 \times \mathrm{maximum}(0, \mathrm{age} - c_1)] + [a_0 + (b_2 - b_1) \times \mathrm{minimum}(\mathrm{age}, c_2)], \quad [1]$$

where a_0 is the intercept (initial BW), b_1 and b_2 represent the different growth slopes, c_1 and c_2 represent the time points that delimit a change in slope, and age is the week of life. A set of dummy variables was created to compare parameters of the BW evolution model between cannulated and noncannulated calves. Digesta pH also was fitted in a broken-line model using the equation

$$\mathrm{pH} = [a_0 + b_1 \times \mathrm{maximum}(0, \mathrm{time} - c_1)] + [(b_2 - b_1) \times \mathrm{maximum}(0, \mathrm{time} - c_2)], \quad [2]$$

where a_0 is the estimate of initial pH at plateau, b_1 and b_2 are the 2 different slopes, c_1 and c_2 are the estimates of the time points where a significant change in slope occurs, and time is the hour postfeeding after the morning meal.

Flow of digesta samples (46.4 ± 0.04 g/h) tended ($P = 0.08$) to increase linearly after feeding, whereas there was a quadratic effect of time ($P < 0.01$) on digesta pH (Figure 1). There were no differences across weeks or between cannulated calves in fresh weight (0.72 ± 0.13 kg/wk), DM content (12.4 ± 1.2%), or pH (7.1 ± 0.6) of digesta. According to the parameter estimates obtained after fitting pH of digesta samples per hour to the model (Figure 1), the initial pH of 7.47 was maintained until 5.45 h after the a.m. feeding. After that point, pH decreased at a rate of 0.34 pH unit/h until 8.69 h postfeeding, when pH began to increase at a rate of 0.33 units/h. A similar fluctuation between meals has been observed in other studies with milk-fed calves (Guilloteau et al., 1986). Despite the relatively low adjusted coefficient of determination of the model (Figure 1) and the inherently high relative error when comparing pH means (Murphy, 1982), this model allowed prediction of fluctuations of ileal pH between meals.

Ileal digesta pH fluctuation can provide information about abomasal digestion of different sources of nutrients in milk replacer (Guilloteau et al., 1986). For instance, the time it takes the first chyme that leaves the abomasum to reach the ileum after feeding could be identified as the break point estimate representing the first decrease in baseline pH ($c_1 = 5.45$ h) because the digesta passing through at that moment carries the acid from gastric digestion. Assuming a constant transit across the small intestine, this parameter would allow us to evaluate differences on timing of the abomasal release of digesta. Factors such as the presence or not of curd formation in the abomasum modify abomasal flow between different milk replacer components (Petit et al., 1987). In addition, considering the relation between fluctuation of abomasal digesta pH and abomasal outflow (Sen et al., 2006), abomasal emptying duration could be roughly estimated by measuring the time that digesta pH remained lower—that is, from the pH at the initial plateau until the end of the negative slope

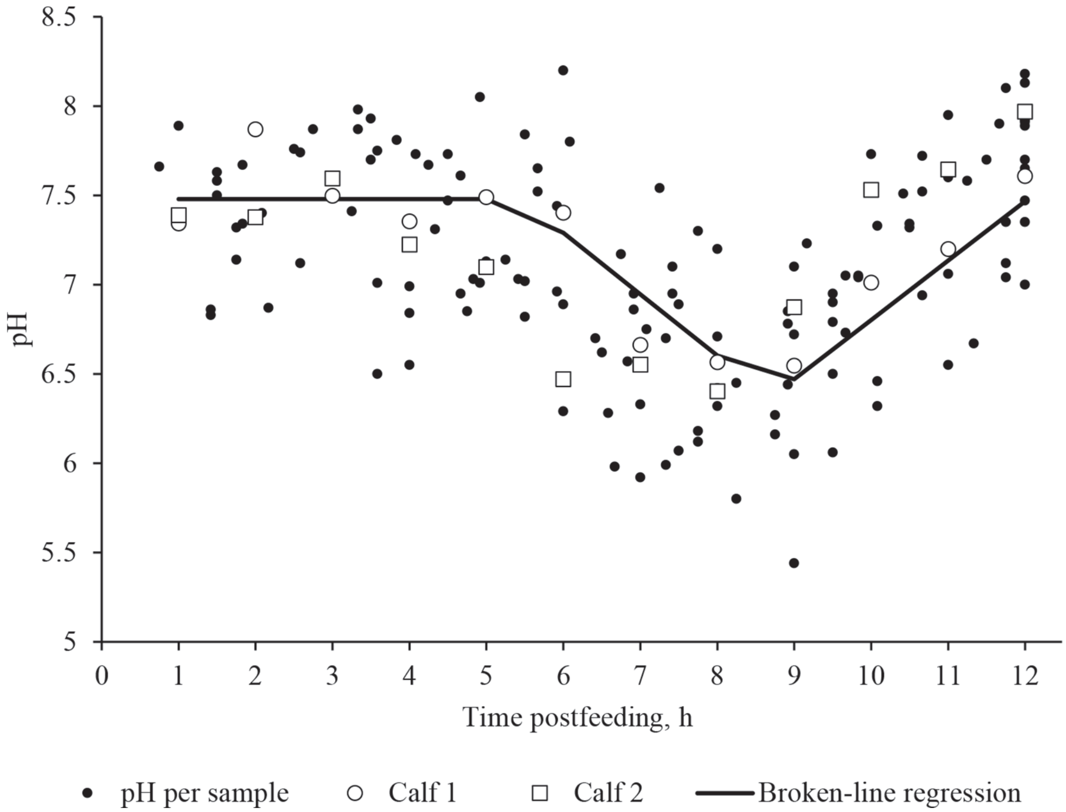

Figure 1. Ileal digesta pH fluctuation during 12 h after the morning feeding (milk replacer only) during 4 weekly collection periods from wk 4 to 8 of life. Digesta pH per sample (●) was averaged by calf and hour (○ = calf 1; □ = calf 2) to estimate the nonlinear broken-line model parameters; pH = $[a_0 + b_1 \times \text{maximum}(0, \text{time} - c_1)] + [(b_2 - b_1) \times \text{maximum}(0, \text{time} - c_2)]$; $R^2 = 0.7$, $P < 0.01$. According to this equation, parameter estimates were as follows: initial pH (a_0) = 7.47; time at pH decrease (c_1) = 5.45 h; decreasing slope (b_1) = −0.34 pH unit/h; time at pH increase (c_2) = 8.69 h; and increasing slope (b_2) = 0.33 pH unit/h.

($c_2 - c_1 = 4.05$ h). According to the literature, abomasal emptying time following 2 L of milk replacer intake is between 3.16 and 3.43 h (Nouri and Constable, 2006; Sen et al., 2006; Marshall et al., 2008). Because feeding larger volumes of milk may slow abomasal emptying (Burgstaller et al., 2017), our estimate of 4.05 h seems appropriate considering we fed an average of 4 L per feeding. This estimate may be a beneficial parameter to record in digestibility experiments because of the effect that different milk replacer ingredients may have in changing abomasal emptying rate and its implications on nutrient digestion and digestive health (Burgstaller et al., 2017).

Even though continuous collection of digesta during 12 h seems to be a good procedure to capture the entire daily fluctuation of digesta pH, restricting the collection of digesta to a reduced interval of time can be sufficient to measure ileal digestibility. Kim et al. (2016) found in ileal-cannulated pigs that 6 h of continuous collection starting 4 or 6 h after feeding provided similar estimates of standard ileal digestibilities of AA compared with a 12-h continuous collection period. A shorter collection period would reduce considerably the labor requirement, but further research is needed to assess whether shorter sampling intervals can be applied to milk-fed calves.

One cannulated calf died on d 4 postsurgery due to a fibrinous peritonitis in the peritoneal cavity. Therefore, at the beginning of the measuring period, there were only 2 calves in each group (n = 2). We acknowledge the limitations of the small number of calves; however, we compared intakes and growth between cannulated calves and paired noncannulated calves. Initial BW, mean BW, ADG, milk replacer intake, and water intake were not affected by cannulation (Table 1). However, final BW was lower ($P = 0.05$) and starter intake tended ($P = 0.06$) to be lower in cannulated calves. Differences in starter intake occurred because one of the cannulated calves did not consume any solid feed during the trial. There were no effects on health (fecal, nasal, respiratory, and ear scores), rectal temperature, or the odds of incurring diarrhea ($P = 0.57$) or being medicated ($P = 0.35$). The broken-line regression model (Equation 1) adequately fit the BW data set, explaining 99% of the

Table 1. Least squares means and parameter estimates for effects of ileal cannulation on rectal temperature, intake, and BW

Item	Treatment[1]		SE	*P*-value				
	Cannulated	Noncannulated		Trt[2]	Day	Week	Trt × day	Trt × week
Rectal temperature, °C	38.35	38.35	0.23	0.99	0.49	—	0.72	—
Water intake, kg/d	1.02	0.85	0.64	0.67	<0.01	—	0.48	—
Milk replacer intake, kg/d	1.30	1.32	0.04	0.73	<0.01	—	0.41	—
Starter intake,[3] kg/d	0.06	0.21	0.01	0.06	0.43	—	0.60	—
DMI, kg/d	1.28	1.36	0.08	0.51	—	<0.01	—	0.38
Initial BW at wk 2, kg	54.4	54.1	1.41	0.86	—	—	—	—
Final BW, kg	89.2[b]	94.6[a]	2.9	0.05	—	—	—	—
Mean BW, kg	73.3	76.1	1.5	0.31	—	<0.01	—	0.27
ADG, kg/d	0.75	0.89	0.07	0.14	—	<0.01	—	0.73
BW model parameters[4]								
Initial BW at plateau (a_0), kg	47.3	46.5	1.37	0.59	—	—	—	—
First slope (b_1), kg/wk	2.68	3.70	0.95	0.31	—	—	—	—
Age at slope change (c_1), wk	2.82	2.52	0.82	0.72	—	—	—	—
Second slope (b_2), kg/wk	6.91	7.77	0.41	0.07	—	—	—	—
Age at growth recess (c_2), wk	7.00	7.00	NA[5]	NA	—	—	—	—

[a,b]Means within a row with different superscripts differ ($P < 0.05$) between main effect treatment.

[1]Two calves were cannulated on d 15 of life, and 2 noncannulated calves were used as controls. Both groups were fed milk replacer at a rate of 2% (DM basis) of BW daily.

[2]Treatment.

[3]Starter was offered from wk 6.

[4]Estimates of the parameters from Equation 1.

[5]NA = not applicable.

variation. Comparison of the model parameters revealed a tendency for a slower rate of growth in cannulated calves after approximately 2.5 wk, which reflected the effect of surgery during their second week of life (Table 1), in agreement with the difference in final BW found with the mixed linear model analysis.

The broken-line regression approach allowed parameterization of both growth and pH models. Broken-line regression models usually have been used to estimate nutrient requirements in dose–response studies (Robbins et al., 2006) because the break point or knot estimates allow identification of when a significant change in slope exists. The same principle can be applied when measuring the response to a continuous variable such as temperature (Hammami et al., 2013) or time, as we did in this analysis. In our study, the broken-line regression was a convenient tool to evaluate the growth rate after surgery and could help identify differences in pH fluctuation and digesta flow between diets in future experiments.

Despite an expected negative effect on final BW and growth rate after surgery, the cannulation and sampling technique permitted collection of sufficient ileal digesta while allowing satisfactory growth and health of the calves. Unlike methodology applied in the past such as re-entrant cannulas (Markowitz et al., 1964) or continuous milk replacer infusion into the abomasum (Montagne et al., 2000), continuous collection of digesta between meals is a convenient technique that reduces labor requirement and minimally disrupts the regular behavior and digestive transit of the calves. In addition, the collection method used in the current experiment seems to provide reliable data for digestive tract transit between meals because it allowed an accurate depiction of fluctuation of pH in the digesta reaching the distal ileum between meals. The pH fluctuation patterns are an important component of understanding dietary effects on digestion and could be used to estimate abomasal emptying rate, among other parameters.

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