Ruminal in situ disappearance and whole-tract digestion of starter feeds in calves before, during, and after weaning

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ABSTRACT

Ten bull calves ($n = 5/\text{diet}$) were cannulated at 3 wk of age and used in a 2×2 factorial design with repeated measures over time to compare rumen and whole-tract degradability of 2 calf starter diets and to describe an in situ technique for estimating ruminal degradability of diets in calves at different ages. Calves received milk replacer and 1 of 2 starter diets through wk 7. Mean birth weight was 38.7 ± 1.3 kg. Weaning occurred in wk 8, and calves received only starter (up to 4,500 g/d) through wk 15. Starter diets were a complete pellet (PEL; 42% starch, 13% neutral detergent fiber, NDF) or texturized feed (TEX; 31% starch, 22% NDF). Portions of each diet were dried and ground through a 2-mm screen, and 1.25 g was inserted into concentrate in situ bags (5 cm $\times$ 10 cm, 50- μm porosity). Each calf received duplicate bags of each diet for a total of 8 bags/calf (2 diets $\times$ 2 time points). All bags were inserted at the time of starter feeding. Half of the bags were removed at 9 h, and the other half were removed at 24 h. After removal from the rumen, bags were rinsed, dried (55°C), and composited by diet and by calf within week for NDF, nitrogen (N), and starch analyses. This process was repeated over 3 d during wk 5, 7, 9, 11, 13, and 15. Daily starter intake and total fecal excretion were recorded during the same 3-d periods. Diets, refusals, and feces were subsampled, dried, ground, composited by calf by week, and analyzed for NDF, N, and starch content. Apparent digestibility coefficients, total intake, and fecal excretion were calculated and analyzed with a mixed models procedure. Intake and fecal excretion of all measured nutrients increased from wk 5 through wk 15 of age and were greater for calves fed TEX, whereas the proportion of dry matter (DM), N, and starch apparently digested through the total tract decreased from wk 5 to 15 and was greater in

calves fed PEL. Ruminal disappearance of DM, N, and starch after 9-h incubations increased linearly with age. Likewise, DM, NDF, and N disappearance after 24-h incubations also increased. Ruminal disappearance of DM and NDF was greater for PEL than for TEX. Ruminal disappearance was estimable for DM, NDF, N, and starch. In addition, changes over time and changes due to rumen environment were clearly demonstrated. Based on these data, there is potential to design specific rations and feed processing methods for calves based on their ability to utilize nutrients.

Key words: calf, digestibility, in situ

INTRODUCTION

Before and for some time after weaning, calves undergo a process of rumen development during which papillae elongate, tissue thickens and becomes metabolically active, and rumen volume increases (Tamate et al., 1962; Sutton et al., 1963). Furthermore, researchers are currently seeking to understand rumen microbiome establishment that happens during this time (Anderson et al., 1987; Kim et al., 2016). In addition to a developing rumen and microbiome, studies directly measuring rumen pH in calves consistently demonstrate a decline in rumen pH during the weaning transition, with mean pH values of approximately 5.0 (Anderson et al., 1987; Beharka et al., 1998; Suarez-Mena et al., 2015, 2016), which contrasts sharply with a mean ruminal pH ≥ 6.0 that is observed commonly in healthy adult cattle (Krajcarski-Hunt et al., 2002; Ganesella et al., 2010; Stefanska et al., 2018). Rumen pH can affect the extent of rumen fermentation by altering microbial populations and tissue absorptive capacity (Sutton et al., 1963). Given these physiological changes occurring in young calves, it is not surprising that their digestive ability also changes over time.

Efforts to describe digestion and nutrient availability in calves have used ruminal, duodenal, and ileal cannulas and blood catheters to measure apparently undigested and digested nutrients (Sutton et al., 1963;

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Quigley et al., 1985; Lalles et al., 1990). Vazquez-Anon et al. (1993) used a ruminal in situ technique to compare protein digestion in calves postweaning with adult dairy cattle in late lactation and reported greater protein disappearance rates in calves 8 wk postweaning compared with mature cows. Since these data were reported, small cannulas suitable for young calves have become commercially available (Bar-Diamond Inc., Parma, ID) and cannulation methods have been refined such that the observed growth and performance of ruminally cannulated calves are similar to that of noncannulated calves (Kristensen et al., 2010). Despite these advances, a detailed ruminal in situ procedure has not been described for calves.

Development of ruminally fistulated cattle and ruminal in situ techniques has been instrumental for quantifying the extent of digestion and nutrient utilization in the rumen (Stoddard et al., 1951; Vanzant et al., 1998). The in situ procedure involves suspending a small portion of feed inside a bag of known porosity in the rumen and measuring nutrient disappearance (Vanzant et al., 1998). Plotting this disappearance over time provides estimates of the rate at which nutrients are degraded in the rumen and potentially available to ruminal microbes or to the host. Models based on these data are used in ration formulation to meet nutrient requirements of heifers and mature cows (National Research Council, 2001), and application of this technique in dairy calves could be beneficial. Two important limitations exist for in situ procedures in young calves. First, rumen capacity limits the number of bags that can be inserted and therefore limits replicates and sampling time points. Second, rumen development is progressive and diet dependent (Tamate et al., 1962; Sutton et al., 1963). Therefore, it is necessary for digestibility curves to be determined in calves in various management systems.

This study was designed to estimate rumen degradation of 2 calf starter diets using an in situ method and to investigate the interaction between basal diet and in situ substrate in calves at different ages. A secondary objective was to compare nutrient intake, output, and whole-tract degradability of 2 calf starter diets in calves at different ages. A highly processed, high-starch diet was hypothesized to be more digestible in situ; however, when fed as a basal diet, digestibility was hypothesized to decrease due to low rumen pH. Digestibility, nutrient intake, and output in all calves were hypothesized to increase with age.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the University of Wisconsin–Madison Institutional Animal Care and Use Committee (IACUC no.

A005848). The authors are aware of 1 study using an in situ technique in calves; therefore, a power analysis was conducted using mean and standard deviation of rumen available DM reported by Vazquez-Anon et al. (1993). Assuming a power of 0.9 and an α -value of 0.05, a minimum of 4 calves were needed per treatment. Ten Holstein bull calves born at the Marshfield Agricultural Research Station (Marshfield, WI) between June 17 and July 5, 2017, were used for this experiment, providing 1 extra calf per treatment to allow for unexpected variability. The overall study design was a completely randomized design with repeated measures. The in situ portion of the study used a 2×2 factorial design with 2 basal diets—pelleted (**PEL**) and texturized (**TEX**)—and 2 substrates evaluated in situ (same as basal diets). Measurements were taken across 3-d periods when calves were 5, 7, 9, 11, 13, and 15 wk of age.

Calves and Diets

Calves received 3.79 L of colostrum within 3 h of birth. Birth weight was (mean $\pm$ SE) 38.7 ± 1.3 kg. Additional feedings of colostrum were offered twice per day until 48 h of age. Blood samples were drawn between 48 and 72 h of age to determine serum total protein (handheld refractometer, ATAGO, Kowloon, Hong Kong); calves were included in the study when serum total protein was >5.5 g/dL. After 48 h, the diet consisted of 2 daily feedings of 1.90 L (227 g of DM) of milk replacer (22% CP, 20% fat; Land O' Lakes Inc., Arden Hills, MN) via nipple bottle at 0700 and 1900 h for 6 wk followed by a single feeding of 1.90 L at 0700 h for 7 d. Calves were completely weaned at 8 wk of age. Starter diets were randomly assigned when calves were approximately 1 wk of age (6.6 ± 3.4 d), and each calf remained on their respective diet for the duration of the study. The diets offered (Table 1) were designed to cause (PEL) or blunt (TEX) rumen acidosis to achieve the objectives of a concurrent study of acidosis in calves. Highly processed, high-starch diets have been shown to induce acidosis in ruminants (Keunen et al., 2002). Therefore, rumen pH was expected to be lower in calves fed PEL compared with TEX diets. Rumen pH, VFA concentration, and tissue measurements are reported in a companion manuscript (S. L. Gelsinger, W. K. Coblenz, G. I. Zanton, R. K. Ogden, and M. S. Akins, unpublished data). Beginning at 1 wk of age, fresh starter (228 g) was offered daily at 0800 h and refusals were weighed at the end of each 24-h period. When a calf refused <200 g for 2 consecutive days, the amount offered was increased up to a maximum of 4,500 g/d per calf. Samples were collected from each new bag of starter and from each refusal and stored at -20°C pending subsequent nutrient analyses. Fresh

starter samples were composited by diet for nutrient analyses.

Calves were housed in individual calf hutches (4.8 m²/calf) from birth through 8 wk of age. Thereafter, calves were housed in divided superhutches (5.0 m²/calf) with nose-to-nose contact between hutchemates. Calves within a superhutch could access only their own feed and water. Rubber mats were placed beneath the entire area of individual hutches and superhutches to prevent bedding consumption and to facilitate fecal collection. Small soft-rubber cannulas (28 mm i.d.) were fitted to each calf at approximately 3 wk of age. These were replaced with larger soft-rubber cannulas (51 mm i.d.; Bar-Diamond Inc., Parma, ID) between 7 and 9 wk of age to accommodate the growth of the fistulas.

In Situ Method

Samples of each starter diet were dried at 55°C for 72 h in a forced-air oven and ground through a 2-mm screen using a model 4 Wiley mill (Thomas Scientific,

Swedesboro, NJ). Dacron in situ concentrate bags (5–10 cm, 50- μ m porosity; Ankom Technology, Macedon, NY) were filled with 1.25 g of dried ground starter and heat sealed. A small bag size was chosen due to the size of the cannula and rumen. The recommended sample-to-surface area ratio for determining in situ digestibility in mature cattle is 10 mg/cm² to reduce variation, although the reviewed recommendation is as high as 20 mg/cm² (Vanzant et al., 1998). Due to a rumen size limitation on the number of replicated bags, a larger ratio was necessary to allow sufficient remaining sample for nutrient analyses after incubation. Therefore, a pilot study was conducted to compare 10 and 12.5 mg/cm² sample-to-surface area ratios. Bags containing 1.00 or 1.25 g of ground starter were inserted into a ruminally cannulated calf that was not part of the study and then removed after 9 and 24 h. The DM disappearance (%) of the 2 sample-to-surface area ratios was compared and found to be identical; therefore, the larger ratio was used for the study.

Dacron bags containing either PEL or TEX substrates were placed inside a nylon mesh bag with a drawstring closure (Medela Inc., McHenry, IL; 12.7 $\times$ 17.8 cm, 23 mm² porosity) and soaked in water (39°C, 10 min) before insertion into the rumen at the time of starter feeding. The drawstring closure of the nylon mesh bag was closed and an additional nylon string (~60 cm) was tied around the drawstring and remained on the outside of the rumen. The mesh bag was folded end over end to allow passage through the smaller cannula (28 mm i.d.). Folding was not necessary for larger cannulas (51 mm i.d.). Two in situ bags of each diet were included per time point. Each calf received a total of 8 Dacron bags/d. Bags (n = 2/diet) were removed after 9 or 24 h by using the external nylon string to pull the mesh bag opening through the cannula. The drawstring was released and Dacron bags were removed individually using blunt needlenose pliers. After larger cannulas were fitted, mesh bags could be easily externalized and Dacron bags removed using gloved hands. After removal from the rumen, bags were rinsed 10 times by manual swirling in a bucket (3.78 L) of clean cold water for 2 min/rinse (Vanzant et al., 1998). Two additional bags containing each diet were soaked in water (39°C, 10 min) and then subjected to the cold-water rinsing procedure without insertion into the rumen to determine nutrient disappearance in response to presoaking and rinsing only. Following the final rinse cycle, bags were squeezed to remove excess water, dried at 55°C, and weighed to determine DM disappearance. This process was repeated in all calves on 3 consecutive days/wk beginning at 5 wk and repeated every other week through wk 15 (n = 6 wk). To ensure sufficient sample residue

Table 1. Nutrient composition of diets¹ designed to cause and prevent rumen acidosis in calves²

Item, % of DM unless noted	PEL	TEX
Ingredient		
Ground corn grain	49.4	—
Whole corn grain	—	37.1
Extruded corn grain	5.0	—
Soybean meal	22.1	—
Protein concentrate mix	—	33.2
Ground oats	6.2	—
Whole oats	—	10.1
Cottonseed hull pellets	—	12.3
Wheat middlings	5.2	—
Canola meal	3.3	—
Soybean oil	—	0.9
Molasses	3.0	3.3
Calcium	1.6	—
Starch	1.0	—
Salt	0.5	—
Yeast	0.1	0.1
Energy and mineral premix	2.8	3.0
Nutrient		
DM, ³ %	86.5	89.0
CP ³	21.7	19.5
Ether extract	3.72	4.48
NDF ³	15.1	25.3
ADF	5.39	13.5
NFC	57.8	48.1
Starch ³	42.7	35.3
Sugar	5.56	6.17
Ash ³	9.02	5.85

¹Starter diets were a complete pellet (PEL; 42% starch, 13% NDF) or texturized feed (TEX; 31% starch, 22% NDF).

²Values are estimates provided by the manufacturer unless otherwise noted.

³Values were determined independent of the manufacturer using described laboratory techniques.

for analyses, dried in situ samples from different days were composited, resulting in 24 samples/calf (2 diets $\times$ 2 time points $\times$ 6 wk). Total fecal collection occurred on the same days on which the in situ procedure was performed. Feces were scraped from the rubber mats into individual collection containers, composited by calf over 24 h, weighed, mixed, subsampled, and stored at -20°C for further analysis.

Sample Analyses

Composited starter samples, individual daily refusals, and fecal subsamples were thawed, dried in a forced-air oven at 55°C for 48 to 72 h, and ground through a 1-mm screen using the Wiley mill described previously. Dried ground fecal samples were composited to provide a single sample per calf per sampling week. Portions of ground feed, feces, and in situ residues were placed in aluminum pans and dried at 105°C for 24 to 48 h to determine DM concentration.

Neutral detergent fiber concentrations in samples of feed, feces, and in situ residues were determined using the filter bag technique and associated batch procedures outlined by Ankom Technology Corp. (Macedon, NY) for an Ankom200 Fiber Analyzer. Briefly, F-57 filter bags (Ankom Technology Corp.) containing 0.5 g of ground sample were subjected to neutral detergent digestion with sodium sulfite and α -amylase (75 min) and 3 subsequent 5-min rinses with boiling water. Thereafter, bags were soaked for 3 min in acetone, dried overnight at 105°C , and weighed. Forage of known NDF concentration and an empty bag were included in each digestion as positive and negative controls, respectively. Samples were rerun when the difference between duplicate values exceeded 3.0 units of NDF (%). In situ samples were analyzed only once due to insufficient sample mass.

Nitrogen concentrations were determined in duplicate samples of feed, feces, and in situ residues by a rapid combustion procedure (method 990.03, AOAC, 1998; model TruMac CN; Leco Corp., St. Joseph, MI). Samples were rerun, as sample mass allowed, when the N concentration between duplicates differed by ≥ 0.15 percentage units.

Starch concentration was determined in samples of feed, feces, and in situ residues using methods described by Hall (2015) with additional 20-min incubations at 100 and 50°C . Final glucose concentration in digested samples was determined using a glucose autoanalyzer (model 2700D; Yellow Springs Instrument Co., Yellow Springs, OH). Pure cornstarch was included as a positive control. Starch recovery based on the cornstarch ranged from 93 to 97%.

Calculations

Whole-tract apparent digestibility coefficients for diet DM, NDF, N, and starch were calculated using the following equation:

$$1 - \frac{\text{mass of nutrient in feces}}{(\text{mass of nutrient offered} - \text{mass of nutrient refused})} \times 100\%.$$

Mass of each nutrient ingested included milk replacer intake for wk 5 and 7. No attempt was made to adjust for endogenous or urinary contributions to fecal N.

In situ ruminal disappearance for DM, NDF, N, and starch was calculated for each time point (0, 9, or 24 h) using the following equation:

$$1 - \frac{(\text{mass of nutrient remaining} / \text{mass of nutrient inserted}) \times 100\%.$$

Statistical Analysis

Following confirmation of normal distribution for each response variable via histograms, nutrient output and digestibility coefficient data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Whole-tract digestibility estimates, nutrient intake, and output data were analyzed as

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij},$$

where Y_{ij} is the dependent variable, μ is the overall mean; α_i is the fixed effect of dietary treatment, where i = PEL or TEX; β_j is the fixed effect of age, where j = every other week from wk 5 through 15; and ε_{ij} is the error associated with the ij measurement taken j week from calves on i treatment. Ruminal degradability estimates were divided into separate data sets by time point (0, 9, and 24 h) and analyzed as

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean; α_i is the fixed effect of substrate, where i = PEL or TEX; β_j is the fixed effect of age, where j = every other week from wk 5 through 15; γ_k is the fixed effect of basal diet, where k = PEL or TEX; and ε_{ijk} is the error associated with the ijk measurement taken from i substrate during j week from calves consuming k diet. Only α_i and β_j effects were included to model

nutrient disappearance at 0 h because these bags were not inserted into calves.

The effect of sampling day on DM disappearance was determined using a model including the fixed effects of sampling day, substrate, age, and basal diet and all 2-way interactions. The interaction of sampling day with age and substrate was tested and removed due to insignificance. Other 3- and 4-way interactions were not included to avoid overspecification of the model and due to a potential lack of meaningful interpretation. Calf was included as a random effect.

Week was included in each whole-tract, 9-h, and 24-h disappearance model as a repeated effect with calf or calf within diet as the subject. Day was included as a repeated effect with calf within the week by substrate interaction as the subject. Variance component structure was used to form covariance matrices. Linear, quadratic, and cubic contrasts were reported to determine the pattern of the week of age effect. Individual comparisons of day within week were made using Tukey-adjusted P -values. Significance was declared when $P \leq 0.05$; tendencies are discussed when $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

General Observations

Several limitations observed during this trial were (1) a small rumen capacity for holding in situ bags, (2) small cannula size dictated by small body size, and (3) significant infiltration of hair into in situ bags during incubation. Small rumen capacity and small cannula size necessitated reduced initial samples and, therefore, limited residues for postincubation analyses. This was exacerbated by the highly digestible nature of the diets. Average 24-h DM disappearance across all weeks was 81.6 and 74.2% for PEL and TEX diets, respectively. Less digestible feedstuffs would result in greater residual sample mass. Increasing the ratio of sample mass: surface area of bags, inserting duplicate bags for removal at each time point, and compositing bags by week (3 d/wk) provided sufficient sample for determination of DM, NDF, N, and starch concentrations. Small cannula size also made removal of bags difficult. Needlenose pliers allowed all bags to be removed; however, the process was time consuming, and several bags were ripped and sample was lost in the process. Insertion of 51-mm i.d. cannulas between wk 7 and 9 alleviated the limitation of cannula size and allowed easy insertion and removal of bags. Use of these cannulas is highly recommended for further studies using this method.

The amount of N contamination from hair was estimated by comparing values from duplicate samples tested with or without manual removal of visible hairs.

Nitrogen concentration was decreased by an average of 12% (maximum = 56%) by removal of visible hairs. Hair contamination may have also inflated the ash value, but this was not estimated. Hair consumption by calves in this study may have been increased due to limited physically effective fiber, especially in the PEL diet. This diet was formulated to cause acidosis to achieve the objectives of a separate study. Physically effective fiber is an important dietary component for regulating rumen function, including the formation of a rumen mat, normal chewing behavior, and flow of saliva, which moderates rumen pH (Zebeli et al., 2012). When rumen acidosis was induced in adult cattle offered a TMR with particles of various sizes, cattle sorted to select for larger particles, thereby increasing physically effective fiber intake (Kmicikewycz and Heinrichs, 2015). Calves in the current study experienced rumen acidosis (rumen pH ≤ 5.6 ; Gozho et al., 2007) per the objectives of a concurrent study. Mean rumen pH $\pm$ standard error (minimum, maximum) was 5.4 ± 0.24 (3.3, 7.2) and 5.6 ± 0.24 (3.5, 6.8) for PEL and TEX calves, respectively (data not shown). Calves had no opportunity to increase physically effective fiber intake and may have resorted to self-grooming to increase saliva flow. No attempts were made to quantify particle size of rumen contents; however, gross observations throughout the study revealed the absence of a rumen mat in calves fed the PEL diet. Rumen contents in these calves appeared to be a homogeneous, thick liquid mixture and variably foamy, whereas, calves fed the TEX diet showed a distinct solid and liquid separation free of foam. Excessive self-grooming may also be a sign of boredom. In a review of animal behavior, Albright (1987) identified excessive coat licking as a concern for calves housed individually without bedding. Hair infiltration may be less dramatic in other environments or with different diets, though future researchers should be aware of the potential for underestimating ruminal N disappearance.

Whole Tract

Total intake and fecal excretion of DM, NDF, N, and starch are given in Table 2 with P -values for the main effect of dietary treatment and contrasts for the age effect. Intake and fecal excretion of all measured nutrients increased from wk 5 through wk 15 and were greater for TEX calves ($P \leq 0.01$). Intake of NDF increased more rapidly in TEX calves ($P < 0.01$), likely due to a greater NDF concentration in the TEX diet (25.3 vs. 15.1%). Quadratic effects of age were observed for intake ($P \leq 0.02$) but not fecal output ($P \geq 0.11$) of each measured nutrient. Fecal excretion of each measured nutrient increased more rapidly in TEX calves ($P \leq$

0.03). A cubic effect was observed for fecal starch excretion ($P < 0.01$) and was most dramatic in TEX calves ($P = 0.03$). Increases in intake and fecal excretion are expected as calf body size also increased throughout the study. Mean BW in wk 5 and 15 was 42.5 and 122.9 kg, respectively, for PEL calves and 44.4 and 149.8 kg, respectively, for TEX calves (data not shown).

Whole-tract apparent digestibility coefficients for DM, NDF, N, and starch and percentage of starch in the feces are given in Table 3. Digestibility of DM, NDF, N, and starch decreased linearly ($P \leq 0.01$) from wk 5 to 15. Decreases occurred or tended ($P \leq 0.09$) to occur more rapidly in calves fed the TEX diet with the exception of apparent N digestibility. Quadratic effects by age were observed for DM digestibility ($P < 0.01$), with a more pronounced quadratic pattern in PEL calves ($P = 0.02$). Data from Malmuthuge et al. (2013) indicated that calves may experience greater gut permeability at the time of weaning, which could explain the loss in DM digestibility observed here.

A recent meta-analysis reported ranges of 67.6 to 85.2%, 63.9 to 84.9%, 56.1 to 70.7%, and 95.1 to 99.0% for DM, CP, NDF, and starch digestibility coefficients, respectively, for calves postweaning through 16 wk of age (Hu et al., 2018). Whole-tract digestibility coefficients reported in Table 3 for DM, apparent N, and starch, with few exceptions in PEL calves, fit within these reported ranges. Digestibility coefficients for NDF in the current study are 20 to 30 percentage units lower than the reported range. This could be due to differences in sources of NDF or estimation methods. Studies included in the meta-analysis used marker systems (Hill et al., 2016; Suarez-Mena et al., 2011), whereas the current study attempted direct collection of NDF in feces and subtraction from NDF intake.

Percentage of starch in the feces was greater ($P < 0.01$) in calves fed the TEX diet and followed quadratic patterns ($P = 0.01$), peaking in wk 9 and then decreasing for both diets. Steele et al. (2017) also observed increased fecal starch percentage in calves immediately postweaning. Cubic effects were also observed for fecal starch percentage in both treatments due to increases observed in wk 15. Fecal starch increased more dramatically for TEX calves, which resulted in a cubic by treatment interaction ($P = 0.05$) in whole-tract starch digestibility. These differences in the percentage of starch in the feces between diets could be related to intake differences and possibly inadequate chewing to break apart the whole grains included in the TEX diet. The range of fecal starch percentage reported for TEX calves is narrow compared with data from Dennis et al. (2017), which ranged from approximately 1 to 18% in calves fed starter diets containing whole grains.

Decreases in whole-tract DM, NDF, and apparent N digestibility over time may be explained by changes in passage rate. Many studies have documented that the extent of digestion decreases as passage rate through the gastrointestinal tract increases (VanSoest, 1983). Liquid passage rate (S. L. Gelsinger, W. K. Coblenz, G. I. Zanton, R. K. Ogden, and M. S. Akins, unpublished data) increased in TEX calves from 9.0 to 18.0%/h between wk 6 and 8. This response coincides with increasing DMI and decreasing whole-tract DM digestibility between wk 7 and 9. Digestibility of DM, NDF, and apparent digestibility of N continued to decrease through wk 15; likewise, liquid passage rate remained high (18.0 to 16.3%/h) through wk 14. Starch digestibility followed quadratic and cubic patterns and was high (≥ 95.3) in all measurement weeks, thus indicating calves' capacity to digest starch despite changes in passage rate. Changes in digestibility over time were less dramatic for calves offered the PEL diet. This could be related to differences in feeding behavior, rumen or intestinal environment, or dietary characteristics, including physical form or ingredient composition.

In Situ

Nutrient disappearance from in situ bags after a 10-min incubation in water at 39°C followed by rinsing is shown in Table 4. Greater proportions ($P = 0.01$) of DM, NDF, and N were lost from the PEL diet, indicating that PEL was generally more soluble, likely due to ingredient differences, compared with TEX. The proportion of starch lost during rinsing did not differ between diets ($P = 0.27$).

Ruminal degradability values for DM, NDF, N, and starch from PEL and TEX substrates after 9- and 24-h incubations are given in Table 5 with P -values for effects of substrate and linear and quadratic contrasts for age. No interactions existed between substrate and basal diet for DM, NDF, N, or starch disappearance after 9- or 24-h incubation ($P \geq 0.11$). Disappearance of DM and NDF after 9- and 24-h incubation in the rumen was greater ($P \leq 0.01$) for the PEL diet. Starch and N disappearance did not differ between diets ($P \geq 0.13$) regardless of incubation time. Disappearance of DM, N, and starch after 9-h incubations increased linearly ($P \leq 0.01$) from wk 5 to 15. Quadratic contrasts were also observed for DM and N disappearance after 9-h incubations ($P \leq 0.01$). Disappearance after 24-h incubations increased linearly from wk 5 to 15 for DM, NDF, N, and starch ($P \leq 0.02$). There were no interactions of diet with linear or quadratic effects of age for any nutrient ($P \geq 0.20$). Cubic effects of age were observed for 9-h DM and NDF disappearance and

Table 2. Least squares means of total intake and fecal excretion of DM, NDF, N, and starch during collection periods made every other week from wk 5 through 15 of age in calves fed 2 starter diets¹

Item	Age, wk						P-value ²							
	5	7	9	11	13	15	SE	Trt	Lin	L × T	Quad	Q × T	Cub	C × T
Nutrient intake, ³ g/d														
DM														
PEL	931	1,394	2,064	2,524	2,430	3,203	174.1	<0.01	<0.01	0.64	0.01	0.23	0.42	0.58
TEX	1,597	2,104	3,156	3,307	3,573	3,863								
NDF														
PEL	69	170	301	368	354	467	40.0	<0.01	<0.01	<0.01	<0.01	0.09	0.28	0.97
TEX	287	457	767	798	848	940								
N														
PEL	32	47	69	84	81	107	5.2	<0.01	<0.01	0.62	0.02	0.28	0.47	0.44
TEX	50	64	94	99	108	115								
Starch														
PEL	196	481	850	1,040	1,002	1,320	64.3	<0.01	<0.01	0.23	0.01	0.40	0.21	0.48
TEX	381	634	1,070	1,124	1,218	1,311								
Fecal nutrient output, ³ g/d														
DM														
PEL	107	202	376	482	452	611	65.6	<0.01	<0.01	<0.01	0.56	0.57	1.00	0.58
TEX	324	410	684	919	976	1,235								
NDF														
PEL	38	85	164	195	201	271	39.8	<0.01	<0.01	<0.01	0.91	0.58	0.88	0.65
TEX	167	239	371	506	574	720								
Apparent N ⁴														
PEL	3	7	12	15	13	17	2.5	<0.01	<0.01	0.03	0.11	0.67	0.60	0.55
TEX	10	13	18	25	23	30								
Starch														
PEL	1	4	14	14	6	9	4.5	<0.01	<0.01	<0.01	0.56	0.09	<0.01	0.03
TEX	5	13	33	31	24	61								

¹PEL = pelleted starter (42.7% starch, 15.1% NDF); TEX = texturized starter (35.3% starch, 25.3% NDF).
²Trt = fixed effect of starter diet; Lin = linear contrast for the effect of week; L × T = interaction between linear contrast for week and starter diet treatment; Quad = quadratic contrast for the effect of week; Q × T = interaction between quadratic contrast for week and starter diet treatment; Cub = cubic contrast for the effect of week; C × T = interaction between cubic contrast for week and starter diet treatment.
³Starter refusals and total fecal production were collected and composited over a 3-d period each sampling week.
⁴Values are not adjusted for possible urine contamination.

Table 3. Least squares means of whole-tract digestibility coefficients for DM, NDF, N, and starch and fecal starch based on collection periods made every other week from wk 5 through 15 of age in calves fed 2 starter diets¹

Item, %	Age, wk						P-value ²							
	5	7	9	11	13	15	SE	Trt	Lin	L × T	Quad	Q × T	Cub	C × T
DM														
PEL	89.0	85.8	81.4	80.9	81.4	81.1	1.68	<0.01	<0.01	0.09	<0.01	0.02	0.66	0.40
TEX	79.6	80.8	78.2	72.2	73.1	68.0								
NDF														
PEL	45.6	50.7	44.1	47.3	43.0	42.7	5.72	0.07	<0.01	0.09	0.09	0.22	0.32	0.61
TEX	41.4	49.9	51.2	36.5	33.7	23.6								
Apparent N ³														
PEL	88.4	84.7	81.7	81.6	84.2	83.5	2.14	<0.01	<0.01	0.52	0.14	0.02	0.60	0.33
TEX	79.5	80.1	79.9	74.5	78.4	74.1								
Starch														
PEL	99.2	99.0	98.4	98.7	99.5	99.3	0.41	<0.01	<0.01	<0.01	0.52	0.13	<0.01	0.05
TEX	98.6	97.9	96.9	97.1	98.2	95.3								
Fecal starch														
PEL	1.36	2.10	3.82	2.84	1.16	1.62	0.63	<0.01	0.16	0.04	0.01	0.26	<0.01	0.14
TEX	1.66	3.23	4.96	3.61	2.25	5.16								

¹PEL = pelleted starter (42.7% starch, 15.1% NDF); TEX = texturized starter (35.3% starch, 25.3% NDF).

²Trt = fixed effect of starter diet; Lin = linear contrast for the effect of week; L × T = interaction between linear contrast for week and starter diet treatment; Quad = quadratic contrast for the effect of week; Q × T = interaction between quadratic contrast for week and starter diet treatment; Cub = cubic contrast for the effect of week; C × T = interaction between cubic contrast for week and starter diet treatment.

³Values are not adjusted for possible urine contamination.

24-h NDF disappearance. In these cases, nutrient disappearance increased through weaning (8 wk) and then decreased for 1 to 2 wk and increased again in wk 15. This could demonstrate a delayed response to weaning, but more data are needed to confirm this observation and determine possible mechanisms.

Vazquez-Anon et al. (1993) also demonstrated increases in rumen available DM and CP in calves from 2 through 8 wk postweaning for each feed ingredient investigated in situ. Likewise, Lalles and Poncet (1990) demonstrated increased ruminal digestion of soybean meal OM as calves aged.

Increasing ruminal disappearance while whole-tract digestibility was concurrently decreasing is likely related to particle size differences between the 2 measurements and the rate of passage of nutrients through the gastrointestinal tract. Smaller particle size increases the

surface area available for digestion, thereby increasing the rate of digestion (Wadhwa et al., 1998). Starter diets were ground through a 2-mm screen before insertion into the rumen. This was done to mimic chewing behavior. It is possible that a larger grind size may be more representative of calf chewing behavior, but no attempt was made to measure rumen particle size in this study. If particle size after chewing was >2 mm, this could explain some of the difference in digestion, especially in calves fed the TEX diet, which included whole, unprocessed grains.

The effects of passage rate on whole-tract digestibility have been discussed. Single-endpoint in situ digestibility estimates are mostly independent of the effects of passage rate because the incubation duration is predetermined; thus, in situ measurements can provide insight into rumen fermentation capabilities largely uncoupled from changes in passage rate. The extent of DM, NDF, N, and starch disappearance in the rumen increased with age. Apparent N digestion from the PEL diet increased most dramatically from 50.0 to 62.6% and 67.7 to 79.6% after 9 and 24 h, respectively. The greatest changes observed for the TEX diet were a 5.3- and 6.9-percentage unit increase in 9-h starch and 24-h N disappearance, respectively. Disappearance of DM increased by 6.3 and 5.1 percentage units for PEL and 4.7 and 5.4 percentage units for TEX after 9 and 24 h of ruminal incubation, respectively. These changes indicate that a calf's ability to digest nutrients increases from 5 to 15 wk of age; therefore, there may be potential to design specific rations for calves based

Table 4. Least squares means of nutrient disappearance of 2 starter diets¹ following 10-min incubation at 39°C and 10 subsequent 2-min rinses in cold water

Item, %	Diet		SE	P-value ²
	PEL	TEX		
DM	48.3	44.1	1.1	0.01
NDF	30.4	20.7	2.7	0.01
N	39.0	31.5	2.1	0.01
Starch	52.8	59.7	3.0	0.27

¹PEL = pelleted starter (42.7% starch, 15.1% NDF); TEX = texturized starter (35.3% starch, 25.3% NDF).

²Effect of starter diet.

Table 5. Least squares means of ruminal disappearance of DM, NDF, N, and starch following 9- and 24-h in situ incubation in calves fed 1 of 2 starter diets¹ during wk 5 through 15 of age

Item, %	Age, wk						SE	<i>P</i> -value ²						
	5	7	9	11	13	15		Trt	Lin	L × T	Quad	Q × T	Cub	C × T
9-h incubation														
DM														
PEL	65.1	69.9	70.1	69.0	69.6	71.4	0.95	<0.01	<0.01	0.85	<0.01	0.59	0.01	0.09
TEX	59.8	60.4	65.7	63.8	63.8	64.5								
NDF														
PEL	42.3	50.1	41.4	47.5	40.0	48.1	2.58	<0.01	0.38	0.57	0.85	0.58	<0.01	0.94
TEX	34.9	36.9	44.4	35.8	36.3	41.4								
Apparent N ³														
PEL	50.0	60.9	61.1	62.1	61.6	62.6	1.79	0.41	<0.01	0.40	<0.01	0.55	0.56	<0.01
TEX	58.7	55.2	63.6	61.5	66.5	60.0								
Starch														
PEL	76.9	75.9	80.5	75.8	81.2	81.7	2.24	0.13	<0.01	0.61	0.91	0.51	0.58	0.40
TEX	77.7	76.9	79.9	82.9	83.0	83.0								
24-h incubation														
DM														
PEL	78.8	80.7	81.5	82.5	82.4	83.9	0.90	<0.01	<0.01	0.76	0.48	0.83	0.22	0.91
TEX	71.6	71.8	75.7	74.0	75.0	77.0								
NDF														
PEL	45.1	47.5	49.8	52.4	45.8	52.4	2.76	<0.01	0.02	0.87	0.27	0.78	<0.01	0.14
TEX	33.6	39.0	47.6	37.3	36.9	44.8								
Apparent N ³														
PEL	67.7	68.6	73.3	76.7	78.6	79.6	2.29	0.37	<0.01	0.26	0.57	0.20	0.26	0.99
TEX	73.3	70.4	75.1	72.1	80.6	80.2								
Starch														
PEL	95.7	96.2	96.8	96.8	97.0	97.7	0.95	0.33	0.02	0.91	0.69	0.80	0.89	0.55
TEX	96.5	95.9	97.9	97.4	97.9	97.7								

¹Diet evaluated in situ; PEL = pelleted starter (42.7% starch, 15.1% NDF); TEX = texturized starter (35.3% starch, 25.3% NDF).

²Trt = fixed effect of starter diet; Lin = linear contrast for the effect of week; L × T = interaction between linear contrast for week and starter diet treatment; Quad = quadratic contrast for the effect of week; Q × T = interaction between quadratic contrast for week and starter diet treatment; Cub = cubic contrast for the effect of week; C × T = interaction between cubic contrast for week and starter diet treatment.

³Values are not adjusted for hair contamination.

on their ability to utilize nutrients. The age response in DM disappearance was relatively small for the diets used in this study; however, greater changes may be observed with more complex diets and inclusion of forages.

The basal diets consumed by calves did not affect 9-h disappearance of any nutrient ($P \geq 0.19$), and interactions between the basal diet and other fixed effects were not significant at any time point ($P \geq 0.06$; data not shown). The main effect of basal diet on 24-h ruminal disappearance of DM, NDF, N, and starch is shown in Table 6. Basal diet did not affect N or NDF disappearance ($P \geq 0.18$), but starch and DM disappearance were both reduced ($P \leq 0.05$) by about 1.0 percentage unit in calves consuming the PEL diet. These data indicate that the rumen environment created by consumption of the PEL diet may have affected the extent of ruminal fermentation, but the magnitude of these responses is small.

Spreading time points across multiple days can allow sufficient characterization of digestibility curves without inundating the rumen with in situ bags, thereby

raising concerns about fermentation being affected. This procedure assumes that the rumen environment remains relatively static across multiple days. Before this study, it was unclear whether increasing physiological development would cause distinguishable differences in in situ nutrient disappearance during a 3-d period. The *P*-values reported in Table 7 are a test of the null hypothesis that the value obtained for DM disappear-

Table 6. Least squares means of the 24-h in situ ruminal disappearance of nutrients in calves consuming different basal diets

Item, %	Diet ¹		SE	<i>P</i> -value ²
	PEL	TEX		
DM	77.4	78.4	0.4	0.05
NDF	43.3	45.4	1.1	0.18
Apparent N ³	74.5	74.8	1.0	0.82
Starch	96.4	97.5	0.4	0.04

¹PEL = pelleted starter (42.7% starch, 15.1% NDF); TEX = texturized starter (35.3% starch, 25.3% NDF).

²Main effect of basal diet on ruminal nutrient disappearance.

³Unadjusted for hair contamination.

Table 7. Least squares means of the in situ ruminal DM disappearance (%) of 2 calf starter diets on each day of a 3-d sampling period in calves consuming 2 different calf starter diets

Time, ¹ h	Day ²			SE	P-value ³	
	1	2	3		Lin	Quad
0	45.7	46.4	46.6	0.54	0.21	0.70
9	65.0	65.7	67.2	0.37	<0.01	0.30
24	76.6	76.7	80.5	0.43	<0.01	<0.01

¹Duration of intraruminal incubation.²Sampling day; sampling occurred during wk 5, 7, 9, 11, 13, and 15.³Lin = linear contrast for effect of day; Quad = quadratic contrast for effect of day.

ance is not different between days. Loss of DM from in situ bags during the rinsing procedure (0-h samples) was not different between days ($P \geq 0.21$); however, the null hypothesis was rejected for 9- and 24-h incubations where DM disappearance increased ($P < 0.01$) linearly from d 1 to 3. This difference in values obtained on different days could indicate that a calf's capacity to digest DM increases at a measureable rate during a 3-d period. However, because each calf received bags on each day, it is not possible from these data to determine what proportion of the observed differences is due to the calves' adaptation to placement of bags in the rumen. Nonsignificant quadratic contrast ($P = 0.30$) indicates that values were not different between d 1 and 2 for 9-h disappearance. The quadratic relationship observed in 24-h DM disappearance is likely caused by the large difference between d 2 and d 3 values. Therefore, it is recommended that subsequent studies limit the spread of bags for a single digestibility curve to within a 2-d period.

Further research is required to (1) determine appropriate end-point incubation intervals after which no change is observed in nutrient disappearance for each nutrient of interest and (2) determine whether carryover effects or progressive rumen development are largely responsible for differences between days within week that were observed in these data. Furthermore, extending these results with common forages offered to calves may be a practically useful next step.

CONCLUSIONS

Whole-tract apparent DM, N, and NDF digestibility decreased over time, whereas ruminal in situ disappearance after 24 h increased for each measured nutrient. Whole-tract DM, N, and starch disappearance were greater for calves fed a pelleted, high-starch diet (PEL) compared with a texturized, whole-grain, moderate-starch diet (TEX). Likewise, 9- and 24-h in situ disappearance of DM and NDF was greater for PEL diets than for TEX diets. Dietary differences and differences

related to the basal diet were measureable using whole-tract and in situ methods in calves. Based on these data, there is potential to design specific rations or feed processing methods for calves based on their ability to utilize nutrients.

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