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Fermentation in the Small Intestine Contributes Substantially to Intestinal Starch Disappearance in Calves[1,2]

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Abstract

Background: The proportion of starch disappearing from the small intestinal lumen is generally lower in ruminants than in monogastric animals, and there are indications that the starch digestion capacity in ruminants is limited.

Objectives: Milk-fed calves were used to study the rate-limiting enzyme in starch hydrolysis and to quantify starch fermentation in ruminants.

Methods: Forty male Holstein-Friesian calves were fed milk replacer containing either lactose (control) or 1 of 4 corn starch products. The following starch products differed in the enzyme ratios required for their complete hydrolysis to glucose: gelatinized starch [α-amylase and (iso)maltase], maltodextrin [(iso)maltase and α-amylase], maltodextrin with α-1,6-branching (isomaltase, maltase, and α-amylase), and maltose (maltase). In the adaptation period, calves were stepwise exposed to an increasing dose of the starch product for 14 wk to allow maximal adaptation of all enzyme systems involved. In the experimental period, apparent total tract and ileal starch product disappearance, total tract starch product fermentation, and α-amylase, maltase, and isomaltase activities were determined at 18% inclusion of the starch product.

Results: Maltase and isomaltase activities in the brush border did not increase for any of the starch product treatments. Luminal α-amylase activity was lower in the proximal (3.9 ± 3.2 and 2.7 ± 1.7 U/mg Co for control and starch product calves, respectively) but greater in the distal small intestine of starch-fed calves than in control calves (0.0 ± 0.0 and 6.4 ± 1.5 U/mg Co for control and starch product calves, respectively; means ± SEs for control and means ± pooled SEMs for starch product treatments). Apparent ileal (61.6% ± 6.3%) and total tract (99.1% ± 0.4%) starch product disappearance did not differ between starch product treatments, suggesting that maltase activity limits starch digestion in ruminants. Total tract starch product fermentation averaged 414 ± 43 g/d, corresponding to 89% of intake, of which half was fermented before the terminal ileum, regardless of starch product treatment.

Conclusion: Fermentation, rather than enzymatic digestion, is the main reason for small intestinal starch disappearance in milk-fed calves. *J Nutr* 2015;145:1147–55.

Keywords: starch digestion, starch fermentation, milk-fed calf, ruminant, maltase, α-amylase, isomaltase

Introduction

Adult ruminants usually absorb only small amounts of glucose from starch, because microbes in the rumen readily degrade starch, resulting in the production of volatile FAs (VFAs)[9]. Required glucose is mainly synthesized de novo from propionic acid or amino acid precursors (1). Mammalian neonates depend for their energy supply mainly on lactose and fat provided by the milk of the mother. After weaning of monogastric animals, the carbohydrate supply shifts from lactose to starch, resulting in a decreased lactase activity and an increased maltase activity (2, 3). In ruminants, weaning causes a shift from lactose to VFAs as

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[9] Abbreviations used: atom%, atom percentage; CON, control; DM, dry matter; GS, gelatinized starch; ISO, International Organization for Standardization; MD, maltodextrin; MDB, maltodextrin with a high level of α-1,6-branching; MT, maltose; SP, starch product; VFA, volatile fatty acid.

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the main energy supply. When glucose demand is high (e.g., in lactating cows), nutritional strategies are applied to maximize the duodenal flow of starch, escaping ruminal degradation. It is assumed that this starch is enzymatically hydrolyzed, resulting in the absorption of glucose from the small intestine. It is, however, questionable whether a large portion of this starch is absorbed as glucose in ruminants, because the portal glucose appearance was substantially lower than small intestinal starch disappearance in steers (4).

To assess small intestinal starch disappearance in ruminants, the flux of starch into the small intestine needs to be quantified. Available methods include measuring the duodenal flux of starch or infusing starch directly into the abomasum, both of which require the use of cannulas. Alternatively, milk-fed calves could be used to administer starch directly into the small intestine. Closure of the esophageal groove of calves allows the milk replacer to flow directly into the abomasum and subsequently into the small intestine (5). By adding starch to the milk replacer fed to preruminant calves, their capacity to digest starch can be assessed. Previous studies in milk-fed calves indicated that ileal disappearance of partially acid-hydrolyzed starch was low (60% of intake) compared with that of lactose (97% of intake) (6). Similarly, the ileal disappearance of corn starch in steers (66% of abomasal infusion) (4) was low compared with that in pigs (99% of intake) (7). In addition, there are indications that fermentation contributes to starch disappearance from the small intestinal lumen of ruminants. For instance, ileal pH decreased linearly with increasing infusion amount of raw corn starch or partially hydrolyzed corn starch in the abomasum of steers (8, 9). Hence, the capacity to enzymatically hydrolyze starch may be limited in ruminants.

Pancreatic α-amylase activity appears to be greater in omnivores (10, 11) than in ruminant steers (12), although the methods for determining α-amylase activity often vary, which complicates comparisons between studies. Maltase activity was 6.5 times greater (10, 11) and isomaltase activity was 44 times greater (11) in omnivores than in preruminant calves fed a milk replacer containing 3% starch (13). There are indications that pancreatic α-amylase activity (as reviewed in 14) or brush border enzyme activities (4) are limiting factors for starch hydrolysis in ruminants.

The objectives of the current study were as follows: *1*) to determine the rate-limiting enzyme in starch hydrolysis and *2*) to quantify starch fermentation in milk-fed calves. Milk-fed calves were also considered as a model for ruminants. Four products derived from starch were selected to be included in the milk replacer. These starch products (SPs) differed in degree of polymerization and branching and consequently required different ratios of starch-degrading enzymes for their complete hydrolysis to glucose. The combination of apparent ileal SP disappearance, intestinal SP fermentation, and activities of starch-degrading enzymes in calves fed these selected SPs was expected to provide insight into the rate-limiting enzyme, after allowing ample time for enzyme systems to adapt to the different SPs.

Methods

Experimental design and animals. Four industrial products derived from starch (hereafter referred to as SPs) were selected; gelatinized starch (GS; Tate & Lyle Europe), maltodextrin (Tereos Syral; dextrose equivalent = ~13), maltodextrin with a high level of α-1,6-branching (MDB; Tereos Syral; dextrose equivalent = ~9), and maltose (Tereos Syral). The SP characteristics have been described in detail (15). These SPs differed in degree of polymerization and branching and consequently required different ratios of starch-degrading enzymes for their complete hydrolysis to glucose. GS requires α-amylase and (iso)maltase; maltodextrin requires (iso)maltase and α-amylase; MDB requires isomaltase, maltase, and α-amylase; and maltose requires maltase only. A proportional increase in α-amylase requirement is expected with an increase in degree of polymerization.

The experiment was submitted to and approved by the Animal Care and Use Committee of Wageningen University. Forty male Holstein-Friesian calves 13 wk of age (104 ± 1.1 kg) received 1 of 5 milk replacer treatments varying in carbohydrate source as their only source of nutrients. The control (CON) treatment contained lactose as the only carbohydrate source. In the other treatments, 1 of 4 SPs was included at 18% in the milk replacer at the expense of lactose.

The experiment consisted of an adaptation and an experimental period. In the adaptation period, calves were exposed to the dietary treatments for 14 wk. Within these 14 wk, the amount of SP inclusion gradually increased (until a maximum of 36%) and fecal dry matter (DM) content and pH were measured to assess the maximum SP inclusion amount without digestive problems for each individual calf (15). The median of this maximum SP inclusion amount was 18% and was applied in the experimental period. The experimental period started when calves were 27 wk of age (217 ± 3.9 kg) and lasted 3 wk. Measurements on total tract and ileal nutrient disappearance, total tract SP fermentation, blood glucose response to a meal, and enzyme activities were performed during the experimental period. It was hypothesized that differences in these measurements between SP treatments could be used to identify the rate-limiting enzyme system in starch digestion.

Diets and housing. Calves were fed individually according to their metabolic weight at twice the metabolizable energy requirements for maintenance, with estimated metabolizable energy content based on the CON treatment. The metabolizable energy requirement for maintenance was set at 460 kJ · $kg^{-0.75}$ · d^{-1} (16). Milk replacer was mixed with water to obtain a concentration of 154 g/kg and was supplied to the calves at a temperature of ~42°C in a bucket at 0600 and 1600 h in 2 equal portions. Ingredient and nutrient compositions have been described (15). In short, milk replacer contained 171 g/kg crude protein, 176 g/kg crude fat, 63 g/kg crude ash, 21 g/kg moisture, and 522 g/kg lactose for the CON treatment. For the SP treatments, 180 g lactose/kg was exchanged for SP on a wt:wt basis. All SPs originated from corn and differed in natural ^{13}C enrichment [1.0931 atom percentage (atom%) for GS, 1.0936 atom% for maltodextrin, 1.0919 atom% for MDB, and 1.0935 atom% for maltose] from lactose (1.0727 atom%) and the remaining part of the diet (1.0767 atom%). All diets included cobalt-EDTA as an indigestible marker (1.3 g Co-EDTA/kg milk replacer). Chromium chloride (7.69 g $CrCl_3$ hexahydrate) was added to the last milk replacer feeding before anesthesia. Solid feed was not provided, because this would lead to difficulties in estimating starch flow into the intestinal tract. Water was provided ad libitum.

Calves were housed in pairs (2.7 m^2/calf) on wooden, slatted floors. During the collection of feces for the measurement of total tract nutrient disappearance, calves were housed individually for 8 consecutive days. During this period, calves could change posture freely but could not turn around, facilitating the collection of feces. Lights were on from 0600 to 1800 h. The stable was mechanically ventilated, and the temperature and humidity were maintained at 5.2 ± 2.63°C and 94% ± 9.3%, respectively (both means ± SDs), during the experimental period.

Sample collection and measurements. Calves were weighed weekly and were habituated to individual housing for 4 d. Feces were collected semiquantitatively during 4 consecutive days to measure total tract nutrient disappearance and to calculate total tract SP fermentation on the basis of fecal ^{13}C excretion (17; see calculations). Feces were collected from the buckets underneath the slatted floor twice a day and were weighed, homogenized, sampled, and stored at −20°C until analysis.

To assess the appearance of glucose from lactose, GS, or maltose in peripheral blood, a pulse dose of naturally ^{13}C-enriched lactose (1.0917 atom%, included at 375 g/kg milk replacer) or naturally ^{13}C-enriched SP

(1.0930 atom%, included at 180 g/kg milk powder) was provided with the regular morning meal to CON, GS, and maltose (MT) calves, respectively. Blood samples were taken from the jugular vein by venipuncture at −30, 30, 60, 120, 180, 240, and 360 min after feeding and collected in heparin tubes. Plasma was harvested after centrifugation. Plasma samples were stored at −20°C until analysis of ^{13}C enrichment in plasma glucose. In addition to the CON treatment, the GS and MT treatments were selected for the measurement of ^{13}C enrichment of plasma glucose because these treatments differed most in degree of polymerization.

Postmortem, digestus, and mucosal scrapings were collected from gastrointestinal sections to assess ileal nutrient disappearance, passage of milk replacer, and enzyme activity. Calves received chromium chloride at 4 h before anesthesia by pentobarbital injection. Calves were lifted by the forelegs to prevent reflux of milk replacer from the abomasum into the rumen, and subsequently killed by exsanguination. After opening the abdominal cavity, the digestive tract was ligated at 5 positions to prevent digesta from flowing between gastrointestinal segments. Clamps were placed before the rumen, between the rumen and abomasum, after the abomasum, ~100 cm before the ileocecal valve, and just before the ileocecal valve; the gastrointestinal tract was then removed from the calf. The last ~100 cm of the small intestine was taken as the ileum. The reticulorumen, abomasum, ileum, and large intestine (colon and cecum) were separated from the small intestine. The length of the small intestine and ileum was recorded. The small intestine without ileum was spread out and divided into 2 equal segments, i.e., small intestine 1 and small intestine 2. The contents of each of the 3 small intestinal segments and of the reticulorumen, abomasum, and large intestine were collected quantitatively, weighed, homogenized, sampled, and stored at −20°C. Then, each small intestinal segment was cut open longitudinally at 50% of its length and rinsed with PBS. Mucosal scrapings were collected from ~80 cm^2 of each small intestinal segment by using a glass slide and were stored at −20°C. This complete process was finished within 20 min for each calf. The slaughter of all calves was performed in 3 d, and treatments were balanced over days.

Analytical procedures. Fecal and ileal digestus samples were thawed, pooled per calf, and homogenized. DM content in fresh feces was determined according to International Organization for Standardization (ISO) 6496 (18). DM content of feed samples was determined by vacuum drying to a constant weight at 80°C.

For the determination of nutrient disappearance, homogenized feces were subdivided and either oven-dried (for crude fat, ash, and cobalt analysis) or freeze-dried (for total glucose analysis). Ileal digesta were freeze-dried. Dry feces and ileal digesta were ground to pass a 1-mm screen and subsequently analyzed for DM content (18). Nitrogen content was determined in fresh feces and feed samples [Kjeldahl method (19)] and in freeze-dried ileal digesta [Dumas combustion method, Association of Analytical Communities 990.03 by using a Thermo Quest NA 2100 Nitrogen and Protein analyzer from Interscience (20)]. Crude fat content was determined after acid hydrolysis (21) in oven-dried feces and in feed samples. Crude ash content was determined in fecal samples according to ISO 5984 (22). For the determination of SP disappearance, total glucose content [Total Starch kit from Megazyme; Association of Analytical Communities 996.11 (20)] was determined in SP samples, freeze-dried feces, and freeze-dried ileal digesta. Total glucose was determined instead of starch because SPs were composed of starch-derived poly- and oligosaccharides and glucose (15). Analyzing starch content only could therefore underestimate the total amount of glucose present in the fecal or ileal samples originating from the SP. However, in these fecal and ileal samples, glucose originating from lactose could be present as well. Therefore, galactose was analyzed and it was assumed that the glucose originating from lactose could be calculated from the galactose content, assuming equimolar quantities of glucose and galactose.

Galactose and lactose concentrations were determined in fresh feces and fresh ileal digesta by using high-performance anion-exchange chromatography (Dionex ICS 3000; Dionex) according to Zhao et al. (23). First, 200 mg sample was suspended in 4 mL Milli-Q water (Merck Millipore), and samples were equilibrated overnight at 4°C. Enzymes were inactivated in a water bath at 100°C for 10 min. Samples were centrifuged for 10 min at 5000 × *g*. Subsequently, the supernatant was filtered through a 0.2-μm filter, diluted as required, and injected (10 μL). For galactose, samples were eluted (0.3 mL/min) by using water during 35 min with the addition of 0.5 mol/L NaOH postcolumn (0.1 mL/min). For lactose, samples were eluted (0.3 mL/min) by using a gradient of 0.1 mol/L NaOH to 0.4 mol/L sodium acetate in 0.1 mol/L NaOH during 15 min followed by 1 mol/L sodium acetate in 0.1 mol/L NaOH for 5 min.

Total carbon and carbon with an atomic mass of 13 g (^{13}C) were determined in the SPs and oven-dried feces after ball milling by using the isotope ratio MS combustion technique (Finnigan Delta V Advantage isotope ratio mass spectrometer; Finnigan MAT). Cobalt concentration in oven-dried feces and in freeze-dried ileal digesta and chromium concentration in the digesta of each gastrointestinal segment was analyzed by atomic absorption spectrophotometry (by using a SpectrAA 300 atomic absorption spectrophotomer; Varian B.V.). The recovery of chromium in each gastrointestinal segment was determined as an indicator of the passage rate of digesta and calculated from the quantity of digesta collected from each segment and its chromium concentration. Total chromium recovery was calculated as the sum of chromium recoveries in all segments.

Digestus samples of small intestine 1 and small intestine 2 were thawed and analyzed for α-amylase activity (Amylase Activity Assay Kit; Biovision). One unit of α-amylase represents the amount of α-amylase that hydrolyzes the substrate of the kit (ethylidene-para-nitrophenol-G7), resulting in 1 μmol nitrophenol/min at 25°C and pH 7.2. Luminal α-amylase activity was expressed as units per milligram of cobalt. Mucosal scrapings of 4 randomly selected calves per treatment were analyzed for lactase, maltase, and isomaltase activity by using the method of Dahlqvist (24). One unit of brush border enzyme represents the hydrolysis of 1 μmol disaccharide/min at 37°C. Protein concentration was analyzed in the mucosal scraping samples by using the bicinchoninic acid procedure (25). Brush border enzyme activity was expressed as units per gram of protein.

Blood plasma samples of CON, GS, and MT calves were analyzed for ^{13}C enrichment in glucose. Samples were derivatized by adding 1 mL ethanol to 100 μL of plasma followed by centrifugation (10 min at 400 × *g* at 4°C). The supernatant was evaporated under a constant stream of nitrogen at 60°C for 20 min. Subsequently, 50 μL pyridine and 100 μL acetic anhydride were added and mixed for 1 min by using a vortex, followed by acetylation overnight. Samples were solubilized with 1250 μL acetone and analyzed for ^{13}C enrichment in glucose by using GC combustion isotope ratio MS (GC Combustion III ThermoQuest Finnigan).

Calculations. Apparent total tract and ileal nutrient disappearance was calculated by using the following equation:

$$\text{Disappearance (\% of intake)} = \left[1-\left(\text{Co}_{\text{feed}}/\text{Co}_{\text{digesta}}\right) \times \left(\text{Nutrient}_{\text{digesta}}/\text{Nutrient}_{\text{feed}}\right)\right]\times 100 \quad (1)$$

where Co_{feed} is the cobalt concentration in the milk replacer (mg/kg DM), $\text{Co}_{\text{digesta}}$ is the cobalt concentration in the feces or digesta (mg/kg DM), $\text{Nutrient}_{\text{digesta}}$ is the nutrient concentration in the feces or digesta (g/kg DM), and $\text{Nutrient}_{\text{feed}}$ is the nutrient concentration in the milk replacer (g/kg DM).

Apparent total tract and ileal DM disappearance was calculated by using the following equation:

$$\text{Dry matter disappearance (\% of intake)} = \left[1-\left(\text{Co}_{\text{feed}}/\text{Co}_{\text{digesta}}\right)\right]\times 100 \quad (2)$$

Total tract SP fermentation was calculated by the method of Gerrits et al. (17) on the basis of the measured ^{13}C enrichment in basal milk replacer, lactose, SP, and feces. In short, in all SP treatments, the SPs were derived from corn, resulting in higher natural ^{13}C enrichment in the SPs than in the basal milk replacer and lactose (see the section "Diets and housing" for details). An increase in fecal ^{13}C enrichment would result from fecal SP excretion or from microbial biomass that has incorporated the ^{13}C from SP. The SP excretion in feces was analyzed (see "Analytical procedures"), and microbial biomass from SP fermentation could be

calculated by the difference. In this calculation, it was assumed that 1 g carbohydrate fermented results in 0.2 g microbial biomass in the feces [derived from 0.3 kJ fecal biomass/kJ carbohydrate (26) and assuming 15.56 kJ/g carbohydrate (27) and 23.13 kJ/g biomass (28)].

The ^{13}C enrichment in glucose was determined in the peripheral blood plasma of the CON, GS, and MT calves. Basal plasma samples (30 min before feeding) were taken as background values. In the postprandial samples, the ^{13}C enrichment in excess of background was calculated for plasma glucose and was expressed per gram of naturally ^{13}C-enriched carbohydrate provided.

Statistical analysis. All statistical analyses were performed with SAS 9.2 (SAS Institute). Mean daily body weight gain, length of the small intestine, intestinal disappearance of nutrients, and SP fermentation variables were analyzed for treatment effects by ANOVA with the use of the general linear model procedure. Day of slaughter did not affect ileal nutrient disappearance and was therefore excluded from the model. Studentized residuals of all models were checked for homogeneity of variance. Apparent total tract crude fat disappearance (square root), apparent ileal nitrogen disappearance [transformed data = (original data^c − 1)/c, with c = 8], atom percentage of the feces (log), and fecal SP excretion (log) were transformed to obtain homogeneity of variance. Visual inspection of studentized residuals revealed 1 outlier for fecal DM output, 1 outlier for fecal nitrogen output, and 1 outlier for SP fermentation variables. There was no biological reason for excluding the data of these calves, and conducting the model with or without these calves did not affect the main treatment effect. Therefore, all calves were included in the data analysis.

Enzyme activities and recovery of chromium were analyzed for treatment, segment, and treatment × segment effects by using the MIXED procedure. Segment was included as a repeated statement, with calf as the subject. On the basis of fit statistics (Akaike and Bayesian information criterion), the heterogeneous first-order autoregressive covariance structure was used for all enzyme activities and for recovery of chromium. Isomaltase and α-amylase activities and recovery of chromium were log-transformed, and lactase activity was square root–transformed to obtain homogeneity of variance. When interaction effects were found, treatment effects were analyzed for each segment separately. Total chromium recovery was analyzed for treatment effects by ANOVA with the use of the general linear model procedure.

The ^{13}C enrichment of plasma glucose in excess of background was analyzed for treatment, time, and treatment × time effects by using the MIXED procedure. Time was included as a repeated statement, with calf as the subject. On the basis of fit statistics (Akaike and Bayesian information criterion), the first-order autoregressive covariance structure was used. When an interaction effect was found, treatment effects were analyzed for each time point separately. Visual inspection of studentized residuals revealed 3 outliers in the ^{13}C enrichment of plasma glucose data. There was no biological reason for excluding the data of these calves, and conducting the model with or without these calves did not affect main effects. Therefore, these calves were included in the data analysis.

Differences were considered significant when $P < 0.05$. When main effects were significant, pairwise comparisons were made by using Tukey's method. Results are expressed as nontransformed means and their pooled SEMs.

Results

One calf from the CON treatment was excluded from the experiment because of persistent milk replacer refusals and ruminal drinking, which resulted in bloat. One calf from the MT treatment died before the end of the experiment, with the cause of death remaining unknown upon autopsy. Mean daily body weight gain of the calves during the experimental period was 1.05 ± 0.05 kg/d. A greater mean daily body weight gain ($P = 0.018$) was found for the GS treatment (1.14 ± 0.05 kg/d) than for the CON (0.96 ± 0.06 kg/d) and MT (0.91 ± 0.03 kg/d) treatments, which was related to the slightly higher DM intake in the GS treatment in the period of feces collection ($P < 0.001$; **Table 1**).

Nutrient disappearance. Apparent total tract and ileal nutrient disappearance values are presented in **Table 2**. Six calves had no or insufficient ileal digesta to perform all nutrient analyses,

TABLE 1 Body weight, feed intake, and fecal output characteristics of, and total tract starch product fermentation in, calves fed a milk replacer containing lactose as the only carbohydrate source or 18% gelatinized starch, maltodextrin, maltodextrin with a high degree of α-1,6-branching, or maltose at the expense of lactose[1]

	Treatment						
	CON	GS	MD	MDB	MT	Pooled SEM	P^2
n	7	8	8	8	7		
Body weight, kg	224	218	235	227	217	4	—
Dry matter intake, g/d	2711	2913	2786	2662	2527	43	—
Starch product intake, g/d	—	507	501	447	416	9	—
Fecal output characteristics							
Dry matter output, g/d	168[b]	259[a]	246[a]	222[a,b]	226[a,b]	16	0.004
Starch product[3] output, g/d	—	5.3	5.4	2.9	5.3	2.2	0.87
^{13}C enrichment, atom%	1.0776[b]	1.0829[a]	1.0832[a]	1.0825[a]	1.0828[a]	0.0004	<0.001
Biomass from starch product fermentation,[4] g/d	—	89	90	77	75	8.6	0.46
Starch product fermentation,[4] g/d	—	447	451	384	373	43	0.46
Starch product fermentation,[4] % of intake	—	89	90	86	90	8.8	0.99

[1] Values are means. Labeled means without a common letter in a row differ, $P < 0.05$. atom%, atom percentage; CON, control calves fed milk replacer containing lactose as the only carbohydrate source; GS, gelatinized starch, calves fed milk replacer containing 18% of gelatinized starch at the expense of lactose; MD, calves fed milk replacer containing 18% of maltodextrin at the expense of lactose; MDB, calves fed milk replacer containing 18% of maltodextrin with a high degree of α-1,6-branching at the expense of lactose; MT, calves fed milk replacer containing 18% of maltose at the expense of lactose.

[2] *P* values apply to the treatment effect. When no mean for the CON treatment is shown, the *P* value applies to differences between the starch product treatments only.

[3] Starch product was calculated by measuring total anhydrous glucose excretion and was corrected for glucose originating from lactose by assuming that the glucose originating from lactose equaled galactose excretion.

[4] Fermentation of starch products estimated from fecal ^{13}C excretion and assuming that 0.2 g fecal microbial biomass requires 1 g starch product to be fermented. For details, see text.

TABLE 2 Apparent total tract and ileal nutrient disappearance in calves fed a milk replacer containing lactose as the only carbohydrate source or 18% of gelatinized starch, maltodextrin, maltodextrin with a high degree of α-1,6-branching, or maltose at the expense of lactose[1]

	Treatment						
	CON	GS	MD	MDB	MT	Pooled SEM	P^2
Apparent total tract nutrient disappearance, % of intake							
n	7	8	8	8	7		
Dry matter	93.7[a]	91.1[b]	91.2[a,b]	91.7[a,b]	91.0[b]	0.6	0.029
Nitrogen	86.1[a]	79.8[b]	81.9[a,b]	79.3[b]	79.9[b]	1.2	0.003
Crude fat	91.0	93.6	95.6	94.9	94.4	1.1	0.07
Lactose	100.0	100.0	100.0	100.0	100.0	0.0	—
Starch product[3]	—	99.0	99.0	99.4	98.8	0.4	0.79
Apparent ileal nutrient disappearance, % of intake							
n	6	6[4]	8	7	7[5]		
Dry matter	88.0[a]	81.9[a,b]	78.7[b]	79.4[b]	78.6[b]	1.2	<0.001
Nitrogen	83.1	83.6	81.5	84.1	82.9	1.8	0.74
Lactose	100.0[a].	99.7[a]	99.5[a,b]	99.5[a,b]	99.0[b]	0.1	<0.001
Starch product[3]	—	64.0	60.5	63.6	58.5	6.3	0.91

[1] Values are means. Labeled means without a common letter in a row differ, $P < 0.05$. CON, control calves fed milk replacer containing lactose as the only carbohydrate source; GS, gelatinized starch, calves fed milk replacer containing 18% of gelatinized starch at the expense of lactose; MD, calves fed milk replacer containing 18% of maltodextrin at the expense of lactose; MDB, calves fed milk replacer containing 18% of maltodextrin with a high degree of α-1,6-branching at the expense of lactose; MT, calves fed milk replacer containing 18% of maltose at the expense of lactose.
[2] *P* values apply to the treatment effect. When no mean for the CON treatment is shown, the *P* value applies to differences between the starch product treatments only.
[3] Starch product disappearance was calculated by measuring total anhydrous glucose intake and excretion and was corrected for glucose originating from lactose by assuming that the glucose originating from lactose equaled galactose excretion.
[4] For the GS treatment, the number of calves for apparent ileal dry matter disappearance was 7; for the other nutrients the number of calves was 6.
[5] For the MT treatment, the number of calves for apparent ileal nitrogen disappearance was 6; for the other nutrients the number of calves was 7.

resulting in incomplete data for these calves. The apparent DM disappearance at the ileum ($P < 0.001$) and total tract ($P = 0.029$) was greater for the CON treatment than for all SP treatments. Apparent total tract disappearance of crude fat was numerically greater for the SP calves than for CON calves ($P = 0.07$). Apparent total tract disappearance of lactose was complete for all calves. Small amounts of lactose were measured in the ileal digesta of the SP calves but not in the ileal digesta of CON calves, resulting in a treatment effect for the apparent ileal disappearance of lactose ($P < 0.001$). Apparent total tract disappearance of nitrogen was greater for CON calves than for GS, MDB, and MT calves ($P = 0.003$). At the ileum, however, nitrogen disappearance was not affected by treatment. Apparent total tract and ileal disappearance of SP did not differ between SP treatments. The length of the small intestine was not affected by treatment ($P = 0.88$) and averaged 29 ± 1.2 m.

SP fermentation. Fecal DM output was greater in SP calves than in CON calves ($P = 0.004$; Table 1). Only small amounts of SP were excreted in the feces of SP calves. The SP calves had a greater ^{13}C enrichment of the feces than did the CON calves ($P < 0.001$; Table 1). Total tract SP fermentation was not affected by SP treatment and averaged 414 ± 43.0 g/d, corresponding to 89% of SP intake (Table 1).

Enzyme activity. Brush border enzyme activity for lactase, maltase, and isomaltase and luminal activity for α-amylase are presented in **Table 3**. Lactase activity did not differ between dietary treatments and was greater in small intestine 1 compared with small intestine 2 ($P < 0.001$) and the ileum ($P < 0.001$). Maltase and isomaltase activity was not significantly greater for any of the SP treatments compared with the CON treatment. Luminal α-amylase activity was measured in small intestine 1 but was not detected in small intestine 2 for CON calves, whereas this activity was higher in small intestine 2 than in small intestine 1 in all SP treatments (treatment × segment, $P = 0.015$).

Passage. Three calves were excluded from the analysis on chromium recovery because of missing samples. Two calves were identified as ruminal drinkers (chromium recovery in the rumen >50% of the pulse dose) and were excluded from the analysis. Total chromium recovery did not differ between treatments ($P = 0.24$) and averaged 85% ± 3.1% of intake. Recovery of chromium per gastrointestinal segment is shown in **Figure 1**. In the CON treatment, almost half of the provided chromium was recovered in small intestine 2, and 4.7% ± 1.92% of the chromium had reached the large intestine. A treatment × segment interaction was found ($P = 0.008$), which was caused by a treatment effect in small intestine 2 ($P = 0.033$) and in the large intestine ($P = 0.033$). The maltodextrin (MD) and MT treatments had a lower chromium recovery in small intestine 2 ($P = 0.029$ and $P = 0.07$, respectively) and a greater chromium recovery in the large intestine ($P = 0.06$ and $P = 0.033$, respectively) compared with the CON treatment, indicating a higher passage rate of the milk replacer in the second part of the small intestine for these calves.

^{13}C in plasma glucose. The response of ^{13}C in excess of background in peripheral plasma glucose of the CON, GS, and MT calves is shown in **Figure 2**. The response of ^{13}C in excess of background in time differed between treatments (treatment × time interaction, $P < 0.001$). At all postprandial time points, the

TABLE 3 Brush border enzyme activity and luminal α-amylase activity in small intestinal segments of calves fed a milk replacer containing lactose as the only carbohydrate source or 18% of gelatinized starch, maltodextrin, maltodextrin with a high degree of α-1,6-branching, or maltose at the expense of lactose[1]

	Treatment						*P*		
	CON	GS	MD	MDB	MT	Pooled SEM	TRT	SEG	TRT × SEG
Brush border enzyme activity, U/g protein									
n	5	4	4	4	4				
Lactase							0.53	<0.001	0.47
Small intestine 1	193	182	163	176	197	12.6			
Small intestine 2	14	5	4	6	4	4.4			
Ileum	0.8	0.7	0.7	0.9	1.1	0.3			
Maltase							0.046	<0.001	0.58
Small intestine 1	9	6	4	3	4	1			
Small intestine 2	46	41	19	21	18	12			
Ileum	32	17	22	18	17	8			
Isomaltase							0.032	<0.001	0.97
Small intestine 1	3	2	1	2	2	0.4			
Small intestine 2	13	13	5	8	10	4.0			
Ileum	11	7	7	8	7	2.6			
Luminal α-amylase activity, U/mg Co							0.074	0.002	0.015
n	7	8	8	8	7				
Small intestine 1	3.9	1.9	3.9	3.2	1.8	2.0			
Small intestine 2	0.0	5.2	8.9	5.7	5.9	1.4			

[1] Values are means. One unit (U) of brush border enzyme represents the hydrolysis of 1 μmol disaccharide/min at 37°C and 1 U of α-amylase represents the amount of α-amylase that hydrolyzes the substrate of the kit (ethylidene-para-nitrophenol-G7), resulting in 1 μmol nitrophenol/min at 25°C and pH 7.2. Co, indigestible marker cobalt, provided continuously as Co-EDTA with the milk replacer; CON, control calves fed milk replacer containing lactose as the only carbohydrate source; GS, gelatinized starch, calves fed milk replacer containing 18% of gelatinized starch at the expense of lactose; MD, calves fed milk replacer containing 18% of maltodextrin at the expense of lactose; MDB, calves fed milk replacer containing 18% of maltodextrin with a high degree of α-1,6-branching at the expense of lactose; MT, calves fed milk replacer containing 18% of maltose at the expense of lactose; SEG, segment, the small intestine without the ileum (i.e., last ~100 cm of the small intestine) was divided equally into small intestine 1 and small intestine 2; TRT, dietary treatment.

enrichment of plasma glucose was substantially greater ($P < 0.001$) after intake of ^{13}C enriched lactose (CON) than after intake of ^{13}C enriched GS or MT. The latter 2 treatments did not differ at any point in time.

Discussion

This experiment was conducted to determine the rate-limiting enzyme in starch hydrolysis and to quantify starch fermentation in milk-fed calves. The greatest maltase activity was 46 U/g protein in small intestine 2 of CON calves. This is very low when compared with other, nonherbivorous species, for example, weaned pigs (180 U/g protein halfway along the small intestine) (11), neonatal pigs of ~22 d of age fed a milk replacer (600 U/g protein in the jejunum, (29) and rats fed a high-maltose diet (178 U/g protein) (10). Similarly, the greatest isomaltase activity in our study was 13 U/g protein in small intestine 2 of CON calves, whereas this amount was ~80 U/g protein halfway the small intestine of weaned pigs (11). Lactase activity averaged 182 ± 12.6 U/g protein in small intestine 1 across all treatments. This

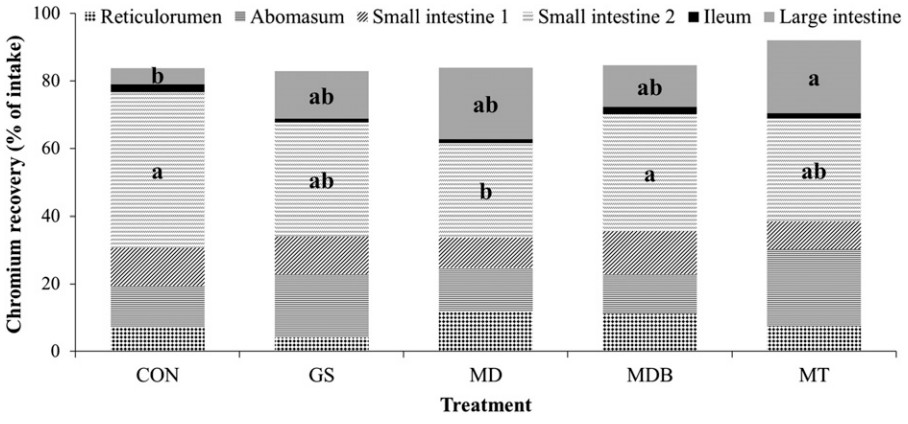

FIGURE 1 Recovery of chromium (provided as chromium chloride hexahydrate) per gastrointestinal segment of calves at 4 h after feeding a control milk replacer or a milk replacer containing 18% of 1 of 4 starch products. Labeled means without a common letter within the same gastrointestinal segment differ, $P < 0.05$; n = 6–7. The pooled SEMs equaled 3.1% for the reticulorumen, 3.3% for the abomasum, 1.3% for small intestine 1, 3.9% for small intestine 2, 0.5% for the ileum, and 4.3% for the large intestine. CON, control calves fed milk replacer containing lactose as the only carbohydrate source; GS, calves fed milk replacer containing 18% of gelatinized starch at the expense of lactose; MD, calves fed milk replacer containing 18% of maltodextrin at the expense of lactose; MDB, calves fed milk replacer containing 18% of maltodextrin with a high degree of α-1,6-branching at the expense of lactose; MT, calves fed milk replacer containing 18% of maltose at the expense of lactose.

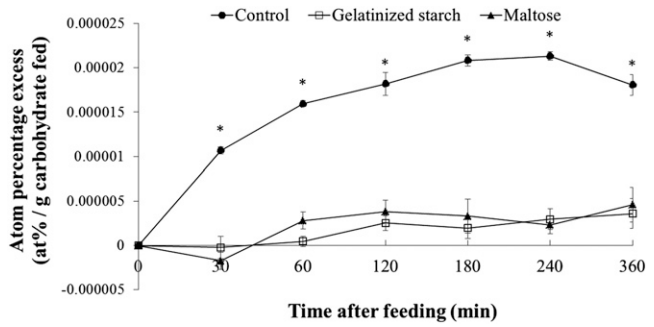

FIGURE 2 The ^{13}C enrichment in excess of background in peripheral plasma glucose of calves fed a milk replacer containing 37.5% naturally ^{13}C-enriched lactose (control) or 18% naturally ^{13}C-enriched gelatinized starch or maltose. The atom percentage in excess of background is corrected for the dose of the naturally ^{13}C-enriched carbohydrate fed. Values are means ± SEMs, n = 7–8. The response of ^{13}C in excess of background in time differed between treatments (treatment × time interaction, $P < 0.001$). *Control is different from other means at that time, $P < 0.05$.

high lactase activity was expected, because lactase activity remains high in calves through the continuous feeding of lactose (30, 31). We measured luminal α-amylase activity in small intestinal digesta. Often, α-amylase activity is measured in pancreatic tissue samples or in pancreatic juice, making comparisons with our study difficult. Abomasal infusion of partially hydrolyzed starch in steers decreased pancreatic α-amylase activity (32, 33) and pancreatic juice α-amylase activity and secretion (33). In steers, α-amylase is probably not the limiting step in small intestinal starch digestion, because infusing amylase into the jejunum of steers fed a diet containing 60% cracked maize did not increase the small intestinal digestibility of starch (34).

The selected SPs used in this study require different ratios of starch-degrading enzymes for their complete hydrolysis to glucose. Differences between treatments in activities of starch-degrading enzymes, and ileal SP disappearance, allow the rate-limiting enzyme for starch hydrolysis in milk-fed calves to be determined. The activity of α-amylase was greater for SP calves than for CON calves in small intestine 2 and is probably not limiting in starch digestion. This is in agreement with studies in steers (4, 34). Maltase and isomaltase activities were not increased in any of the small intestinal segments after providing their specific substrate for 14 wk in the MT and MDB treatments, respectively. In addition, ileal SP disappearance did not differ between treatments. Because all SPs require maltase activity to achieve complete hydrolysis to glucose, our data indicate that maltase limits starch digestion in milk-fed calves.

The possibility that nutrients from the milk replacer (i.e., fat or lactose) inhibit brush border enzyme activity cannot be completely excluded in this study. For example, Lee et al. (35) showed an inhibitory effect of sucrose on maltase-glucoamylase activity in vitro. In rabbits, however, replacing dietary starch for lactose did not affect apparent ileal starch disappearance (36). To the best of our knowledge, such studies have not been performed in ruminants. Surprisingly, the maltase activity was greatest for the CON calves. Similar negative effects of partially hydrolyzed starch on pancreatic α-amylase activity have been shown before in steers (32, 33).

In piglets, greater luminal α-amylase activity (U/g DM digesta) was found in the duodenum than in the jejunum and ileum (37). This is in contrast with our results, in which greater α-amylase activity was found in small intestine 2 than in small intestine 1 for SP calves, whereas no α-amylase activity was found in small intestine 2 for CON calves. This may indicate that a substantial portion of the amylase found in the digesta in small intestine 2 of SP calves is of microbial origin or that the amylase moves with the undigested substrate more rapidly from small intestine 1 to small intestine 2, as confirmed by the chromium recovery data, which indicate an increased rate of passage in the SP treatments compared with the CON treatment. The chromium recovery in the reticulorumen averaged 10% ± 4.7%, indicating that the esophageal groove reflex was still functioning.

The lack of sufficient starch-degrading enzyme activity in the brush border resulted in a decreased apparent ileal disappearance of DM in the MD, MDB, and MT treatments compared with the CON treatment. Therefore, greater amounts of substrate were available for colonic fermentation in calves fed SPs. On the basis of apparent ileal (62%) and apparent total tract (99%) disappearance, 37% of the ingested SP was fermented in the large intestine. Total tract SP fermentation, estimated from ^{13}C excretion in feces, averaged 89% of SP intake, regardless of the SP source, indicating that 52% of the SP intake was fermented before the terminal ileum. Milk replacer that leaked into the reticulorumen averaged 11% ± 5.1% for SP-fed calves. This indicates that 41% of the SP intake was fermented in the small intestine. This 41% is calculated by the difference and is based on an indirect method for quantifying microbial biomass in the small intestine.

As an alternative approach, total tract SP fermentation can be estimated from the extra DM output of SP calves compared with CON calves, assuming that the extra DM output in SP calves, corrected for differences in undigested fecal SP, fat, or ash, would be largely microbial biomass. SP calves produced more feces than did CON calves (≥70 g DM/d), but fecal fat excretion was lower in SP calves than in CON calves (−16 g fat/d). This results in an increase of 86 g nonfat fecal DM/d when feeding SPs to calves. The difference in fecal DM output between SP and CON calves was partly caused by SP excretion directly (5 g SP/d) but could not be explained by increased ash excretion in SP calves. This leaves 81 g DM/d that is unaccounted for, which is hypothesized to be undigested microbial mass resulting from SP fermentation. Assuming 1 g SP fermented per 0.2 g fecal microbial biomass (26), this would require 405 g SP to be fermented, corresponding to 87% of SP intake. Both methods for quantifying total tract SP fermentation indicate that, regardless of the SP source, the majority of SP intake is fermented in calves. This is in agreement with the greater apparent ileal compared with apparent total tract nitrogen disappearance in SP calves, indicating a net influx of nitrogen into the large intestinal lumen. A lower apparent ileal nitrogen disappearance for SP calves could be expected after substantial small intestinal starch fermentation as well; however, apparent ileal nitrogen disappearance did not differ between SP treatments and the CON treatment. Most probably, a considerable portion of the microbial biomass produced in the small intestine is digested before the terminal ileum. We quantified SP fermentation at a SP inclusion amount of 18%. A linear decrease in fecal DM content and pH was observed with increasing SP inclusion amount during the adaptation period in these calves, irrespective of SP source (15), showing that fermentation contributes to SP disappearance at lower inclusion amounts as well.

A clear increase in ^{13}C enrichment of plasma glucose was found after feeding naturally ^{13}C-enriched lactose to CON calves. In contrast, only a marginal increase was found after

feeding naturally ^{13}C-enriched GS or maltose, indicating that only small amounts of glucose in peripheral blood originate from GS or maltose. This corresponds with the result that 66% of the small intestinal SP disappearance is attributed to fermentation, which results in the production of VFAs, and leaves only 34% of the small intestinal SP disappearance for enzymatic digestion, which results in the release of glucose; however, propionate could also have been used as a precursor for gluconeogenesis and could have contributed to the low ^{13}C enrichment in plasma glucose in GS and MT calves.

The low brush border enzyme activity, the large amount of total tract and small intestinal SP fermentation, the reduced retention time of digesta in the small intestine, and the small increase in ^{13}C enrichment in plasma glucose after SP feeding show that calves are not able to enzymatically hydrolyze starch in substantial amounts. Portal glucose flux studies (4, 8) have evaluated starch digestion in the small intestine of ruminants as well but are often inconclusive. Fifty-five percent of the infused starch (60 g/h) into the abomasum of steers disappeared before the ileocecal junction. However, only 43% of this small intestinal disappearance could be accounted for as net portal glucose uptake, after correction for the negative portal glucose uptake found with water infusion to account for glucose use by the portal-drained viscera (8). This leaves 57% of the small intestinal starch disappearance unaccounted for. The arterial use of glucose by the portal-drained viscera was increased after infusion of partially hydrolyzed starch in the abomasum compared with infusion in the rumen (38). This unexpected increase indicates that the arterial glucose use by the portal-drained viscera is greater when luminal starch/glucose is present, suggesting that correction after water infusion in portal glucose flux studies is insufficient. However, infusing this partially hydrolyzed starch (800 g/d) into the rumen instead of into the abomasum did not alter the flux of VFAs across the portal-drained viscera (38). This confuses the message of these studies and corresponds to our observations on small intestinal fermentation of starch. Direct quantification of enzymatic starch hydrolysis in ruminants remains to be studied and could be studied by measuring a response in ^{13}C glucose in the portal circulation after abomasal infusion of ^{13}C-enriched starch.

In translating our results obtained with milk-fed calves to functional ruminants, we assume that a functioning rumen and the resulting type of digesta does not affect the capacity of the ruminant small intestine to enzymatically hydrolyze starch and absorb its end products. Possible matrix effects of the milk replacer were discussed above. The possibility that rumen microbes, flowing through the small intestinal tract, interfere with the nonmicrobial enzymatic hydrolysis of starch cannot be excluded, but this is considered unlikely.

Our data lead to the hypothesis that maltase is the rate-limiting enzyme in starch digestion in milk-fed calves, and that the largest part of small intestinal starch disappearance is due to fermentation rather than enzymatic hydrolysis to glucose. Such extensive fermentation in the small intestine has not been demonstrated before and could occur in ruminants as well.

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References

1. Danfaer A, Tetens V, Agergaard N. Review and an experimental study on the physiological and quantitative aspects of gluconeogenesis in lactating ruminants. Comp Biochem Physiol 1995;111:201–10.
2. Marion J, Petersen YM, Romé V, Thomas F, Sangild PT, Le Dividich J, Le Huërou-Luron I. Early weaning stimulates intestinal brush border enzyme activities in piglets, mainly at the posttranscriptional level. J Pediatr Gastroenterol Nutr. 2005;41:401–10.
3. Reddy BS, Wostmann BS. Intestinal disaccharidase activities in growing germfree and conventional rats. Arch Biochem Biophys 1966;113:609–16.
4. Kreikemeier KK, Harmon DL. Abomasal glucose, maize starch and maize dextrin infusions in cattle: small intestinal disappearance, net portal glucose flux and ileal oligosaccharide flow. Br J Nutr 1995;73:763–72.
5. Abe M, Iriki T, Kondoh K, Shibui H. Effects of nipple or bucket feeding of milk-substitute on rumen by-pass and on rate of passage in calves. Br J Nutr 1979;41:175–81.
6. Coombe NB, Smith RH. Digestion and absorption of starch, maltose and lactose by the preruminant calf. Br J Nutr 1974;31:227–35.
7. Lin FD, Knabe DA, Tanksley TD Jr. Apparent digestibility of amino acids, gross energy and starch in corn, sorghum, wheat, barley, oat groats and wheat middlings for growing pigs. J Anim Sci 1987;64:1655–63.
8. Kreikemeier KK, Harmon DL, Brandt RT Jr., Avery TB, Johnson DE. Small intestinal starch digestion in steers: effect of various levels of abomasal glucose, corn starch and corn dextrin infusion on small intestinal disappearance and net glucose absorption. J Anim Sci 1991;69:328–38.
9. Branco AF, Harmon DL, Bohnert DW, Larson BT, Bauer ML. Estimating true digestibility of nonstructural carbohydrates in the small intestine of steers. J Anim Sci 1999;77:1889–95.
10. Deren JJ, Broitman SA, Zamcheck N. Effect of diet upon intestinal disaccharidases and disaccharide absorption. J Clin Invest 1967;46:186–95.
11. Kidder DE, Manners MJ. The level and distribution of carbohydrases in the small intestine mucosa of pigs from 3 weeks of age to maturity. Br J Nutr 1980;43:141–53.
12. Russell JR, Young AW, Jorgensen NA. Effect of dietary corn starch intake on pancreatic amylase and intestinal maltase and pH in cattle. J Anim Sci 1981;52:1177–82.
13. Le Huerou I, Guilloteau P, Wicker C, Mouats A, Chayvialle JA, Bernard C, Burton J, Toullec R, Puigserver A. Activity distribution of 7 digestive enzymes along small intestine in calves during development and weaning. Dig Dis Sci 1992;37:40–6.
14. Huntington GB. Starch utilization by ruminants: from basics to the bunk. J Anim Sci 1997;75:852–67.
15. Gilbert MS, Van den Borne JJGC, Berends H, Pantophlet AJ, Schols HA, Gerrits WJJ. A titration approach to identify the capacity for starch digestion in milk-fed calves. Animal 2015;9:249–57.
16. Van Es AJH, Nijkamp HJ, Van Weerden EJ, Van Hellemond KK. Energy, carbon and nitrogen balance experiments with veal calves. In: Blaxter KL, Kielanowski J, Thorbek G, editors. Energy metabolism of farm animals. Newcastle-upon-Tyne (United Kingdom): Oriel Press; 1967. p. 197–201.
17. Gerrits WJJ, Bosch MW, van den Borne JJGC. Quantifying resistant starch using novel, in vivo methodology and the energetic utilization of fermented starch in pigs. J Nutr 2012;142:238–44.
18. International Organization for Standardization. Animal feeding stuffs: determination of moisture and other volatile matter content. Geneva (Switzerland): International Organization for Standardization; 1999. (ISO publication 6496.)

19. International Organization for Standardization. Animal feeding stuffs: determination of nitrogen content and calculation of crude protein content. Part 2: block digestion/steam distillation method. Geneva (Switzerland): International Organization for Standardization; 2005. (ISO publication 5983-2.)
20. AOAC International. Official methods of analyses. 18th ed. Arlington (VA): AOAC International; 2005.
21. International Organization for Standardization. Animal feeding stuffs, animal products, and faeces or urine: determination of gross calorific value—bomb calorimeter method. Geneva (Switzerland): International Organization for Standardization; 1998. (ISO publication 9831.)
22. International Organization for Standardization. Animal feeding stuffs: determination of crude ash. Geneva (Switzerland): International Organization for Standardization; 2002. (ISO publication 5984.)
23. Zhao J, Schols HA, Chen Z, Jin Z, Buwalda P, Gruppen H. Substituent distribution within cross-linked and hydroxypropylated sweet potato starch and potato starch. Food Chem 2012;133:1333–40.
24. Dahlqvist A. Method for assay of intestinal disaccharidases. Anal Biochem 1964;7:18–25.
25. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85.
26. Livesey G. Calculating the energy values of foods: towards new empirical formulae based on diets with varied intakes of unavailable complex carbohydrates. Eur J Clin Nutr 1991;45:1–12.
27. Blaxter KL. Energy metabolism in animals and man. Cambridge (United Kingdom): Cambridge University Press; 1989.
28. Cordier JL, Butsch BM, Birou B, Von Stockar U. The relationship between elemental composition and heat of combustion of microbial biomass. Appl Microbiol Biotechnol 1987;25:305–12.
29. Oliver WT, Mathews SA, Phillips O, Jones EE, Odle J, Harrell RJ. Efficacy of partially hydrolyzed corn syrup solids as a replacement for lactose in manufactured liquid diets for neonatal pigs. J Anim Sci 2002;80:143–53.
30. Huber JT, Keith JM, Rifkin RJ. Effect of level of lactose upon lactase concentrations in small intestines of young calves. J Dairy Sci 1964;47:789–92.
31. Toofanian F, Hill FWG, Kidder DE. The mucosal disaccharidases in the small intestine of the calf. Ann Rech Vet 1973;4:57–69.
32. Swanson KC, Matthews JC, Woods CA, Harmon DL. Postruminal administration of partially hydrolyzed starch and casein influences pancreatic α-amylase expression in calves. J Nutr 2002;132:376–81.
33. Walker JA, Harmon DL. Influence of ruminal or abomasal starch hydrolysate infusion on pancreatic exocrine secretion and blood glucose and insulin concentrations in steers. J Anim Sci 1995;73:3766–74.
34. Remillard RL, Johnson DE, Lewis LD, Nockels CF. Starch digestion and digesta kinetics in the small intestine of steers fed on a maize grain and maize silage mixture. Anim Feed Sci Technol 1990;30:79–89.
35. Lee BH, Quezada-Calvillo R, Nichols BL Jr., Rose DR, Hamaker BR. Inhibition of maltase-glucoamylase activity to hydrolyze α-1,4 linkages by the presence of undigested sucrose. J Pediatr Gastroenterol Nutr 2012;55: Suppl 2:S45–7.
36. Gutiérrez I, Espinosa A, García J, Carabaño R, De Blas JC. Effect of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned rabbits. J Anim Sci 2002;80:1029–37.
37. Feng J, Liu X, Xu ZR, Lu YP, Liu YY. Effect of fermented soybean meal on intestinal morphology and digestive enzyme activities in weaned piglets. Dig Dis Sci 2007;52:1845–50.
38. Harmon DL, Richards CJ, Swanson KC, Howell JA, Matthews JC, True AD, Huntington GB, Gahr SA, Russell RW. Influence of ruminal or postruminal starch on visceral glucose metabolism in steers. In: Chwalibog, A, Jakobsen, K, editors. Energy metabolism in animals. Proceedings of the 15th International Symposium on Energy Metabolism; September 11–16, 2000; Snekkersten, Denmark. Wageningen (The Netherlands): Wageningen Pers; 2001. p. 273–6.