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Nutrient digestibility and endogenous protein losses in the foregut and small intestine of weaned dairy calves fed calf starters with conventional or enzyme-treated soybean meal

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ABSTRACT

The aims of this experiment were (1) to compare the effects of a soybean meal with an enzymatic treatment (ESBM) to reduce the concentration of antinutritional factors versus a standard soybean meal (SBM) on foregut and small intestine digestion in weaned dairy calves and (2) to estimate the endogenous losses of crude protein (CP) in the small intestine. Our hypothesis was that a diet containing ESBM instead of SBM would improve ruminal and small intestine digestion and absorption of nutrients. A T-cannula was placed in the duodenum, and a second T-cannula was installed in the distal ileum of 12 Holstein calves at approximately 3 wk of age. Calves were weaned on d 42, and on d 50 they were assigned randomly to a quadruplicated 3 × 3 Latin square with 10-d periods. Digesta samples were collected on d 7 and 8 from the ileum and d 9 and 10 from the duodenum. The diets were fed for ad libitum intake and consisted of a calf starter (CS) of 20% CP with SBM as the main source of protein (CTRL), and an isonitrogenous CS with an ESBM instead of SBM (ENZT). A third diet with a low content of CP (10%) and no soy protein was fed to estimate endogenous N losses and digestibilities of test ingredients. Flows and digestibilities of nutrients were compared between CTRL and ENZT and their test ingredients (SBM vs. ESBM, respectively). Duodenal net flows of CP and total AA as well as ruminal microbial protein synthesis per kilogram of digested CP were greater, and flow of nonprotein N and CP true (corrected by endogenous and microbial flows) foregut digestibility were lower with ENZT than CTRL. The apparent small intestine digestibilities of CP and total AA were greater for ESBM than SBM, but there were no differences between the CTRL and ENZT diets. We observed no differences in digestibilities at the duodenum or ileum of starch or NDF, but true small intestine digestibilities of CP and all AA were greater with ENZT than CTRL. Total endogenous protein losses in the small intestine estimated from calves fed the low-CP with no soy protein diet were 37 ± 1.5 g of CP and 29 ± 1.4 g of AA/kg of DMI. These values may be considered the basal endogenous losses as they are similar to values obtained with the regression method, which estimates N losses when dietary N is null. Our results indicated that the inclusion of an ESBM improved the efficiency of ruminal microbial protein synthesis per digested kilogram of organic matter and CP, and increased CP and AA absorption in the small intestine despite a greater proportion of undigested dietary protein entering the duodenum.

Key words: amino acid, digesta, digestive tract, microbial protein

INTRODUCTION

Antinutritional factors in soybeans may cause a significant reduction in feed digestibility, and thus a negative effect on calf performance. Antinutritional factors interfere with regular gastrointestinal function by reducing the action of proteases, altering the morphology of the epithelium and transport of nutrients, supplying indigestible di- and oligosaccharides, and triggering a gastrointestinal and systemic antigenic response, among others (Ansia and Drackley, 2020a). During the preweaning period, older calves better resist the effects of antinutritional factors (Barratt and Porter, 1979) and maintain a greater growth rate than younger calves (Akinyele and Harshbarger, 1983) due to their lower permeability of the mucosal barrier (Steele et al., 2016) and the rapid increase in microbial and enzymatic activity since birth (Rey et al., 2012). Ruminal fermentation in weaned calves increases the vulnerability of these

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factors to complete hydrolysis (Lynch et al., 1988; Mir et al., 1989), reducing the appearance of negative effects. However, antinutritional factors also may inhibit ruminal microbial and enzymatic activity (D'Mello, 2006). Ruminal fermentation in weaned calves did not completely counteract the negative effects of protease inhibitors in raw untreated soybeans (Daniels et al., 1972; Abdelgadir et al., 1984), which emphasizes the importance of feeding properly processed soy protein sources during the weaning phase (Drackley, 2008).

Processing of soybeans by heating, ethanol extraction, protein isolation, and microbial treatment can reduce the adverse effects caused by antinutritional factors (Lallés, 1993). In addition to the reduction and inactivation of antinutritional and antigenic factors, microbial treatment (i.e., fermentation) of soybean meal (**SBM**) activates the release of bioactive compounds and peptides that enhance the immune system response and can provide other health benefits (Chatterjee et al., 2018). Inclusion of a microbially treated SBM in calf starter (**CS**) promoted better growth rates, decreased fecal and health scores, and enhanced the immune system response during weaning and microbial infection (Kim et al., 2010, 2012). In piglets, microbial-treated SBM reduced severity of diarrhea; increased DMI, ADG, and gain:feed ratio (Kiers et al., 2003); and increased villus height of the small intestine in comparison with heated SBM (Feng et al., 2007).

Therefore, an enzymatically treated SBM (**ESBM**) may be an interesting ingredient in calf diets to improve nutrient digestibility. We have previously found that an ESBM can replace 50% of the CP from whey protein in milk replacer with no differences in small intestine digestibility of CP and total AA in young calves (Ansia at al., 2020b), and that the use of ESBM instead of conventional SBM can increase apparent dietary nutrient foregut digestibility and improve microbial protein (**MCP**) synthesis efficiency in weaned calves at 3 mo of age (Ansia et al., 2020c). Seo et al. (2013) also reported greater DM digestibility and MCP synthesis during in vitro digestion with ruminal fluid when corn and SBM were enzymatically treated.

Amino acids are primarily absorbed in the small intestine, and fermentation in the large intestine alters AA profile and N content in feces; therefore, small intestine digestibility provides a more accurate estimation of AA and N bioavailability than total-tract digestibility (Stein et al., 2007). In ruminants, microbial N accounts for most of the total duodenal N (Marini et al., 2008); therefore, duodenal N supply must be studied to accurately determine dietary N digestibilities. To our knowledge, only a few experiments have been performed to determine the small intestinal digestibility of solid feed and digestive tract function in young postweaned calves (Khorasani et al., 1990; Lallés and Poncet, 1990). Analysis of apparent digestibility of CP or AA can lead to misleading conclusions because a fraction of the digesta N reaching the end of the small intestine may be synthesized endogenously. Therefore, endogenous losses (basal + diet specific or nonbasal) of N must be also quantified to obtain an accurate estimation of dietary N digestibility in the small intestine (Montagne et al., 2001).

To the best of our knowledge, no comparison between SBM and ESBM for ruminal fermentation or small intestinal nutrient digestibility in weaned dairy calves has been reported. Our objectives were to estimate endogenous N losses and to compare nutrient flow and digestibility measured at the duodenum and ileum in weaned dairy calves fed CS based on either SBM or ESBM. Our hypothesis was that, due to the enzymatic treatment of SBM, a diet containing ESBM instead of SBM would improve ruminal digestion, MCP synthesis, and small intestinal disappearance of dietary nutrients, including CP and AA.

MATERIALS AND METHODS

Animals and Treatments

The Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign approved all procedures (protocol #18146). Twelve Holstein calves (11 males and 1 freemartin female) were transported at approximately 2 d of age from a commercial dairy farm to the Nutrition Field Laboratory at the University of Illinois, Urbana. Calves were housed individually in outdoor polyvinyl chloride hutches placed on crushed limestone and bedded with straw. Calves were fed twice daily (0630 and 1830 h) a commercial milk replacer (28.5% CP, 15% fat; Excelerate, Milk Specialties Global Animal Nutrition, Eden Prairie, MN) reconstituted to 15% solids and fed at a rate of 1.5% (DM) of BW for wk 1, 2% of BW thereafter until wk 4, and 1.5% of BW during wk 5. Milk replacer was reduced gradually over 1 wk and calves were weaned on d 42. Calves had ad libitum access to water and to an SBM-based CS, which was later used as the control diet. The CS was multitextured and was offered in a bucket and in a bottle with nipple (Braden Start Bottle, Coburn Braden, Whitewater, WI) until d 49. After that, the 3 experimental CS were only offered in a bucket mixed with 5% (as-fed basis) of chopped grass hay until the end of the experiment.

On d 23 after arrival, calves were fitted with a T-cannula in the proximal duodenum and with a second

Table 1. Ingredient composition of calf starters

Ingredient, % DM	Diet[1]		
	CTRL	ENZT	LOCP
Cracked corn	22.50	22.50	22.50
Whole oats	16.50	16.50	16.50
Molasses	5.20	5.20	5.20
Pellet	55.85	55.85	55.80
Beet pulp shreds	9.28	20.08	45.04
Wheat middlings	12.13	13.99	37.54
Soybean meal 48%	51.54	—	—
HP-RumenStart[2]	—	39.90	—
Ground corn	18.44	17.33	8.80
Vitamin-mineral premix[3]	7.98	7.97	7.98
Titanium dioxide	0.72	0.72	0.72

[1]Calves were fed for ad libitum intake a control calf starter (CS) with conventional soybean meal solvent-extracted (SBM) as the main source of protein (CTRL), an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT), or a CS with low content of CP (LOCP).

[2]Enzyme-treated soybean meal (Hamlet Protein, Horsens, Denmark).

[3]Calcium carbonate, 5.4%; monocalcium phosphate, 1.4%; magnesium oxide, 0.12%; sodium chloride, 0.7%; trace mineral and vitamin mix, 0.3% (ADM Animal Nutrition, Decatur, IL); 26 kIU of vitamin A/kg; 6 kIU of vitamin D/kg; and 30 IU of vitamin E/kg.

T-cannula in the terminal ileum during the same surgical procedure. Pre-surgery and surgical procedures were as described elsewhere (Ansia et al., 2019). Cannulated calves were returned to full feeding of their milk allowance gradually over a period of 5 d. At 50 d of age, calves were blocked by BW and randomly assigned to a quadruplicated 3 × 3 Latin square with 10-d periods. The 3 experimental diets (Tables 1 and 2) were as follows: a control CS of 20% CP with SBM as the main source of protein (**CTRL**), an isonitrogenous CS with an ESBM (HP-RumenStart, Hamlet Protein A/S, Horsens, Denmark) as the main source of protein (**ENZT**), and a CS with a low content of CP (10%) and no added soy protein (**LOCP**). The ESBM was produced by treating dehulled SBM with a mixture of proprietary enzymes, which reduced the concentrations of trypsin inhibitor (1,300 ± 500 mg/kg), β-conglycinin (2.0 ± 1.0 mg/kg), oligosaccharides (10,000 ± 5,000 mg/kg; Jiang et al., 2006), and phytate (2,000 ± 500 mg/kg) in comparison with conventional SBM. The purpose of the inclusion of a low-CP diet was to extrapolate digestibilities of the test ingredients in the other 2 diets (Kong and Adeola,

Table 2. Chemical composition of calf starters and test ingredients[1] (g/kg of DM)

Item[2]	Diet			Test ingredient	
	CTRL	ENZT	LOCP	SBM	ESBM
OM	911	910	913	917	929
CP	199	206	104	528	626
NDF	196	241	280	90	104
NPN	1.72	1.73	2.07	—	—
Fatty acids	37.0	34.8	35.8	—	—
Starch	332	316	322	—	—
Ash	88.6	90.4	87.3	82.8	70.6
Ca	14.9	15.0	14.9	2.8	3.9
P	5.71	5.49	5.49	8.2	10.1
Ala	9.21	9.14	5.2	22.5	27.2
Arg	12.42	11.69	5.32	37.3	42.7
Asx	19.2	19.1	7.5	57.9	70.5
Cys	2.99	3.15	1.95	6.92	9.32
Glx	33.2	33.2	15	93.8	110
Gly	8.43	8.31	4.82	21.8	25.8
His	5.1	4.9	2.76	13.7	15.6
Ile	8.65	8.52	3.71	24.8	30.4
Leu	15.48	15.12	7.37	39.8	48.1
Lys	10.95	9.97	4.75	33.1	36.3
Met	2.81	2.69	1.52	7.14	8.36
Phe	9.51	9.4	4.25	26.4	32.4
Pro	10.48	10.47	5.97	25.6	31.2
Ser	7.91	7.98	3.88	22.5	28.6
Thr	7.13	7.03	3.49	20.3	24.0
Trp	2.23	2.01	1.07	6.81	7.61
Tyr	6.28	6.06	3.29	16.8	20.3
Val	9.25	9.11	4.49	27.0	32.4
Total AA	181	177	86	504	601

[1]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal solvent-extracted (SBM) as the main source of protein (CTRL), an isonitrogenous CS with an enzyme-treated SBM (ESBM) as the main source of protein (ENZT), or a CS with low content of CP (LOCP).

[2]Asx = Asn + Asp; Glx = Glu + Gln.

2014) and to estimate basal (minimum inevitable N loss not influenced by diet composition) endogenous losses in the small intestine by linear regression between the total and digestible CP and AA in duodenal contents (Stein et al., 2007). Titanium dioxide (0.4% DM basis) and acid insoluble ash (**AIA**) were used as external and internal indigestible markers for digesta, respectively.

Sampling, Measurements, and Chemical Analysis

On d 7 and 8 of each period, a 250-mL plastic bag (Nurser standard liners, Playtex, North Bergen, NJ) was attached to the ileal cannula using an autolocking cable tie after removing the cap. Bags were removed when full of digesta or approximately every 30 min. Digesta was collected continuously during 12 h (between the a.m. and p.m. meals). The same procedure was used on d 9 and d 10 when digesta was collected from the duodenal cannula, with the exception that collection was restricted to intervals of 2 h with 2-h periods of no collection between them. This method was applied to avoid an excessive loss of water and electrolyte flow into the small intestine because of the considerable flow of digesta out of the duodenal cannula. Starting time of collection alternated between days to cover the entire timeframe within a day. For each bag, digesta pH was measured immediately with a portable pH meter (Accumet AP110, Fisher Scientific, Atlanta, GA), and the content was frozen thereafter. Samples were pooled by calf and period. Calf starter samples were collected every second day starting on d 4 of each period and composited by period before analysis. Samples of freeze-dried feed and digesta were analyzed at the Agricultural Experimental Station Laboratory of the University of Missouri–Columbia as follows: AOAC International (2006) methods for the complete AA profile (982.30), CP (990.03), AIA (942.05), fatty acids (996.06), Ca and P (985.01), and ADF (973.18); AACC International Method (AACC, 2010) for starch (76–13.01); NDF (Van Soest et al., 1991); soluble NPN (Prigge et al., 1976); and titanium (Myers et al., 2004). Dry matter (method 930.15; AOAC International, 2010) and ash (method 942.05; AOAC International, 2010) were determined for all CS and freeze-dried digesta samples by drying in a forced-air oven at 135°C for 2 h and at 600°C for 2 h 45 min, respectively.

Body weight averaged 68 ± 8 (±SD) kg at the beginning of the experimental period (8 wk of life). Body weight and body frame measurements (heart girth, body length, withers height, hip height, and hip width) were recorded at the beginning and at the end of each 10-d period, while water and solid feed intake were measured individually twice daily (0630 and 1830 h). Respiratory health (McGuirk and Peek, 2014) and fecal scores (1 = normal, solid; 2 = semiformed, pasty; 3 = loose, but stays on top of bedding; 4 = watery, sifts through bedding) were recorded twice daily.

Flow of Nutrients

The flow of any nutrient expressed in gram per kilogram of DMI was calculated by multiplying its concentration (DM basis) in digesta (duodenal or ileal) by the flow of DM (g/kg of DMI) calculated according to the following equation:

$$\text{DM flow} = \frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{digesta}}} \times 1{,}000, \quad [1]$$

where $\text{Marker}_{\text{diet}}$ and $\text{Marker}_{\text{digesta}}$ are the Ti or AIA concentrations in gram per kilogram of DM in diet and digesta, respectively. External markers behave differently than internal markers and may yield shorter mean retention times in the rumen (Faichney et al., 1989). Therefore, the average of the DM flows calculated with each marker individually was used for all nutrient flows and digestibility calculations. Even though these 2 markers do not interact with each other (Guzman-Cedillo et al., 2017), results must be interpreted cautiously because flow through the gut of chromic oxide infused directly into the rumen is not associated with any digesta phase (Merchen, 1988) and may not be representative of the flow of all nutrients across the digestive tract.

Foregut and Small Intestine Digestibility

The apparent foregut digestibility measured at the duodenum (**AFD**) of any given nutrient was calculated using the following equation:

$$\text{AFD (\%)} = \left[1 - \left(\frac{\text{Nutrient}_{\text{duodenal}}}{\text{Nutrient}_{\text{diet}}}\right) \times \left(\frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{duodenal}}}\right)\right] \times 100, \quad [2]$$

where $\text{Nutrient}_{\text{duodenal}}$ and $\text{Nutrient}_{\text{diet}}$ are the concentrations (g/kg) in digesta at the duodenum and diet (DM basis), respectively, and $\text{Marker}_{\text{diet}}$ and $\text{Marker}_{\text{duodenal}}$ are the Ti or AIA concentrations (g/kg) in diet and digesta at the duodenum (DM basis), respectively (Stein et al., 2007).

The apparent small intestine digestibility measured at the ileum (**AID**) of any given nutrient was calculated using the following equation:

$$\text{AID } (\%) = \left[1 - \left(\frac{\text{Nutrient}_{\text{ileal}}}{\text{Nutrient}_{\text{duodenal}}}\right) \times \left(\frac{\text{Marker}_{\text{duodenal}}}{\text{Marker}_{\text{ileal}}}\right)\right] \times 100, \quad [3]$$

where $\text{Nutrient}_{\text{duodenal}}$ and $\text{Nutrient}_{\text{ileal}}$ are the concentrations (g/kg) in digesta at the duodenum and ileum (DM basis) respectively, and $\text{Marker}_{\text{ileal}}$ and $\text{Marker}_{\text{duodenal}}$ are the Ti or AIA concentrations (g/kg) in digesta at the ileum and duodenum (DM basis), respectively (Stein et al., 2007).

Digestibilities of CP and AA in the test ingredients were estimated using the difference method with the LOCP diet as a basal diet. In the test diets (CTRL and ENZT), a portion of the basal diet was replaced by the test ingredients (SBM and ESBM, respectively). Digestibility (%) of a nutrient in the test ingredient (D_{ti}) was calculated as follows (Kong and Adeola, 2014):

$$D_{ti} = D_{bd} + \frac{D_{td} - D_{bd}}{P_{ti}}, \quad [4]$$

$$P_{ti} = 1 - P_{bd}, \quad [5]$$

where D_{bd} and D_{td} are the digestibility in the basal diet and test diet, respectively, and P_{ti} and P_{bd} are the proportional contribution of the nutrient by the test ingredient and the basal diet to the test diets, respectively.

The apparently true foregut digestibility (**ATFD**) of CP and AA was estimated by using the total duodenal N concentration minus the concentration of microbial N (endogenous N is not considered) in equation 2. The true foregut (**TFD**) and small intestine (**TID**) digestibility were estimated by the percentage disappearance between the ingested and foregut undigested, and between foregut and small intestine undigested fractions, respectively, of dietary protein (estimation of these digesta fractions described in the following subsection); therefore, they are adjusted by microbial and endogenous protein flows.

The linear relationship between CP or AA absorbed in the small intestine (e.g., digestible CP_{duo}) and the respective contents of duodenal flow (CP_{duo}) can be expressed according to the following equation:

$$\text{Digestible CP}_{\text{ileo}} = \text{CP}_{\text{endo}} + \text{a} \times \text{CP}_{\text{duodenal}}, \quad [6]$$

where the intercept of the regression (CP_{endo}) is the flow of intestinal basal endogenous CP losses (i.e., flow of CP at 0 duodenal CP flow), and the slope (a) is another estimate of the TID of CP (Mariz et al., 2018).

Flows of Microbial, Endogenous, and Undigested Dietary Protein

As a marker of microbial N, total DNA was extracted from freeze-dried duodenal digesta in duplicate (4.2% intraassay CV) using a QIAamp PowerFecal DNA kit (catalog no. 12830–50; Qiagen, Hilden, Germany). Sample DNA concentration was quantified in duplicate (0.7% intraassay CV) using a Nanodrop spectrophotometer (Nyxor Biotech, Paris, France). Microbial cells were extracted by digesta fractionation (Montoya et al., 2015). Freeze-dried digesta was first reconstituted (1:20, wt:vol at room temperature) in saline solution (0.15 mol/L) and centrifuged at 250 × *g* for 15 min at 4°C. Supernatant was then centrifuged at 14,500 × *g* for 30 min at 4°C. This second precipitate contained microbial cells, and CP and DNA concentrations were measured in it to use the coefficient DNA/total N to estimate total microbial N per kilogram of digesta DM in duodenal samples.

Flows of microbial, endogenous, and undigested dietary protein in digesta (at duodenum and ileum), were also estimated by the AA profile method (**AAP**) proposed by Duvaux et al. (1990). This method uses multiple regression analysis to estimate the theoretical proportions of each protein that minimize the χ^2 distance between their composited AA profile and the AA profile of the digesta. The χ^2 distance between 2 proteins i and j was calculated according to the following equation:

$$\chi^2 = \left(\frac{AA_{ij}}{\sqrt{AA_{ij}}} - \frac{AA_{ik}}{\sqrt{AA_{ij}}}\right)^2, \quad [7]$$

where AA_{ij} and AA_{ik} are the percentages of AA_k in the sum of all the assayed AA in this study (k = 18) in proteins i and j, respectively. The lower the χ^2 distance, the greater is the similarity between the proteins involved in each comparison.

Reference AA profiles found in the literature were used for each of the digesta proteins. For the endogenous protein flow to the duodenum, the average of the mean AA profile of adult cows (Larsen et al., 2000; Richter et al., 2010) and growing goats (Zhou et al., 2008, 2009) was used, whereas only that of the adult cow (Larsen et al., 2001) was used for the flow to the distal ileum. For MCP, the AA profile of ruminal microbial protein (83.5% bacteria + 16.5% protozoa) as detailed by Sok at al. (2017) was used for both sites. The AA profile of the diets was used as reference for undigested dietary protein entering the duodenum and leaving the ileum.

Statistical Analysis

Data for pH were analyzed independently for each site of the small intestine and composited by hour. Comparisons of mean pH per diet were obtained using the PROC MIXED procedure in SAS version 9.4 (SAS Institute, Cary, NC) with the REPEATED statement. The covariance structure (compound symmetry for duodenal and autoregressive moving average for ileal samples) that resulted in the smallest Bayesian and Akaike information criterion was chosen. Period, treatment, and postfeeding hour plus its interaction with treatment were included as fixed effects, and time nested within calf and day was included as a random factor. The SLICE statement was used to find differences in the interaction between treatments and time. Initial pH values (pH of the first sample collected each day) and a set of dummy variables representing the sequence of treatments to account for the carryover effect were included as covariates. To describe the diurnal fluctuation of pH, hourly least squares means for digesta pH were obtained when the time effect was significant, and were fitted to the best possible broken-line model according to the adjusted R^2 value, significance of the model parameter estimates, and visual appraisal of the residuals using NLREG [v. 6.5, Brentwood, TN; Sherrod (1991)]. Digesta pH fluctuation was fitted using the following equation:

$$\text{pH} = \{a_0 + [b_2 \times \max(0, \text{time} - x_1)]\} + [b_1 \times \max(0, \text{time})] + [b_3 \times \max(0, \text{time} - x_2)], \quad [8]$$

where a_0 is the estimate of initial pH, b_1 and b_3 are the 2 negative slopes and b_2 the positive slope, x_1 and x_2 are the estimates of the time points where a significant change in slope occurs (knots), and time is the hour postfeeding after the morning meal.

Comparisons between treatments for mean digesta pH, ADG and body frame measurements (both actual changes and increments as a percentages of the initial value), solid feed and water intake, and fecal scores were analyzed as mixed-effect models with treatment, period, and day (only for variables measured daily) as fixed effects, square and calf within square as random, and the dummy carryover variables and the DMI average per period (as a percentage of BW) to account for the potential effect of passage rate on nutrient digestibility as covariates within the PROC MIXED procedure in SAS. Comparisons of all digestibility measures and flow of nutrients between diets were performed using PROC MIXED, with diet and period as fixed factors and square and calf within square as random effects. The CONTRAST statement was used to compare CTRL and the ENZT diets. A 1-sided t test was performed on the AFD and AID for each diet to determine if a net outflow or inflow existed (i.e., means by treatment different from 0) using the TTEST procedure with a 95% confidence interval. Assumptions about the normality and homogeneity of residuals derived from all the analyses of variance were checked using the PROC UNIVARIATE procedure and the INFLUENCE option within PROC MIXED in SAS. Statistical significance was declared when $P \leq 0.05$ and tendency when $0.05 < P \leq 0.10$.

Table 3. Least squares means of intakes, ADG, body frame measurements change, and fecal scores in weaned calves fed a conventional or enzyme-treated soybean meal-based calf starter

	Diet[1]			*P*-value
Item	CTRL	ENZT	SEM	Diet[2]
Intake, kg/d				
Starter	2.17	2.22	0.11	0.61
Water	6.73	7.11	0.43	0.38
ADG, g/d	501	466	103	0.74
Change,[3] cm				
Heart girth	1.65	2.28	0.54	0.25
Body length,	1.94	2.87	0.96	0.34
Withers height	1.28	1.14	0.67	0.83
Hip height	0.80	0.94	0.43	0.76
Hip width	0.31	0.50	0.18	0.31
Fecal scores[4]	1.78	1.44	0.27	0.56

[1]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal solvent-extracted (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT).

[2]*P*-value for the contrast between CTRL and ENZT diet.

[3]Difference between the measurement value on the last day (d 10) and first day (d 1) on each diet.

[4]Fecal scores were assigned twice per day based on the following values: 1 = normal, solid; 2 = semiformed, pasty; 3 = loose, but stays on top of bedding; 4 = watery, sifts through bedding.

RESULTS

Body Growth, Feed Intake, and Health Scores

We found no differences in ADG, body frame measurements, CS, and water intake between the CTRL and ENZT diets (Table 3). Fecal and health scores were also not different (Supplemental Table S1, http://dx.doi.org/10.17632/m22d8mtp9r.1).

Digesta pH

Mean duodenal pH tended ($P = 0.07$) to be greater for CTRL than for ENZT (Table 4). On the other hand, ileal digesta pH was lower ($P < 0.01$) with the CTRL diet in comparison with the ENZT diet. Although duodenal pH was not affected by the time postfeeding,

Table 4. Least squares means of digesta pH of samples collected at the duodenum and ileum in weaned calves fed a conventional or enzyme-treated soybean meal-based calf starter

	Diet[1]			*P*-value		
Digesta pH	CTRL	ENZT	SEM	Hour	Diet × hour	Diet[2]
Duodenal	2.98	2.89	0.06	0.43	0.14	0.07
Ileal	7.29	7.52	0.07	0.05	0.51	<0.01

[1]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal solvent-extracted (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[2]*P*-value for the contrast between CTRL and ENZT diet.

digesta pH at the end of the ileum fluctuated according to the broken-line model parameters with no difference between diets (Supplemental Table S2, http://dx.doi.org/10.17632/m22d8mtp9r.1). In brief, the pH dropped after the morning meal until approximately 9 h later and then increased rapidly until reaching preprandial values 12 h after feeding.

Flows of Ileal and Duodenal Digesta

The duodenal flows of DM ($P = 0.02$), total AA ($P = 0.01$), and NDF ($P < 0.001$) were greater with the ENZT diet, whereas the NPN ($P < 0.001$) and ash ($P < 0.01$) flow were lower when compared with CTRL (Table 5). Digesta net flows (i.e., relative to the intake of each nutrient) of CP and AA were greater ($P < 0.01$), whereas the flow of NPN was lower ($P < 0.001$) with ENZT compared with CTRL. Only the ileal flows of DM ($P = 0.05$) and ash ($P < 0.01$) were greater with ENZT than with CTRL. A tendency ($P = 0.06$) for a greater flow of NDF also was observed with the ENZT diet.

Apparent Foregut Digestibility

The AFD of OM and ash were greater ($P < 0.01$) for CTRL than for ENZT (Table 6). However, neither of the AFD values of ash were different from zero, indicating a balance between inflow and outflow of mineral content. Negative AFD values of ash and fatty acids reflected the addition of minerals from the gut endogenous secretions, and synthesis of fatty acids from microbial fermentation in the rumen, respectively. Similarly, the AFD of CP and total AA was not different from 0 for CTRL, and only that of CP was different from 0 for ENZT. Only ENZT had a positive net outflow of AA. The balance between in- and outflows or strictly negative values of AFD for AA and CP indicated a substantial contribution of de novo synthesized MCP relative to the flow of undigested dietary protein in the duodenum. The AFD was greater with the CTRL diet for all AA, except Ala, Cys, Gly, Met, Ser, and Thr compared with the ENZT diet. The AFD of CP and total AA also was greater ($P < 0.001$) for SBM than for ESBM, but AFD of CP and AA was not different from zero for ESBM, indicating a greater ability of ESBM compared with SBM to support MCP synthesis

Table 5. Least squares means of digesta flows of nutrients at the duodenum and ileum in weaned calves fed a conventional or enzyme-treated soybean meal-based calf starter

	Diet[1]			*P*-value
Digesta flow, g/kg of DMI	CTRL	ENZT	SEM	Diet[2]
Duodenal				
DM	613	652	16	0.02
CP	200	205	5	0.34
Net CP[3]	952	1,058	28	<0.001
Total AA	171	179	3	0.01
Net AA[3]	925	1,027	30	<0.01
NPN	7.76	6.46	0.3	<0.001
Net NPN[4]	37.1	33.6	1.9	0.07
Fatty acids	42.8	41.5	1.4	0.34
Starch*	93.6	87.8	8.3	0.88
NDF*	129	161	8	<0.001
Ash*	97.3	91.1	3	<0.01
Ileal				
DM*	396	427	16	0.05
CP	83.3	81.5	3.1	0.55
Total AA	68.7	66.8	2.9	0.52
NPN*	5.60	5.30	0.33	0.42
Fatty acids*	25.7	27.2	1.35	0.27
Starch*	45.5	46.7	5.4	0.82
NDF*	119	136	9	0.06
Ash	64.7	75.1	3.8	<0.01

[1]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[2]*P*-value for the contrast between CTRL and ENZT diet.

[3]Flow of a nutrient relative to the dietary intake of that specific nutrient.

[4]Flow of NPN relative to dietary protein N intake.

*Variables where the covariate of intake as a % of BW had a $P \leq 0.05$.

Table 6. Least squares means of apparent foregut digestibilities (AFD) percentages in weaned calves fed a conventional or enzyme-treated soybean meal-based calf starter

Item,[1] % of intake	Diet[2]		SEM	*P*-value
	CTRL	ENZT		Diet[3]
OM†	43.5	38.2	1.7	<0.01
Ash*	−10.1[4]	−0.2[4]	2.8	<0.01
Ca	19.1	17.0	2.9	0.49
P	−15.6	−21.7	4.1	0.15
NDF*	33.4	32.9	3.2	0.89
Fatty acids*	−14.9	−20.2	4.0	0.19
Starch	70.5	71.8	2.5	0.61
NPN	−383	−281	18	<0.001
Ala	−17.1	−18.5	4.5	0.74
Arg†	26.0	13.4	2.9	<0.001
Asx	5.6[4]	−7.0	3.7	<0.01
Cys	6.1	9.5	2.4	0.16
Glx	26.3	14.4	2.4	<0.001
Gly	−7.7	−12.3	4.3	0.29
His	21.8	12.0	2.4	<0.001
Ile	−3.7	−16.1	3.7	<0.01
Leu	5.3	−5.5[4]	3.1	<0.01
Lys	−14.2	−28.1	4.6	<0.01
Met	1.9[4]	−1.4[4]	3.2	0.31
Phe	9.6	−2.9[4]	3.2	<0.001
Pro*	24.7	19.0	2.4	0.02
Ser	5.7[4]	1.7[4]	3.6	0.26
Thr	−17.0	−23.0	4.0	0.14
Trp	14.9	5.6[4]	3.4	<0.01
Tyr	−2.0	−9.0	3.9	0.08
Val	−12.0	−21.5	3.8	0.02
ΣAA	6.5[4]	−1.0[4]	3.6	0.04
Test ingredient[5]	11.1	1.6[4]	4.9	<0.001
CP	4.8[4]	−6.0	2.9	<0.001
Test ingredient[5]	7.5	−3.4[4]	4.7	<0.001

[1]Asx = Asn + Asp; Glx = Glu + Gln.

[2]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[3]*P*-value for the contrast between CTRL and ENZT diet.

[4]Values are not different from zero.

[5]AFD of the components (CP and total AA) of the test ingredient (SBM in the CTRL diet and enzyme-treated SBM in the ENZT diet) as calculated by the difference method (Kong and Adeola, 2014).

*Variables where the covariate of intake as a % of BW had a $P \leq 0.05$ or † if $0.05 < P \leq 0.10$.

or a greater flow of undigested dietary protein to the duodenum.

Apparent Small Intestine Digestibility

Digestibility in the small intestine (Table 7) of ash ($P < 0.001$) and Ca ($P < 0.001$) were greater with the CTRL diet compared with ENZT. We observed a greater AID of Arg ($P = 0.03$), Glx ($P = 0.03$), Ile ($P = 0.04$), Leu ($P = 0.02$), Phe ($P < 0.001$), and Tyr ($P = 0.02$) and a tendency for greater AID of Gly ($P = 0.08$) for ENZT when compared with CTRL. The AID of CP and total AA in ESBM also was greater ($P = 0.01$) than in SBM.

Duodenal Flows of MCP and ATFD

The MCP flow were not different between diets, but the efficiency per kilogram of CP digested was greater ($P < 0.01$) with ENZT diet than CTRL. Accordingly, the percentage of microbial N relative to total or protein N flow was lower with ENZT (Table 8). Daily amounts of OM ($P < 0.01$), non-N fraction (OM% − CP% − fatty acids%; **NNF**; $P < 0.001$), CP ($P < 0.01$), starch

Table 7. Least squares means of apparent small intestine digestibility (AID) percentages in weaned calves fed a conventional or enzyme-treated soybean meal-based calf starter

Item,[1] % of duodenal flow	Diet[2]		SEM	*P*-value
	CTRL	ENZT		Diet[3]
OM†	29.7	35.7	3.8	0.12
Ash*	29.6	17.1	4.2	<0.01
Ca	16.3	−13.6	5.6	<0.001
P*	56.4	59.1	4.1	0.51
NDF*	10.2[4]	10.7	7.1	0.94
Fatty acids*	34.3	32.1	4.7	0.64
Starch	45.0	46.1	4.8	0.82
NPN	25.3	16.6	6.1	0.16
Ala*	54.2	58.9	3.3	0.19
Arg*	72.3	77.2	2.4	0.03
Asx*	56.8	58.8	3.2	0.53
Cys*	23.2	25.9	5.4	0.61
Glx*	51.1	57.9	3.1	0.03
Gly*	42.5	46.6	4.9	0.08
His*	56.6	58.5	3.4	0.44
Ile*	65.9	71.0	2.4	0.04
Leu*	64.2	70.0	2.5	0.02
Lys*	62.8	60.9	2.8	0.22
Met*	64.5	67.7	2.7	0.32
Phe*	63.6	70.9	2.7	<0.01
Pro*	52.0	55.0	3.6	0.28
Ser*	53.4	59.3	3.4	0.19
Thr*	49.7	54.2	3.6	0.34
Trp*	58.1	60.2	3.1	0.51
Tyr*	62.5	68.1	2.5	0.02
Val*	60.6	65.2	2.9	0.15
ΣAA*	56.8	60.7	3.0	0.20
Test ingredient[5]	66.1	71.6	2.7	0.01
CP*	55.0	59.3	3.1	0.17
Test ingredient[5]	66.2	71.8	3.0	0.01

[1]Asx = Asn + Asp; Glx = Glu + Gln.

[2]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[3]*P*-value for the contrast between CTRL and ENZT diet.

[4]Values are not different from zero.

[5]AID of the components (CP and total AA) of the test ingredient (SBM in the CTRL diet and enzyme-treated SBM in the ENZT diet) as calculated by the difference method (Kong and Adeola, 2014).

*Variables where the covariate of intake as a % of BW had a $P \leq 0.05$ or † if $0.05 < P \leq 0.10$.

($P < 0.001$) digested before the duodenum per day, and flows of NPN per day ($P < 0.001$) were lower with ENZT than with CTRL. After correction for MCP flow, the ATFD of CP ($P < 0.001$) and AA ($P < 0.01$) were lower for the ENZT diet than for the CTRL diet, and for the ESBM than for SBM ($P = 0.02$).

Basal Endogenous N Losses and True Small Intestine Digestibility

According to the regression method, basal endogenous CP and AA losses at the end of the ileum were 35 ± 5 and 25 ± 4 g/kg of DMI (Table 9), respectively, and accounted for approximately 50 and 45% of the total ileal flow. In addition to Glx and Asx, Leu (2.4 g/kg of DMI), Ser, Val, and Pro (1.8 g/kg of DMI each) were the AA with the greatest contribution to those endogenous AA losses. True small intestine digestibility estimates for CP and AA were 78 ± 3 and 77 ± 3%, respectively. Among individual AA, Arg (93 ± 2%) had the greatest and Cys (52 ± 6%) the lowest TID, whereas the mean for the remaining of the AA was 79%.

Flows of Microbial, Total Endogenous, Undigested Dietary Protein, and True Foregut and Small Intestine Digestibility

The AAP method yielded percentages of endogenous, microbial, and undigested dietary protein relative to the total CP duodenal flow of 10 ± 5% ($P = 0.06$), 52 ± 7% ($P < 0.001$), and 38 ± 7% ($P < 0.001$) with LOCP; 6 ± 4% ($P = 0.10$), 45 ± 6% ($P < 0.001$), and 48 ± 6% ($P < 0.001$) with ENZT; and 7 ± 4% ($P = 0.12$), 52 ± 7% ($P < 0.001$), and 41 ± 6% ($P < 0.001$) with CTRL, respectively. For the ileal flow,

Table 8. Least squares means for digesta flows and efficiency of microbial synthesis, and apparently true foregut digestibility of CP and AA in weaned calves fed a conventional or enzyme-treated soybean meal-based calf starter

	Diet[1]			*P*-value
Item	CTRL	ENZT	SEM	Diet[2]
Microbial N,† g/kg of DMI	16.6	17.5	1.3	0.49
Microbial N,[3] g/kg of OM	39.4	35.8	2.5	0.48
Microbial N,[3] g/kg of NNF	49.3	48.4	4.2	0.73
Microbial N,[3] g/kg of CP	156	190	9	<0.01
Microbial N,[4] % of total N	57.8	45.6	3.0	<0.001
Microbial N,[4] % of protein N	76.1	56.7	4.5	<0.01
Microbial N,* g/d	35.8	36.6	2.7	0.78
NPN,* g/d	17.2	14.2	0.7	<0.001
OM digested,[5]* kg/d	1.17	1.06	0.03	<0.01
NNF digested,[5]* g/d	893	776	32	<0.001
CP digested,[5]† kg/d	254	186	22	<0.01
Starch digested,[5]* kg/d	538	488	19	0.01
NDF digested,[5] kg/d	145	158	20	0.5
Ratio of CP:NNF[6]	0.2	0.26	0.03	0.11
Total AA ATFD[7]*	58.2	35.3	3.4	<0.001
Test ingredient ATFD[8]	55.8	46.7	8.5	0.01
CP ATFD[7]*	60.5	36.8	3.7	<0.001
Test ingredient ATFD[8]	58.4	48.5	10.4	0.01

[1]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[2]*P*-value for the contrast between CTRL and ENZT diet.

[3]Grams of microbial N per kilogram of OM, N-free fraction (NNF; OM − CP − fatty acids), CP, or starch truly disappeared before reaching the duodenum.

[4]Grams of microbial N as a percentage of total duodenal N or only protein N.

[5]Amounts of OM, NNF, CP, starch, and NDF disappeared before reaching the duodenum expressed in kilograms per day.

[6]Ratio of CP to NNF disappeared before reaching the duodenum.

[7]Apparently true foregut digestibility (ATFD) of CP and total AA was estimated by correcting total flows by duodenal microbial protein flow assuming a microbial AA-N content of 80% of the total N.

[8]The ATFD of the components (CP and total AA) of the test ingredient (SBM in the CTRL diet and enzyme-treated SBM in the ENZT diet) as calculated by the difference method (Kong and Adeola, 2014).

*Variables where the covariate of intake as a % of BW had a $P \leq 0.05$ or † if $0.05 < P \leq 0.10$.

Table 9. Linear relationship between flows of duodenal and absorbed CP and individual AA across the small intestine of weaned calves fed a conventional or enzyme-treated soybean meal-based or low-CP calf starter[1]

	Regression parameter[3]					
Item[2]	Intercept	Slope	AdjR^2	Root MSE	P_i	P_s
Ala	−1.58	0.75	0.92	0.48	<0.001	<0.001
Arg	−1.53	0.93	0.97	0.43	<0.001	<0.001
Asx	−2.03	0.72	0.87	1.38	0.001	<0.001
Cys	−0.60	0.52	0.51	0.33	<0.001	<0.001
Glx	−3.03	0.69	0.75	2.68	0.01	<0.001
Gly	−1.16	0.64	0.53	1.19	0.07	<0.001
His	−0.75	0.80	0.88	0.28	<0.001	<0.001
Ile	−1.23	0.84	0.97	0.35	<0.001	<0.001
Leu	−2.36	0.85	0.96	0.60	<0.001	<0.001
Lys	−1.58	0.77	0.93	0.62	<0.001	<0.001
Met	−0.38	0.83	0.93	0.13	<0.001	<0.001
Phe	−1.53	0.87	0.96	0.38	<0.001	<0.001
Pro	−1.76	0.80	0.91	0.49	<0.001	<0.001
Ser	−1.82	0.84	0.93	0.42	<0.001	<0.001
Thr	−1.57	0.75	0.91	0.46	<0.001	<0.001
Trp	−0.36	0.83	0.87	0.15	<0.001	<0.001
Tyr	−0.94	0.82	0.96	0.25	<0.001	<0.001
Val	−1.79	0.82	0.96	0.40	<0.001	<0.001
Total AA	−25.5	0.77	0.92	9.65	<0.001	<0.001
CP	−35.2	0.78	0.92	10.6	<0.001	<0.001

[1]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT), or a CS with low content of CP (LOCP). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[2]Asx = Asn + Asp; Glx = Glu + Gln.

[3]The intercept (in absolute value) of the regression (CP_{endo}) represents the flow of basal intestinal endogenous CP losses, and the slope (a) is an estimate of the true small intestine digestibility of CP according to the following equation: digestible CP_{ileo} = CP_{endo} + a × $CP_{duodenal}$, where digestible CP_{ileo} is the apparent absorbed concentrations of CP or AA across the small intestine, and $CP_{duodenal}$ is the duodenal flow of CP or AA, both expressed in grams per kilogram of DMI. AdjR^2 = adjusted R^2; root MSE = root mean square error; P_i = *P*-value for the intercept of the regression equation; P_s = *P*-value for the slope of the regression equation.

coefficients were 42 ± 11% ($P < 0.01$), 22 ± 13% ($P = 0.11$), and 34 ± 10% ($P < 0.01$) with LOCP; 68 ± 12% ($P < 0.001$), 0%, and 27 ± 13% ($P = 0.05$) with ENZT, and 65 ± 11% ($P < 0.001$), 0%, and 30 ± 11% ($P = 0.01$) with CTRL for endogenous, microbial, and undigested dietary protein, respectively. Applying these percentages to the total N flows at each site, we found duodenal flows of endogenous CP of 11.5 g of CP/kg of DMI, 9.4 g of N/kg of DMI of microbial N, and 46 g of CP/kg of DMI of dietary undigested CP with LOCP (Table 10). Duodenal flows of endogenous CP were not different between CTRL and ENZT. However, flows into the duodenum of microbial N were greater ($P < 0.001$), and those of dietary undigested were smaller ($P < 0.001$) for CTRL than ENZT. At the end of the ileum, we found flows (g of CP/kg of DMI) of 24.7 g of endogenous and 19.6 g of dietary undigested CP with LOCP. Endogenous protein flows at the end of the ileum were not different between CTRL and ENZT, but those of dietary protein were greater ($P < 0.01$) with CTRL than with ENZT. Similar differences were observed when comparing AA flows. Total (microbial + endogenous) protein losses with LOCP were 37.2 ± 1.5 g of CP and 28.9 ± 1.4 g of AA/kg of DMI, representing 64% of the total N flow reaching the end of the ileum. Comparing the duodenal and ileal undigested fractions of dietary undigested protein (Table 11), we observed a greater TFD ($P < 0.001$) of CP and AA for CTRL than for ENZT. On the other hand, the TID of CP and AA, respectively, was greater ($P < 0.001$) with ENZT (78 and 78.7%) than with CTRL. The ESBM diet had lower ($P < 0.001$) TFD and greater ($P < 0.001$) TID than SBM for CP and AA.

DISCUSSION

Despite a lower disappearance before the duodenum of CP and AA, the net flow of both nutrients was greater with the ENZT diet than with CTRL. Feeding a CS with ESBM instead of SBM as the only source of protein increased the efficiency of MCP synthesis and the flow of undigested dietary protein into the duodenum. The AID was greater only for the ESBM ingredient, but no differences were observed between

Table 10. Flows (g/kg of DMI) of endogenous, microbial, and undigested dietary protein estimated by the AA profile method[1] at the duodenum and ileum of weaned dairy calves

	Diet[2]				*P*-value	
Item	CTRL	ENZT	LOCP	SEM	CTRL vs. ENZT	CTRL + ESBM vs. LOCP
Duodenum						
Endogenous CP	13.1	13.3	11.5	0.41	0.54	<0.001
Microbial N	16.6	14.7	9.9	0.44	<0.001	<0.001
Dietary CP	82.6	100.1	46.0	2.63	<0.001	<0.001
Endogenous AA	11.3	11.3	10.1	0.37	0.97	<0.001
Microbial AA-N	14.2	12.8	8.4	0.4	<0.001	<0.001
Dietary AA	70.7	86.3	38.6	2.38	<0.001	<0.001
Ileum						
Endogenous CP	53.6	55.3	24.7	1.47	0.40	<0.001
Microbial N	—	—	2.05	—	—	—
Dietary CP	25.5	21.7	19.4	0.68	<0.001	<0.001
Endogenous AA	44.1	45.3	19.0	1.4	0.52	<0.001
Microbial AA-N	—	—	1.60	—	—	—
Dietary AA	20.8	18.2	15.2	0.64	<0.01	<0.001

[1]Coefficients applied to the total flow of CP to estimate the flows of undigested dietary, microbial, and endogenous protein at the duodenum and ileum of weaned calves were estimated by multiple regression analysis and χ^2 distance according with Duvaux et al. (1990). For the endogenous protein flow at the duodenum, the mean AA profile of adult cows (Larsen et al., 2000; Richter et al., 2010) and growing goats (Zhou et al., 2008, 2009) was used, whereas only that of the adult cow (Larsen et al., 2001) was used for the flow at the ileum. For microbial N, the AA profile as detailed reviewed by Sok et al. (2017) was used for both sites, and the AA profile of the diets was used as reference for dietary undigested protein at both sites as well.

[2]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), an isonitrogenous CS with an enzyme-treated SBM (ESBM) as the main source of protein (ENZT), or a CS with low content of CP (LOCP). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

diets. However, when corrected by the estimated total endogenous protein losses, the TID of CP and AA was greater for the ENZT diet.

Digesta pH

The pH values obtained for all diets were within the range of values observed in healthy adult cows (Van Winden et al., 2002). Among other benefits, enzymatic treatment of SBM reduces the presence and activity of ANF in SBM (Reddy and Pierson, 1994). The greater content of protease inhibitors in the CTRL diet compared with the other diets may have reduced the capacity for pH regulation by the acinar-ductal units of the exocrine pancreas as digesta passes across the duodenum, explaining the lower digesta pH at the end of the ileum (Lallés, 1993; Hegyi et al., 2011).

Fully functional ruminants have a near constant flow of digesta through the abomasum, and diurnal fluctuations of pH are not as prominent as those in young milk-fed calves (Constable et al., 2006), even though ingestion of solid feed stimulates acid production in the rumen and abomasum (Kim et al., 2016). However, the pH fluctuation observed in ileal digesta resembled that in young milk-fed calves (Ansia et al., 2019, 2020b), with a decrease after the fresh starter was offered until approximately 9 h postfeeding, when pH started to recover toward prefeeding values.

Effects of SBM and ESBM

Enzymatic treatment of feed ingredients can promote MCP synthesis by increasing its degradability without modifying its chemical composition (Seo et al., 2013). Unlike in calves 1.5 mo older on average than the calves used in this study (Ansia et al., 2020c), we observed, however, a lower foregut digestion for the ESBM and the ENZT diet. Previous chemical analyses of both protein sources demonstrated that ESBM has less soluble (9 vs. 27% CP) and degradable (42 vs. 49% DM) protein, and more neutral detergent insoluble CP (8.3 vs. 6.4% DM) compared with SBM. These differences may indicate that the more readily degradable fraction of CP was already digested during the enzymatic treatment, resulting in a greater concentration of the less degradable fraction in the final product. Foregut digestibilities of the test ingredients must be considered cautiously because the difference method assumes no interaction between the digestibilities of components in the test ingredients and the basal diet. The effect of the lower CP content of the basal diet on ruminal fermentation might cause this assumption not to be true. Nevertheless, this method should still perform satisfactorily to compare digestibility values between the test ingredients on both soy-based diets because the same limitation applies to both.

Assuming that most of the true digestion of OM occurred in the rumen (Ipharraguerre et al., 2002), less

OM was available per day for MCP synthesis with ENZT than with CTRL. However, we found no differences in MCP daily flow or its efficiency. Unlike the efficiency of MCP synthesis per kilogram of OM digested (**EMPS**), which is a reliable indicator of the use of energy by microbes, the greater efficiency of MCP synthesis per kilogram of dietary CP available or efficiency of dietary N utilization (**EDNU**) indicates a better utilization of the available N to create MCP with the ENZT diet (Bach et al., 2005). Less degradable proteins can increase microbial growth by means of a more efficient capture of dietary N (Beever et al., 1987; Harun, 2019). Even though we did not consider the available N for microbial growth from endogenous proteins in the rumen, we found no differences in duodenal NPN flow per kilogram of digested CP or in the endogenous protein flows estimated by the AAP method. Urea N recycling also could have compensated the deficiency of RDP and become an important source of N available for ruminal microbes, despite not finding differences in NPN duodenal flow, because dietary CP content does not alter the microbial efficiency of ruminal ammonia use (Agle et al., 2010). In addition, microbial enzymatic hydrolysis of soy protein increases the proportion of small-size molecules and reduces the presence of antinutritional factors, which can also promote ruminal microbial activity (Reynal and Broderick, 2003; D'Mello, 2006).

Estimates of the proportion of MCP derived with the AAP method agree closely with values measured quantitatively. The comparison of estimated flows with this method identified a greater proportion of MCP with CTRL and of dietary undigested CP at the duodenum with ENZT relative to total duodenal CP. Because the same estimated coefficient was applied to all individual flows in the AAP method, the smaller SEM allowed for detection of differences in microbial flows between the soy-based diets.

Carbohydrate fermentation is the most important factor limiting microbial synthesis (Hoover and Stokes, 1991; Seo et al., 2013). The actual balance between energy and available protein likely was the reason the maximal microbial efficiency was obtained with the

Table 11. True foregut (TFD) and small intestine (TID) digestibilities of CP and total and individual AA in weaned calves fed a conventional or enzyme-treated soybean meal-based or low-CP calf starter

	TFD[1]				TID[1]			
	Diet[2]			*P*-value	Diet[2]			*P*-value
Item	CTRL	ENZT	SEM	Diet[3]	CTRL	ENZT	SEM	Diet[3]
Ala**	52.3	41.7	1.6	<0.001	67.5	77.7	1.9	<0.001
Arg	69.8	57.4	1.1	<0.001	80.5	87.6	1.5	<0.001
Asp**	61.1	48.3	1.5	<0.001	69.3	77.9	1.8	<0.001
Cys††	62.2	54.8	1.1	<0.001	46.8	58.4	3.0	<0.001
Glx**	69.6	58.6	1.0	<0.001	63.4	74.1	2.2	<0.001
Gly**	56.0	44.8	1.7	<0.001	58.4	67.5	2.3	<0.001
His**	67.8	57.4	1.0	<0.001	69.2	77.6	1.8	<0.001
Ile**	57.3	43.8	1.6	<0.001	75.8	84.2	1.5	<0.001
Leu††	61.0	48.9	1.3	<0.001	74.6	83.1	1.6	<0.001
Lys**	53.0	38.2	1.9	<0.001	74.5	79.0	1.8	0.02
Met††	60.2	50.1	1.4	<0.001	74.9	82.4	1.8	<0.001
Phe††	62.8	50.2	1.3	<0.001	74.2	84.2	1.7	<0.001
Pro†**	69.2	60.2	0.9	<0.001	66.1	75.7	2.1	<0.001
Ser**	61.5	51.5	1.4	<0.001	66.9	78.0	2.0	<0.001
Thr**	51.8	40.5	1.7	<0.001	64.2	75.3	2.1	<0.001
Trp**	65.1	53.6	1.4	<0.001	70.3	78.5	2.0	<0.001
Tyr**	58.3	46.5	1.5	<0.001	73.4	82.7	1.4	<0.001
Val**	53.8	41.2	1.6	<0.001	72.0	81.0	1.7	<0.001
Total AA**	61.9	50.2	1.3	<0.001	69.4	78.7	1.7	<0.001
Test ingredient[4]	61.7	46.1	2.2	<0.001	77.1	91.2	1.9	<0.001
CP**	60.8	48.7	1.2	<0.001	68.0	78.0	1.8	<0.001
Test ingredient[4]	60	42.8	2.0	<0.001	77.7	93	2.2	<0.001

[1]True foregut and small intestine digestibilities were calculated by comparing the estimated flows of undegraded protein at the duodenum with dietary protein, and flows of undigested protein at the ileum and undegraded protein at the duodenum, respectively, with the AA profile method.

[2]Calves were fed during 10 d for ad libitum intake a control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein (CTRL), or an isonitrogenous CS with an enzyme-treated SBM as the main source of protein (ENZT). Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum.

[3]*P*-value for the contrast between CTRL and ENZT diet.

[4]True foregut or small intestine digestibility of the components (CP and total AA) of the test ingredient (SBM in the CTRL diet and enzyme-treated SBM in the ENZT diet) as calculated by the difference method (Kong and Adeola, 2014).

*TFD values for which the covariate of intake as a % of BW had a $P \leq 0.05$ or † if $0.05 < P \leq 0.10$, or ** and †† for TID, respectively.

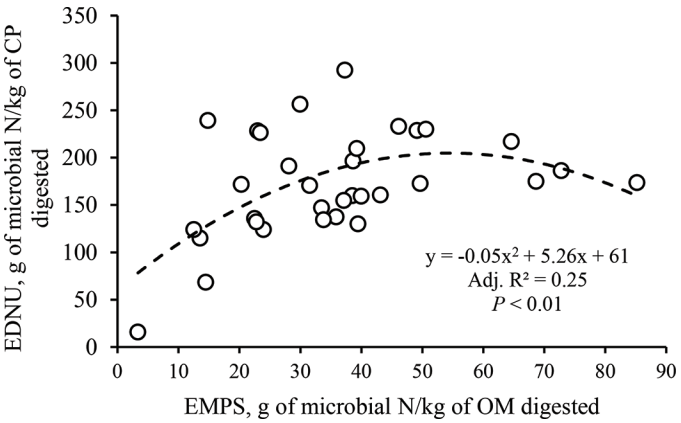

Figure 1. Relationship between efficiency of microbial protein synthesis (EMPS) and efficiency of dietary N utilization (EDNU) of weaned calves at the duodenum. Calves were fed ad libitum intake for 10 d with control calf starter (CS) with conventional soybean meal (SBM) as the main source of protein, an isonitrogenous CS with an enzyme-treated SBM as the main source of protein, or a CS with low content of CP. Digesta samples were collected on d 7 and 8 from the duodenum and on d 9 and 10 from the ileum. Root mean square error = 48.13; the coefficient of EMPS^2 ($P = 0.01$) was significantly different from 0. Adj. = adjusted.

ENZT diet (Clark et al., 1992). An excess of degradable carbohydrates may reduce microbial growth efficiency by energy spilling (dissipated as heat) and by synthesis of reserve carbohydrate (Hackmann and Firkins, 2015). As proposed by Bach et al. (2005), EMPS and EDNU are complimentary indicators of microbial nutrient uptake. Accordingly, we established a quadratic relationship (Figure 1) between these values, from which we calculated that the optimal efficiency of microbial growth occurred with an EMPS of 54 g of microbial N/kg of OM digested and an EDNU of 205 g of microbial N/kg of CP digested. In the most optimal conditions in this experiment, uptake by the microbes would be 128% of the dietary N available, which means that in addition to the complete utilization of dietary N, endogenous N contributed as well. Accordingly, the ENZT diet had values of EMPS and EDNU closest to those of the calculated maximum efficiency. The CTRL diet was the only diet where not all the dietary N (97%) was used for MCP synthesis.

In the present study, starch AFD was not as high (about 10% lower) as observed in 1.5-mo older calves (Ansia et al., 2020c) or in adult cows (Herrera-Saldana et al., 1990; Panah et al., 2020). Likewise, the AID of starch observed in the present experiment was less than reported for adult cows, which usually is between 60 and 70% and is quite consistent, regardless of whether it occurs through microbial fermentation or enzymatic hydrolysis (Gilbert et al., 2015; Panah et al., 2020). Foregut disappearance of NDF also was lower in our study than in adult cows (~70% decrease) or older calves (~30% decrease). These observations indicate that although already functioning as ruminants, recently weaned calves lack the full capacity to digest complex carbohydrates in the rumen, although ruminal digestion capacity improves at a fairly quick pace with time (Gelsinger et al., 2019). Our NDF and starch AFD values agreed with those reported by Gelsinger et al. (2019), who used in situ 9-h ruminal incubations. Although in their study NDF digestibility values did not vary between 9-h and 24-h incubation times, those of starch increased substantially with incubation time, which indicates that its digestibility must be greatly influenced by the passage rate through the rumen. Fractional ruminal passage rate does not incur important changes after weaning (Vazquez-Anon et al., 1993; Gelsinger et al., 2020); however, the rapid increase in rumen volume markedly increases the particle mean retention time in the rumen (Vazquez-Anon et al., 1993). In addition, rumination time increases with age and DMI (Swanson and Harris, 1958), and consequently could contribute to the greater dietary nutrient digestibility in older animals. A confounding effect of the greater NDF concentration in ENZT on fermentation and digestion parameters cannot be dismissed. However, there was no difference in the amount of NDF digested at the duodenum; therefore, we would not expect a substantial effect. A greater concentration of digestible nutrients or more economic ingredients in the diet are opportunities provided by using a protein source with higher CP and AA content.

Even with a greater flow of undigested dietary CP at the duodenum, the ESBM ingredient and the ENZT diet had a greater TID of CP and total AA across the small intestine, according to the estimates from the AAP method. Enzymatic hydrolysis of soy protein may improve its small intestinal digestion due to the greater proportion of smaller peptides (Kiers et al., 2000; Feng et al., 2007) and likely due to greater action of ruminal fermentation over the less rumen degradable protein fraction (Hvelplund and Hesselholt, 1987). True small intestine digestibility of CP with the AAP method in the soy-based diets (73.4 ± 4%) was lower than the value obtained with the regression method (78.2 ± 3%). Nevertheless, when the regression method was applied to CTRL (75.7 ± 3%) and ENZT (79.9 ± 3%) separately, we found a similar difference between methods that was again favorable to the ENZT diet. Similar to Khorasani et al. (1990), both methods used in the present experiment revealed that Arg was the AA with the greatest AID and TID among all diets. The TID values of CP with either method were close to that usually reported in adult cows (75%; Marini et al., 2008; Mariz et al., 2018).

Results of the present experiment did not indicate that there was any flow of MCP at the end of the ileum, which reflected the high digestibility of this protein. Microbial protein TID averages 85%, but can be 87% as directly determined in sheep (Schwab and Broderick, 2017). Due to likely differences between the AA profiles of MCP used as reference (rumen microbial protein) and the MCP at the end of the ileum, the model used in the present experiment may have introduced small inaccuracies because the *P*-values of the estimates for MCP at the duodenum were all significant, whereas those at the ileum showed a tendency for significance with the LOCP diet. Nevertheless, estimates for undigested dietary protein and endogenous protein were all significant and accounted for 95% of the total protein flow. Therefore, MCP was most likely present in very small amounts in ileal digesta.

Endogenous Protein Losses

The AAP method permitted us to estimate the proportions of duodenal CP originated from endogenous sources, which were close to those reported in adult cows (Kim et al., 2001; Marini et al., 2008). Despite the differences in diet characteristics between adult and young ruminants (high vs. low forage inclusion), dietary fiber concentration does not alter the flow of endogenous CP into the duodenum (Larsen et al., 2000; Ouellet et al., 2002; Zhou et al., 2008). At the end of the ileum, estimated total endogenous CP losses were strictly endogenous protein secretions from the animal (nonmicrobial N) with both CTRL and ENZT diets, whereas endogenous CP losses with LOCP included 33% of MCP. The greater proportion of undigested starch and NDF flowing into the small intestine with the LOCP diet (data not shown) may have promoted microbial fermentation, and thus proliferation of microbial mass in the lower small intestine (Klusmeyer et al., 1990; Gilbert et al., 2015). Similarly, basal endogenous losses (N losses at 0% dietary CP) in milk-fed calves also are composed of microbial and endogenous CP losses (Montagne et al., 2000, Ansia, et al., 2020b).

Basal endogenous N losses calculated with the regression method [4.94 ± 1.24 (SD) g of N/kg of duodenal OM] were slightly lower than those reported in adult dairy cows (5.60 ± 0.53 g of N/kg of duodenal OM) as estimated by Marini et al. (2008) and than those from 1.5-mo older calves (5.60 ± 2.72 g of N/ kg of duodenal OM; Ansia at al., 2020c). The flow of basal endogenous losses (35 ± 5 g of CP and 25 ± 5 g of AA/kg of DMI) estimated with the regression method were similar to the flow of total CP losses estimated from the LOCP diet with the AAP method (37 ± 1.5 g of CP and 31 ± 1.5 g of AA/kg of DMI). This observation indicated that the ileal CP and AA losses with a 10% CP diet may be considered the basal endogenous losses. Because MCP synthesis is maximized when dietary CP is between 11 and 12% of DM, a diet containing less than 10% CP may not be able to sustain the same microbial efficiency. Therefore, endogenous and MCP flows with a diet with less than 10% CP cannot be extrapolated to diets with increasing CP contents. At least, data would not fit in a linear equation (Fan et al., 1995). Recycling of endogenous urea can compensate to a certain extent for the lack of degradable protein or for the asynchrony between carbohydrates and N available in the rumen (Reynolds and Kristensen, 2008). However, microbial uptake of N from endogenous urea is limited (Marini et al., 2008; Reynolds and Kristensen, 2008), and microbial growth efficiency is greater when microbes (especially cellulolytic and amylolytic bacteria) utilize N from AA and peptides instead of from ammonia (Bach et al., 2005). Data from the present experiment indicate that total (endogenous + microbial) protein losses from calves fed a 10% CP diet may be considered a close estimate of the basal endogenous CP losses obtained in diets with up to 20% CP if dietary concentrations of starch are similar.

CONCLUSIONS

The use of ESBM as the only supplemental source of CP in CS enhanced microbial efficiency in the rumen per kilogram of dietary CP, the flow of dietary undigested protein into the duodenum, and the small intestine digestibility of CP and AA when compared with SBM. Endogenous protein losses represented 66% of the total CP reaching the end of the ileum; therefore, they must be accounted for when estimating small intestine digestibilities of CP and AA in diets and ingredients fed to young calves. Total endogenous protein losses of a diet with 10% CP may be considered the basal endogenous losses for weaned calves.

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