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A Casein Hydrolysate Does Not Enhance Ileal Endogenous Protein Flows Compared With the Parent Intact Casein When Fed to Growing Pigs

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ABSTRACT

Background: The form of dietary nitrogen (free peptides or intact proteins) may influence the amount of endogenous amino acids found at the terminal ileum of the pig, and it has been speculated that hydrolyzed dietary protein may lead to increased endogenous amino acids.

Objective: To compare the effect of dietary free peptides on ileal endogenous nitrogen and amino acid flows [ileal endogenous nitrogen flow (ENFL), ileal endogenous amino acid flow (EAAFL)] with that of peptides released naturally from dietary protein during digestion, from the same intact parent protein source.

Methods: Six pigs (mean body weight: 34 kg) were equipped with a postvalve T-caecum cannula. Semisynthetic test diets contained the same ^{15}N-labeled intact casein (C) or hydrolyzed casein (HC). Pigs received the test diets every sixth day and the corresponding unlabeled diets in the intervening 5-d periods. Digesta were pooled from 4 to 10 h postprandially. EAAFL and ENFL, calculated with reference to the dietary marker titanium dioxide, were determined by isotope dilution for C and HC.

Results: Ileal EAAFL and ENFL (mean flows $n = 5$ of 1828 and 1912 μg/g of dry matter intake for diets HC and C, respectively) did not differ ($P > 0.05$) between pigs fed HC and C. Centrifugation and ultrafiltration of the HC digesta allowed an estimation of label recycling into gut endogenous proteins. Some 20% of ileal endogenous protein (diet HC, ultrafiltered digesta) was ^{15}N-labeled due to tracer recycling.

Conclusions: The administration of a casein hydrolysate had no effect on ileal endogenous protein flows compared with C. There was no evidence of enhanced ileal endogenous protein losses with the HC diet. *Curr Dev Nutr* 2018;3:nzy083.

Introduction

After the ingestion of a protein-containing meal, undigested dietary and nondietary (endogenous) proteins accumulate at the terminal ileum. It is important to be able to determine the amount of ileal endogenous amino acids (AAs) in the terminal ileal digesta, because such a measure is required to determine true ileal AA digestibility (1, 2), and the endogenous AAs are also an important component of the daily AA requirement (3, 4).

Although protein-free diets have traditionally been used in the determination of digesta endogenous protein (5), such an approach has been criticized as leading to a physiologically abnormal metabolism (6). The enzyme-hydrolyzed protein/ultrafiltration technique (7–9) was developed as an alternative approach whereby animals or human subjects are fed an array of peptides [usually casein-derived, molecular weight (MW) <5 kDa], mimicking the breakdown products of natural digestion. After digesta centrifugation and ultrafiltration (10 kDa MW cutoff), endogenous protein is determined in the high MW fraction. Any undigested dietary AA or peptides (MW <10 kDa) are discarded. The method is commonly used in practice in both animal

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Abbreviations used: AA, amino acid; BW, body weight; C, intact casein; DEB, dietary electrolyte balance; DMI, dry matter intake; EAAFL, ileal endogenous amino acid flow; ENFL, ileal endogenous nitrogen flow; HC, hydrolyzed casein; MW, molecular weight; PVTC, postvalve T-caecum; TiO_2, titanium dioxide.

and human digestion studies (10). It has been questioned, however, as to whether potentially bioactive peptides present in the hydrolysate used with this method may enhance the loss of AA from the small bowel (11–13), over and above that which may be found with the corresponding intact protein. The breakdown products in the gut of the hydrolyzed casein may differ in type and rate of production, which may in turn influence the endogenous proteins.

A previous study has reported greater ileal endogenous AA losses in pigs fed enzymatically hydrolyzed casein than in those fed normal casein (12). However, in the latter study, different protein sources and different methodologies were used for determining endogenous losses between dietary treatments, and dietary electrolyte balance (DEB, $Na^+ + K^+ - Cl^-$) was not controlled. The latter may have an influence on the ileal endogenous losses, because it has been reported to impact apparent ileal nitrogen digestibility in pigs (14).

We have demonstrated, in a controlled study using the ^{15}N-isotope dilution method with the growing rat and a single source of isotopically labeled protein, that intact and hydrolyzed casein have a similar influence on ileal endogenous protein flow (15). The latter findings, however, were tentative because of a high degree of recycling (~65%) of dietary ^{15}N encountered in the frequently fed rat.

Our objective here, therefore, was to assess in the meal-fed growing pig, in which the extent of the dietary ^{15}N recycling was expected to be lower than in the frequently fed rat, the influence of a casein hydrolysate [diet hydrolyzed casein (HC)] compared with the parent intact casein (diet C) on endogenous ileal AA flow. DEB was adjusted between diets, and AA composition was controlled. Both forms of casein were ^{15}N-labeled (same source of protein) so that endogenous protein flows could be determined and compared for both diets (C and HC) using the isotope dilution technique. Specifically, the work addressed the hypothesis that feeding a hydrolyzed casein-based diet would lead to heightened endogenous ileal protein loss compared with an intact protein counterpart. The work also afforded estimation of the degree of recycling of the ^{15}N label at the terminal ileum. Isotope recycling at the lower ileum is known (16) to occur in the pig but to date has not been quantified.

Methods

Animals and housing

Six 10-wk-old Large White × Duroc entire male pigs were housed individually in steel metabolism crates in a room maintained at 24°C ± 1°C. Ethics approval was received from the Massey University Animal Ethics Committee (protocol 05/29).

Surgery

The mean ±SE body weight (BW) on the day of surgery (day 0 of the experimental period) was 34.4 ± 2.0 kg. The pigs were fasted for 12 h before surgery. A postvalve T-caecum (PVTC) cannula was inserted into the caecum of each pig for the collection of ileal digesta, according to the method of van Leeuwen et al. (17). The cannulae were made of medical grade silastic tubing with an internal diameter of 24 mm and external diameter of 32 mm. Before the start of surgery, the pigs were given analgesics: carprofen (Pfizer Laboratories Ltd; 3 mg/kg BW) administered by intravenous injection, and methadone (David Bull Laboratories; 0.2 mg/kg BW) administered by deep intramuscular injection. Anesthesia was induced with an intramuscular injection of midazolam (Roche Products Ltd; 1 mg/kg BW) and ketamine (Parnell Laboratories Ltd; 10 mg/kg BW) followed by an intravenous injection of propofol (Gensia Laboratories Ltd; 2 mg/kg BW). The anesthesia was maintained via inhalation of isoflurane (Merial Ltd; 1.5–2%) in oxygen. Intravenous crystalloids were infused throughout the anesthesia period (5–10 mL · kg BW^{-1} · h^{-1}) to maintain hydration. Immediately after surgery, the pigs received an intramuscular injection of antibiotic (Duplocillin LA, Intervet International B.V.; 2 mL). For the following 4 d, antibiotic powder (Mamyzim, Boehringer Ingelheim Ltd) was dusted on the wound site daily. The site where the cannula was exteriorized was washed with water, and zinc ointment was applied daily throughout the experiment. The pigs regained consciousness within 1 h of surgery and were standing 7–8 h after surgery. There was a 14-d recovery period before the experimental diets were given.

Diets

In total, there were 5 diets: a basal diet, 2 adaptation diets, and 2 test diets (**Table 1**). The test diets contained as the sole source of nitrogen, either uniformly ^{15}N-labeled native phosphocaseinate (C diet) or a ^{15}N-labeled casein-derived hydrolysate (derived from the same native phosphocaseinate, HC diet). A highly digestible basal diet (Table 1) was prepared as described previously (18). The adaptation diets contained the same (but unlabeled) nitrogen sources as those included in the test diets (Table 1). All diets were formulated to meet the nutrient requirements of the growing pig (19). Sodium bicarbonate was added to diet C to equalize the DEB to that of diet HC (20), resulting in DEB values of 199 and 207 mEq/kg of dry matter for C and HC diets, respectively.

Titanium dioxide was added to the test diets (3 g/kg of diet air-dry weight) as an indigestible marker. The determined total nitrogen and AA compositions of the diets are given in **Table 2**.

The ^{15}N-casein was extracted by microfiltration followed by diafiltration (INRA Rennes) of ^{15}N-labeled milk (21). The resulting casein was freeze-dried. Its isotopic enrichment was 0.5450%. There was a homogenous distribution of the ^{15}N-label among individual AA for which enrichment ranged from 0.4942 to 0.5653 atom%. An aliquot of ^{15}N-casein was hydrolyzed with pig pancreatin as previously detailed (20). The MW profile, determined by HPLC gel filtration (20), indicated that 21% of the peptides were between 1 and 5 kDa in size, and 79% were <1 kDa.

Experimental design

For 14 d postsurgery, all pigs were fed the basal diet, which was gradually introduced over the first 7 d up to a daily feeding level of 0.08 metabolic BW ($BW^{0.75}$). This level of feed intake was maintained for the remainder of the trial. Except on digesta collection days, the pigs received 3 meals/d (at 0800, 1200, and 1600) in equal portions. The diets were mixed with water (1:1, w/w), and water was freely available between meals. On digesta collection days, the pigs received (at 0900) the test diet (one-third of the daily portion) mixed with water (2.3:1, w/w), and 200 mL water was given every 30 min. The pigs received the rest of their daily portion at 1900. The pigs were weighed every sixth day, and feed intake was adjusted accordingly.

TABLE 1 Ingredient compositions of the diets fed to the growing pigs[1]

Ingredient	Adaptation diets, g/kg air dry weight		Basal diet, g/kg air dry weight	Test diets,[2] g/kg air dry weight	
	C	HC		C	HC
Cooked wheat[3]	—	—	485.8	—	—
Sucrose	—	—	168	—	—
Casein[4]	—	—	159	—	—
Skim milk powder[4]	—	—	80	—	—
Soybean oil	—	—	80	—	—
Dicalcium phosphate	—	—	22	—	—
Vitamin-mineral mix[5]	—	—	2.5	—	—
Sodium chloride	—	—	1.6	—	—
Synthetic methionine	—	—	0.7	—	—
Calcium carbonate	—	—	0.2	—	—
Antioxidant[6]	—	—	0.2	—	—
Maltodextrin[7]	369.3	364	—	453	448
Sucrose	161	161	—	161	161
Soybean oil	154	154	—	154	154
Casein[8]	211	—	—	211	—
HC[8]	—	234	—	—	234
Purified cellulose[9]	50	50	—	—	—
Vitamin-mineral mix[5]	2.5	2.5	—	—	—
Dicalcium phosphate	25	25	—	—	—
Potassium bicarbonate	4	4	—	—	—
Magnesium sulfate	4	4	—	—	—
Sodium bicarbonate	18	—	—	18	—
Sodium chloride	1.2	—	—	—	—
Potassium chloride	—	1.5	—	—	—
Titanium dioxide	—	—	—	3	3

[1] C, intact casein; HC, hydrolyzed casein.
[2] Some ingredients in the adaptation diets were excluded from the test diets because the test diets were subsequently given to human subjects to allow an interspecies comparison.
[3] Weet-bix, Sanitarium, Auckland, New Zealand.
[4] NZMP, Palmerston North, New Zealand.
[5] Vitalean, Vitec Nutrition Ltd., Auckland, New Zealand. Vitamins provided: (g/kg of diet) vitamin A 3; (mg/kg diet) cholecalciferol 500.0, choline 83.3, niacin 12.5, pantothenic acid 8.3, riboflavin 2.1, vitamin B6 1.7, vitamin E 41.7, vitamin K 1.7; (μg/kg of diet) biotin 8.3, folic acid 417, thiamine 833, vitamin B-12 8.3. Minerals provided: (mg/kg diet) copper 104, iron 83, manganese 38, zinc 100; (μg/kg diet) iodine 833, cobalt 417, selenium 250.
[6] Ethoxyquin, Kemin Industries Ltd., Auckland, New Zealand.
[7] Fieldose 17, Penford NZ Ltd., Auckland, New Zealand.
[8] Nonlabeled proteins in the adaptation diets (NZMP, New Zealand). ^{15}N-labeled proteins in the test diets (INRA Rennes, France).
[9] Avicel. Commercial Minerals Ltd., Auckland, New Zealand.

After postsurgery recovery (day 14), the pig mean ±SE BW was 39.8 ± 0.9 kg. Between day 14 and day 38, an acute feeding protocol was conducted, but this was not the central objective of the present report. At the start of the experimental period (day 38), the mean BW was 61.3 ± 2.9 kg. The experimental period lasted for the following 18 d (3 × 6-d periods).

Feeding and digesta collection

The test diets (C, HC) and a third diet, which was not the subject of the present study, were administered using a duplicated 3 × 3 Latin square design. The pigs were randomly allocated to the Latin square and were fed their respective test diet every sixth day after having been fed the corresponding adaptation diet (containing similar, but unlabeled nitrogen) during the intervening 5-d periods. On the sixth day of each test period, ileal digesta were collected continuously for 10 h following ingestion of the test diet, using plastic bags attached to the cannula. The bung of the cannula was removed 2 h before the collection commenced, as described by van Leeuwen et al. (17), to allow the ileocaecal valve to move so that it was protruding into the lumen of the cannula instead of the intestinal lumen. Digesta collection commenced 30 min before the ingestion of the test diet in order to determine the basal ^{15}N-enrichment of the digesta. Plastic bags were removed every 30 min, and digesta were immediately frozen (–20°C) after addition of benzoic sodium (2.3 mol/L) and phenylmethylsulfonyl fluoride (70 mmol/L). This

TABLE 2 Determined amino acid and nitrogen contents of the test diets

	Diet, g/kg air dry weight	
Amino acid	Intact casein	Hydrolyzed casein
Threonine	7.7	7.2
Valine	12.3	11.1
Isoleucine	9.4	9.0
Leucine	18.2	16.6
Phenylalanine	10.1	9.0
Tyrosine	11.0	9.9
Lysine	15.1	13.8
Histidine	5.5	4.3
Serine	9.2	8.8
Glutamic acid	42.5	40.3
Alanine	7.9	6.6
Proline	21.7	19.6
Arginine	6.6	6.2
Aspartic acid	13.2	12.3
Glycine	3.5	3.2
Cysteine	0.6	0.6
Methionine	5.8	5.4
Tryptophan	2.5	2.2
Nitrogen	32.6	31.8

procedure was adopted to prevent bacterial and protease activity, respectively, in the digesta samples (22).

Chemical analysis

Digesta were freeze-dried, ground, and pooled for each diet and pig over the collection time period between 4 and 10 h after ingestion of the test diet (16, 23). Pooled digesta were analyzed for titanium dioxide (TiO_2), total nitrogen, AAs, and ^{15}N-enrichment of the total nitrogen and single AAs. Pooled digesta from pigs fed diet HC were divided into 2 portions to be analyzed "as is" or after centrifugation and ultrafiltration with a 10-kDa MW cutoff (ultrafiltered digesta = precipitate + retentate) as described previously (20). The ultrafiltered digesta were analyzed for total nitrogen, AAs, the ^{15}N-enrichment of total nitrogen and single AA, and TiO_2. Diets were analyzed for TiO_2, total nitrogen, AA, and ^{15}N-enrichment of total nitrogen and single AAs.

TiO_2 was determined using a colorimetric assay after ashing the sample and digestion of the minerals (24). AA were determined in diets and digesta samples after acid hydrolysis (HCl, 6 mol/L containing 0.1% phenol) using a Waters ion-exchange HPLC (25). In the diets, cysteine and methionine were measured as methionine sulfone and cysteic acid after performic acid oxidation (26), and tryptophan was determined after alkaline hydrolysis (27). Cysteine, methionine, and tryptophan were not determined in ileal digesta due to limited sample size. ^{15}N-enrichment and total nitrogen contents were measured on an isotopic ratio mass spectrometer (Optima, Fisons Instruments) coupled to an elemental nitrogen analyzer (NA 1500 series 2, Fisons Instruments) (28).

The ^{15}N-enrichments of individual AA were determined using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS, Finnigan Delta S; Thermo Fisher Scientific Inc.) as described previously (20, 29, 30).

Data analysis

Total nitrogen (N) and AA flows [μg/g dry matter intake (DMI)] were calculated as follows:

$$\text{Total N or AA flow} = \frac{\text{N or AA in digesta} \times TiO_2 \text{ in diet}}{TiO_2 \text{ in digesta}} \quad (1)$$

Dietary N flows and ileal endogenous nitrogen flow (ENFL) (μg/g DMI) determined according to isotope dilution were calculated as described in Equation *2* and Equation *3*, respectively (23):

$$\text{Dietary N flow} = \text{Total N flow} \times \frac{(E_s - E_0)}{(E_{diet} - E_0)} \quad (2)$$

$$ENFL_{ID} = \text{total N flow} - \text{dietary N flow} \quad (3)$$

where E_{diet} is the ^{15}N-enrichment in the diet (expressed as atom%), E_s is the ^{15}N-enrichment in the digesta sample, and E_0 is the basal enrichment in the digesta or diet sample. Dietary and endogenous flows of single AA determined using the isotope dilution method were calculated as described above for N.

ENFL and ileal endogenous amino acid flow (EAAFL) (μg/g DMI) determined in the ultrafiltered digesta (diet HC) were determined as follows:

$$ENFL_{UF} \text{ or } EAAFL_{UF} = \frac{\text{N or AA in ultrafiltered digesta} \times TiO_2 \text{ in diet}}{TiO_2 \text{ in ultrafiltered digesta}} \quad (4)$$

In using isotope dilution to determine endogenous nitrogen, ^{15}N recovered in the digesta was assumed to trace unabsorbed dietary nitrogen. However, some ^{15}N was detected in the >10-kDa fraction of digesta following centrifugation and ultrafiltration, suggesting that some dietary nitrogen had been absorbed, incorporated into protein, and then recycled into the gut lumen. The latter ^{15}N would be falsely considered as unabsorbed dietary nitrogen tracer. An estimate of the amount of ^{15}N-labeled endogenous protein present due to tracer recycling was calculated based on the ^{15}N-measurements in the endogenous nitrogen as determined in the ultrafiltered digesta and was expressed as a proportion of endogenous nitrogen (R, %) as follows:

$$R = 100 \times \frac{(E_{UF} - E_0)}{(E_{diet} - E_0)} \quad (5)$$

where E_{UF} is the ^{15}N-enrichment in the ultrafiltered (MW > 10 kDa) digesta (diet HC).

The underestimation (U, %) of the endogenous nitrogen losses when determined using the isotope dilution method and due to tracer recycling was calculated using the following equation:

$$U = 100 \times \frac{ENF\,L_{UF} \times \frac{R}{100}}{ENFL_{ID} + ENFL_{UF} \times \frac{R}{100}} \quad (6)$$

Statistical analysis

All the statistical analyses were performed using SAS (version 9.1, SAS Institute Inc.). The data set was subjected to the outlier test of Dixon (31, 32) with $P < 0.05$, and data were analyzed within each experiment

TABLE 3 Total ileal amino acid and nitrogen flows in growing pigs fed diets C or HC[1]

	Diet, μg/g dry matter intake			
	C	HC	Pooled SE	Significance[2]
Threonine	1322	1319	68	NS
Valine	740	959	87	NS
Isoleucine	583	622	64	NS
Leucine	822	947	90	NS
Phenylalanine	312	442	74	NS
Tyrosine	470	445	52	NS
Lysine	476	518	67	NS
Histidine	393	644	118	NS
Glutamic acid	2034	2634	168	NS
Proline	858	1082	65	NS
Glycine	752	704	65	NS
Alanine	741	769	73	NS
Aspartic acid	1232	1443	122	NS
Serine	1025	1116	37	NS
Nitrogen	2488	2752	435	NS

[1]Values are means, $n = 5$. C, intact casein; HC, hydrolyzed casein.
[2]Pig and day effects were NS ($P > 0.05$).

TABLE 4 Ileal endogenous amino acid and nitrogen flows in pigs fed diets C or HC[1]

	Diet, μg/g dry matter intake			
Amino acid	C[2]	HC[2]	Pooled SE	Significance[3]
Threonine[4]	995	910	28	NS
Valine[4]	507	587	35	NS
Isoleucine	392	399	27	NS
Leucine	673	681	49	NS
Phenylalanine	256	310	44	NS
Tyrosine	464	323	45	NS
Lysine	421	361	39	NS
Histidine	369	468	89	NS
Glutamic acid[4]	1170	945	62	NS
Proline[4]	582	565	11	NS
Glycine	608	522	38	NS
Alanine	531	481	37	NS
Aspartic acid	950	879	50	NS
Serine	631	584	21	NS
Nitrogen	1912	1828	248	NS

[1]Values are means, $n = 5$. C, intact casein; HC, hydrolyzed casein.
[2]Flows determined using the isotope dilution method.
[3]Statistical significance: $^*P < 0.05$. Pig and day had no significant effect ($P > 0.05$).
[4]Pig had a significant effect ($P < 0.05$).

using the following general linear model (which accommodates an unbalanced data set):

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijk} \quad (7)$$

where α_i, β_j, and δ_k represented the effects due to pig, day of collection, and diet, respectively. Diet (C or HC) was a fixed effect, and pig and day of collection were random effects. When the effect of the diet was significant ($P < 0.05$), Tukey's test was used for multiple comparisons of the means.

Pooled SE was calculated as follows:

$$\text{Pooled SE} = \text{RMSE}/\sqrt{n} \quad (8)$$

with the root mean squared error (RMSE) determined after application of the general linear model.

Results

The pigs remained healthy and grew normally throughout the study. Minimal leakage occurred during digesta collections. The mean pig live weight at the completion of the trial (day 56) was 73.9 ± 2.2 kg. At dissection postmortem, no signs of adverse effects due to the cannulation were observed.

For both diets C and HC, the data for 1 animal were removed from the data set, as detected by a statistical outlier test ($P < 0.05$). The data removed ranged from 60% to 150% higher than the corresponding mean values. The discrepancy was due to low (2.5-fold lower than mean) amounts of the marker TiO_2 in the digesta. Removal of the data had no effect on the main conclusion of the study of no effect of diet on endogenous ileal nitrogen flow.

Generally, the pigs ate the test diet completely within 15 min. Ileal total AA and N flows were not significantly different ($P > 0.05$) between pigs fed diets C and HC (Table 3). Ileal EAAFL and ENFL were not significantly different ($P > 0.05$) for the pigs fed diets HC and C (Table 4). Endogenous nitrogen was a similar ($P > 0.05$) proportion of the total nitrogen in ileal digesta for pigs receiving diets C and HC.

The amount of ^{15}N-labeled dietary nitrogen recycled within endogenous protein (i.e., present in the >10 kDa fraction of the ultrafiltered digesta) for the pigs given diet HC ($n = 5$) was 21.3% ± 3.1% of the nitrogen collected in this fraction. It was calculated that this would lead to an underestimation of ileal ENFL (diet HC, isotope dilution method) due to the tracer recycling of 13.2% ± 2.7%.

Discussion

Although a possible heightened stimulatory effect of dietary peptides on gut protein secretions with the enzyme-hydrolyzed protein/ultrafiltration method has been discussed (11–13), this was not found in a previous study of the growing rat conducted within our laboratory (15). The latter findings are confirmed by the present work, in which no statistically significant differences in ileal endogenous protein and AA flows (determined by isotope dilution) were found in meal-fed pigs receiving either a casein hydrolysate or the intact parent casein. This is further confirmatory evidence for the validity of the enzyme-hydrolyzed protein/ultrafiltration method for the determination of ileal endogenous protein loss in the pig. Some of the AA flow data were highly variable, such that some large (>20%) numerical differences in endogenous losses were found to be nonsignificant, statistically. However, for 7 of the 14 AAs, differences (between C and HC) were within 10% of each other. For 4 further AAs, the C flows were numerically higher (on average 19%) than the HC flows. Only for valine, phenylalanine, and histidine were the HC endogenous flows numerically but not statistically significantly higher than the C values

(on average 17% higher). These practically important (but statistically nonsignificant) differences found for 7 of the AAs should be investigated further using a greater number of animals. However, there was no overall directional trend in the data set. Taking the data together, there is no evidence that HC is associated with a heightened ileal endogenous protein flow.

The PVTC cannulation (17) used in the present study offers an advantage over other cannulation methods of allowing collection of ileal digesta without transection of the small intestine, thus minimizing effects on small intestinal muscle function. Previous studies have reported that long-term PVTC cannulation (12 wk) in the pig does not greatly alter metabolism (33, 34). In the present study, pigs remained healthy and grew normally. Representative samples of digesta were assumed to be collected through the PVTC-cannula, as the method allows collection of relatively large samples, and dietary marker recoveries determined over a 24-h period have been routinely reported to range from 70% to 100% (35–37). In the present work, in which recovery was calculated for 6 h of flow only, a recovery value of 37% ± 2% was found. Digesta were pooled between 4 and 10 h of collection so as to maximize the recovery of the dietary ^{15}N and to limit the extent of tracer recycling (16). Moreover, the amount of ileal endogenous nitrogen moving with feed dry matter flow has been reported to be relatively constant from 4 h following ingestion of a hydrolyzed casein-based meal in PVTC-cannulated pigs, based on an observed constant ratio of endogenous nitrogen/dietary marker (38). In the present study and as expected (38, 39), only small quantities of digesta were collected during the first 3 h following meal ingestion.

The present values for ENFL and EAAFL are in the range of previously published estimates (1, 11, 40). The present findings show that ileal endogenous protein flows are not enhanced by peptides supplied directly from a dietary hydrolysate of casein in comparison with the parent C, and this finding corroborates earlier results obtained in the rat using similar diets (C and HC) and the same isotope dilution method (15). To the contrary, Yin et al. (12) reported higher ileal endogenous protein flow in growing pigs given HC compared with C. Their comparison, however, was somewhat biased, because different methods (ultrafiltration method compared with homoarginine method) and different dietary markers (TiO_2 compared with chromium III oxide) were used between diets. When intact and hydrolyzed pea proteins were compared in ^{15}N-labeled pigs, similar ileal endogenous protein flows were reported (41), a finding in support of the present result.

The ileal ENFL and EAAFL determined using the ultrafiltration method (data not shown) in the currently reported study were similar to those previously reported (1, 42), except for the present endogenous flow of glutamic acid which was 2 times lower than previous determinations (1, 42). There was no apparent reason for this lower flow for glutamic acid. Ileal endogenous AA values were significantly ($P < 0.05$) lower than for the isotope dilution method for leucine, lysine, histidine, and glycine. There was no difference between methods for ileal endogenous nitrogen. Rapid recycling of the ^{15}N label from the diet to intestinal proteins has been reported (16). The present study was designed (pig as animal model, meal feeding, and defined digesta collection period) to minimize the extent of such recycling. Interestingly, the proportion of ^{15}N-labeled ileal endogenous protein in the high-MW digesta fraction (>10 kDa) was 3 times lower than that reported earlier (15). Although in the present work pigs received a single ^{15}N-labeled meal, in the previous studies rats and pigs had access to the ^{15}N-labeled meal 5–6 times/d for 10 min each hour (continuous feeding) (1, 15). In the present study, recycling may have contributed to an underestimation of ENFL of ∼13% with the isotope dilution method. In using the isotope dilution method here to determine ileal endogenous nitrogen, it was assumed that the degree of ^{15}N recycling was similar between the 2 diets.

Experimental protocols adopted for humans for the determination of ileal endogenous AA losses often require an acute feeding regimen, which raises the question of gut adaptation to the diet (43–46). Therefore, in the currently described study, 5 animals were given the test diets acutely (no adaptation compared with 5-d adaptation, data not shown), although the comparisons were not contemporaneous. The acute feeding regimen led to the same conclusion as for the adapted pigs of no statistically significant ($P > 0.05$) differences between diets C and HC for ileal endogenous nitrogen and AA flows.

In conclusion, a mixture of dietary casein-derived peptides prepared to simulate the natural products of casein digestion (EHP/UF method) did not give rise to higher endogenous protein flows at the terminal ileum of the pig than with the parent C. The study also provides a novel estimate of the dietary (HC) ^{15}N-recycling within ileal endogenous protein (21%) after ingestion of a single ^{15}N-labeled meal in the growing pig. It is estimated that this degree of label recycling would lead to an underestimation of endogenous ileal protein flow of ∼13%, when the isotope dilution method is applied in pigs.

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