The amount of endogenous, as opposed to undigested dietary, protein in digesta is a measure of fundamental interest related to gut physiology and function.

**Objective:** The objective of this study was to determine whether alimentation with proteins having differing amino acid compositions influenced endogenous ileal amino acids (EIAAs) and true ileal amino acid digestibility (TIAAD) values.

**Methods:** Male rats (*n* = 8) were fed a purified diet containing 100 g/kg of 1 of 5 protein hydrolysates, each derived from a different semipurified intact protein source [gelatin, beef muscle (BM), casein, soy protein isolate (SPI), and lactalbumin] devoid of antinutritional factors or fiber. The rats were fed their respective hydrolysate-based diet for 1 d after receiving the same diet but containing the corresponding intact protein source for 7 d. Titanium dioxide was used as an indigestible marker. Ileal digesta were collected after the rats were killed, and EIAAs were determined (precipitate + retentate) after centrifugation and ultrafiltration of the digesta. The TIAAD values of the intact protein sources were determined using EIAA flows based on each protein hydrolysate.

**Results:** Mean EIAA flows differed (*P* < 0.05) across protein hydrolysates for most amino acids, with the mean ± SEM EIAA flows across amino acids being 262 ± 17, 253 ± 12, 248 ± 18, 226 ± 14, and 191 ± 20 mg/kg dry matter intake for the gelatin, BM, casein, SPI, and lactalbumin hydrolysates, respectively. The only difference (*P* < 0.05) for the mean EIAA flows across amino acids within each protein hydrolysate was observed between gelatin (262 ± 17 mg/kg) and lactalbumin (191 ± 20 mg/kg) hydrolysates. Except for Trp (*P* < 0.001) in gelatin and lactalbumin hydrolysates, EIAA flows determined using the casein hydrolysate were not different (*P* ≥ 0.05) from EIAA flows determined using the other protein hydrolysates. TIAAD values were not generally different (*P* ≥ 0.05) regardless of the hydrolysate used to determine the EIAA flows.

**Conclusions:** Protein source affected EIAA flows, although the differences had little effect on TIAAD. Enzyme hydrolyzed casein is a suitable model hydrolysate for determining TIAAD with the enzyme-hydrolyzed protein-ultrafiltration technique.  

**Keywords:** amino acid, endogenous, hydrolysate, ileal digestibility, protein

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**Introduction**

Nondietary protein found as part of the digesta at the terminal ileum comprises endogenous proteins secreted into the gut lumen, sloughed gut epithelial cells, and microbial bodies (1–4). This flow of material, often collectively referred to as the endogenous protein flow, is of fundamental interest because it reflects gut metabolism and is a measure used to adjust apparent estimates of ileal protein and amino acid digestibility. The total endogenous ileal amino acids (EIAAs) from the upper digestive tract can be considered in 2 parts: 1) basal and 2) specific. Basal EIAAs are defined as the endogenous amino acids found in terminal ileal digesta related to food dry matter (DM) flow, whereas the variable component, the specific losses, are the endogenous amino acids found in terminal ileal digesta related to the effects of specific dietary components, such as fiber or antinutritional factors (ANFs) (5).
Several methods have been used to determine basal EIAAs, including using a protein-free diet (6–12), the regression method (13, 14), and the isotope dilution method (15–18), along with methods that are based on alimentation with diets containing synthetic amino acids (6, 12, 19), highly digestible proteins (20), guanidinated proteins (11, 16, 20), proteins devoid of specific amino acids (6), and enzyme-hydrolyzed proteins, followed by ultrafiltration of the digesta (6–11, 15, 16, 20–23). The latter approach allows the basal endogenous losses of all amino acids to be determined simultaneously.

For animals fed protein-free diets, gut protein secretion (24–26) and gut protein turnover (27) may be reduced but the digestibility of some endogenous proteins may be increased (28, 29), leading to underestimated basal EIAAs when compared with alimentation with diets containing peptides (5–11, 21, 30) or protein (5–9, 11, 21, 30). Thus, the feeding of food containing protein or peptides to an animal increases the EIAAs over and above concentrations found when a protein-free food is ingested, and, given that foods normally contain protein, the basal EIAAs can be regarded as the endogenous flows associated with protein-containing (i.e., food) DM. It has been considered that, for food proteins not containing fiber or ANFs, there is no effect of the protein source on the influence of protein-containing DM on the EIAAs. It has been assumed that the different amino acid compositions of protein (i.e., primary structures) and differences in secondary and tertiary protein structures would not influence EIAAs, and there is some limited published data that would support that assumption (31). However, this is an assumption that needs to be fully tested, because it is now well established that the digestion of proteins can give rise to bioactive peptides, some of which influence gut secretions and other gut functions. The amount of endogenous vs. undigested dietary protein in digesta and the factors influencing it are of fundamental interest to gut physiology and function.

The first aim of this study was to determine whether proteins with differing amino acid composition (i.e., different primary structures) and differing secondary and tertiary structures sustain different EIAAs. The secondary aim was to determine whether potential differences in EIAAs translated to differences in true ileal amino acid digestibility (TIAAD). EIAAs were determined in the growing rat using the enzyme-hydrolyzed protein/ultrafiltration (EHP/UF) method (23), using protein hydrolysates for a range of semipurified protein sources, chosen specifically to not contain ANFs or fiber.

**Methods**

**Materials.** The protein sources were obtained as described by Rutherfurd et al. (32). Alcalase and flavorzyme enzymes were obtained from Nutorta New Zealand Limited. Vitamin and mineral mixes for the rat diets were obtained from Plant and Food Research Limited. Laboratory rats (160 rats of 200 g body weight) were supplied by the Small Animal Production Unit, Massey University, Palmerston North, New Zealand.

**Protein hydrolysates and diets.** Hydrolysates of each protein source were prepared by digestion with alcalase 2.4 L at pH 7.5 at 55°C and flavozyme at pH 6.0 at 50°C. The enzyme-to-substrate ratio for alcalase was 5 Anson units/100 g protein source DM and for flavozyme was 40 Leu amino peptide units/g protein source DM. A lesser amount of alcalase (3 Anson units/100 g protein source DM) and flavozyme (25 Leu amino peptide units/g protein source DM) was used for casein and beef muscle (BM) because these proteins digest readily (J Cui, unpublished data, 2008). The incubation times used for alcalase and flavozyme were 2 and 20 h, respectively. After incubation, the enzymes were inactivated by heating at 90°C for 3 min. The resulting hydrolysates were freeze-dried and ground to <1-mm particle size. The molecular-weight distribution of the peptides was determined using LCMS (Agilent 6520 quadrupole time-of-flight mass spectrometer; Agilent Technologies).

Ten semisynthetic powdered test diets were formulated such that each diet contained 1 of the intact proteins [lactalbumin, gelatin, casein, soy protein isolate (SPI), or BM] or 1 of the protein hydrolysates (lactalbumin, gelatin, casein, SPI, or BM hydrolysates) as the sole nitrogen source. The ingredient composition of the diets is shown in Supplemental Table 1. The diets were formulated to contain the same nitrogen content (16 g/kg), which equates to a crude protein content of 100 g/kg based on the generic nitrogen-to-protein conversion factor of 6.25. All diets met the nutritional requirements of the growing rat for all nutrients except protein (33). Titanium dioxide (3 g/kg) was included in each diet as an indigestible marker.

**Animal trial.** Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University.

The trial was conducted as described by Rutherford et al. (32) except that protein hydrolysates were used in place of oxidized protein sources. The unoxidized intact lactalbumin, gelatin, casein, SPI, or BM diets used by Rutherfurd et al. (32) were the same as for the intact protein sources referred to here. The rats allocated to the dietary treatments comprising the protein hydrolysates were fed their respective hydrolysate-containing test diets as described by Rutherford et al. (32). The rats were fed their respective hydrolysate-containing test diets for 1 d only because the amount of hydrolyzed material was limited. The latter “acute feeding” regimen was reported previously for highly purified synthetic diets when fed to rats (11, 16, 20), pigs (10, 16, 34), and humans (10, 15, 34), and Moughan et al. (34) showed that apparent ileal nitrogen digestibility was similar when pigs were fed for either 1 d (acute feeding) or 14 d (adaptation feeding), demonstrating that an acute feeding regimen is suitable for determining apparent ileal protein digestibility. For the currently reported study, it was assumed that an acute feeding regimen would also be suitable for examining EIAAs. The rats were killed and digesta were sampled as described by Rutherfurd et al. (35) and pooled across pairs of rats as described by Rutherford et al. (32), giving 8 digesta samples per treatment (n = 8). The endogenous protein fraction was obtained as described by Rutherford and Moughan (22) except that ultrafiltration was performed using an Amicon Centriprep 3K ultrafiltration device (EMD Millipore).

**LCMS analysis.** Hydrolysates were subjected to LCMS analysis to determine molecular weights. The freeze-dried protein hydrolysate samples (~4 mg) were dissolved in 1 mL of MS-grade water containing 0.1% formic acid and centrifuged at 13,000 × g for 10 min, and 20 μL was autoinjected into an Agilent 1100 LC system (Agilent Technologies) equipped with a Jupiter 5-μm C18 300 HPLC column (Phenomenex). Data were collected with an Agilent 6520 quadrupole time-of-flight mass spectrometer in positive ion mode over a 0–70% acetonitrile/water gradient over 150 min with a flow rate of 0.350 mL/min. The intensity of ions was recorded in the range of 50–3200 m/z and analyzed using MassHunter Workstation Qualitative Analysis software version B03.01 (Agilent Technologies).

**Chemical analysis.** Amino acid contents were determined using HCl hydrolysis and derivatization with o-phthalaldehyde as described by Rutherfurd et al. (36). Trp was determined as described by Rutherford et al. (36). Cys was not determined because it is partially destroyed during HCl hydrolysis, and Pro was not determined because it is a secondary amino acid and therefore does not react with o-phthalaldehyde. The determined Asp values represent the sum of Asp and Asn, and the determined Glu values represent the sum of Glu and Gln. Titanium dioxide contents of the diets and digesta samples were determined using absorbance at 405 nm following the method of Short et al. (37).

**Data analysis.** EIAA flows at the terminal ileum, apparent ileal amino acid digestibility, and TIAAD were calculated as described by Awati et al. (20), in which basal EIAA flow values, used to correct apparent digestibility values to true digestibility values, were based on the ultrafiltered digesta for rats given the hydrolysate-based diets. Daily food
intakes, daily body-weight gains, EIAA flow, and TIAAD values in the text are means ± SEMs.

**Statistical analysis.** The number of replicates (digesta were pooled from 2 rats per replicate) required for each treatment group (n = 8) was estimated with a power >80% at a 2-tailed 5% significance level and based on published EIAA flow values (mean and SEM) (22). The basal EIAA flows were compared across the protein sources (protein hydrolysates) using 1-factor ANOVA (General Linear Model procedure in SAS) (38). TuIAAD values were compared across protein hydrolysates for each protein source separately using 1-factor ANOVA (General Linear Model procedure in SAS) (38). When overall significant (P < 0.05) differences across treatments were observed, individual treatment means were compared using the Tukey’s test. The correlations between daily feed intake or growth rate and basal EIAA flows were analyzed for each amino acid separately using the CORR procedure of SAS (38).

**Results**

The degree of hydrolysis (determined based on the presence of free amino groups) for the hydrolysates ranged from 55% to 71% across the 5 protein hydrolysates, suggesting that extensive, but not complete, hydrolysis was achieved. Based on LCMS analysis, all of the final hydrolysates appeared to contain no peptides >2000 Da (Supplemental Figure 1), although the presence of peptides >3200 Da cannot be excluded because the mass range examined was from 50 to 3200 Da. The detected ions were generally between 100 and 800 Da across all hydrolysates, suggesting the presence of mainly free amino acids and peptides up to ~8 residues in length. There were also a smaller number of larger peptides (800–1300 Da) present in the gelatin hydrolysate and to a lesser degree in the casein hydrolysate.

The food intakes for rats on the second to last day of the study were not different (P ≥ 0.05) between the casein (19.6 ± 0.6 g) and BM (18.9 ± 1.2 g) basal diets, but the latter intakes were higher (P < 0.01) than for the other 3 dietary treatments. The food intakes were also all different (P < 0.05) between the SPI (15.6 ± 0.6 g), lactalbumin (13.8 ± 1.2 g), and gelatin (9.7 ± 0.7 g) basal diets. There was no difference (P ≥ 0.05) in body-weight gain for the rats between the lactalbumin (5.8 ± 0.7 g), casein (5.2 ± 0.3 g), and BM (4.6 ± 0.8 g) diets. The growth rate for rats receiving the SPI diet (2.5 ± 0.3 g) was lower (P = 0.002) than for the rats on the former 3 diets but higher (P < 0.001) than for rats on the gelatin diet (0.01 ± 0.3 g). The rats fed the gelatin-based diet gained little weight over the last 7 d of the study, which may reflect the poor amino acid balance and possibly the poor palatability, leading to a lower food intake for the latter diet compared with the SPI-, lactalbumin-, BM-, and casein-based diets. There was no correlation (P ≥ 0.05) between daily feed intake or growth rate and the basal endogenous ileal flows for any amino acid except Trp for feed intake (P < 0.01) and Met for growth rate (P < 0.01).

The basal EIAA flows determined using the 5 protein hydrolysates are presented in Table 1. Flows for Gly are not presented because basal endogenous ileal Gly flows may be underestimated when using the EHP/UF method because basal endogenous Gly-conjugated bile salts are likely to be present in the smaller undigested dietary peptide fraction after ultrafiltration. There was no difference (P ≥ 0.05) in basal EIAA flows across protein hydrolysates for Asp, Ser, and Thr. For the remaining amino acids, there were differences in basal endogenous ileal flows (P = <0.0001–0.0264) across the 5 protein hydrolysates. The basal endogenous ileal flows for all of the remaining amino acids, with the exception of Val, Ile, Leu, Tyr, Phe, and Trp, were lower (P = <0.0001–0.0471) for lactalbumin hydrolysate when compared with the gelatin hydrolysate. Moreover, with the exception of Val, Tyr, and Lys, the basal EIAA flows were not different (P ≥ 0.05) between the BM, casein, and SPI hydrolysates for any of the amino acids examined. The basal EIAA flow pattern varied across protein hydrolysates depending on the amino acid in question. For example, for Lys, there was no difference (P ≥ 0.05) in the basal endogenous ileal flows between gelatin, BM, and casein hydrolysates nor between casein, SPI, and lactalbumin hydrolysates, but the flows for gelatin and BM hydrolysates were higher (P = 0.0024–0.0097) than those for SPI and lactalbumin hydrolysates. For Trp, there was no difference (P ≥ 0.05) in the basal

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Basal EIAA flows determined in growing male rats using the EHP/UF method with different protein hydrolysates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protrued EIAA, mg/kg DMI</td>
<td>Gelatin</td>
</tr>
<tr>
<td>Asp</td>
<td>529</td>
</tr>
<tr>
<td>Thr</td>
<td>344</td>
</tr>
<tr>
<td>Ser</td>
<td>236</td>
</tr>
<tr>
<td>Glu</td>
<td>561</td>
</tr>
<tr>
<td>Ala</td>
<td>275</td>
</tr>
<tr>
<td>Val</td>
<td>233</td>
</tr>
<tr>
<td>Met</td>
<td>59</td>
</tr>
<tr>
<td>Ile</td>
<td>181</td>
</tr>
<tr>
<td>Leu</td>
<td>293</td>
</tr>
<tr>
<td>Tyr</td>
<td>168</td>
</tr>
<tr>
<td>Phe</td>
<td>174</td>
</tr>
<tr>
<td>His</td>
<td>115</td>
</tr>
<tr>
<td>Lys</td>
<td>198</td>
</tr>
<tr>
<td>Arg</td>
<td>262</td>
</tr>
<tr>
<td>Trp</td>
<td>70</td>
</tr>
</tbody>
</table>

1 Values are means, n = 8. Labeled means in a row without a common letter differ, P < 0.05. BM, beef muscle; DMI, dry matter intake; EHP/UF, enzyme-hydrolyzed protein/ultrafiltration; EIAA, endogenous ileal amino acid; SPI, soy protein isolate.
2 NS, P ≥ 0.05; *0.05 > P ≥ 0.01; **0.01 > P ≥ 0.001; ***P < 0.001.
endogenous ileal flows between gelatin and lactalbumin hydrolysates nor between BM, casein, and SPI hydrolysates, but the flows for the gelatin and lactalbumin hydrolysates were higher ($P < 0.0001–0.0113$) than those for the BM, casein, and SPI hydrolysates. The difference between the highest and lowest basal EIAA flows across all protein hydrolysates ranged from 1.3-fold for Arg to 1.8-fold for Trp.

The apparent ileal amino acid digestibility values for the 5 intact protein sources are given in Supplemental Table 2. The TIAAD values (corrected from apparent ileal amino acid digestibility values using the EHP/UF method based on the 5 different protein hydrolysates) for SPI are shown in Table 2, and the TIAAD values for the other 4 protein sources are presented in Supplemental Tables 3–6. The true ileal digestibility of Trp was not determined for gelatin because gelatin contains negligible amounts (<2 g/kg as determined in the current study) of Trp. There were no differences ($P > 0.05$) in true ileal digestibility for all of the amino acids determined in casein, BM, gelatin, and lactalbumin and the majority of the amino acids in SPI when digestibility was corrected for basal EIAA flows using the EHP/UF method and based on the casein, SPI, BM, gelatin, and lactalbumin hydrolysates. For SPI, there was a difference ($P = 0.0001–0.0462$) in the true ileal digestibility of Val, Met, Ile, Tyr, His, Arg, and Trp determined across protein hydrolysates. However, the actual differences in the TIAAD values across hydrolysates were small (<2.3%) for most of the latter amino acids. The largest difference was 4.4% for Trp.

### Discussion

That the source of dietary protein influences basal EIAA flows is an important finding, hitherto unconsidered. Given that the currently used proteins were semipurified and did not contain nonstarch polysaccharides or ANFs, the observed differences in endogenous flows appeared to be related to differences in the primary, secondary, or tertiary structures of the proteins. It is conceivable that, during digestion, different proteins give rise to different amounts and types of bioactive peptides that may influence gut protein metabolism and thus basal EIAA flows, and this warrants more detailed investigation.

An important assumption in the currently reported study, which used the EHP/UF method for determining EIAA flows at the end of the ileum, is that the mixture of peptides and free amino acids administered to the rats reflected the natural products of protein digestion in the gastrointestinal tract. Accordingly, a casein hydrolysate prepared with trypsin (6) and pancreatin (15) has been used previously. However, pepsin-like proteases, such as alcalase, have also been used to prepare protein hydrolysates for determining EIAA flows (19). In the currently reported experiment, alcalase and flavorzyme were used to prepare the hydrolysates rather than pepsin, trypsin, and chymotrypsin. Alcalase has a specificity similar to pepsin and chymotrypsin (39) whereas flavorzyme possesses exopeptidase activity, which is also present in the gastrointestinal tract. Relatively long incubation times were used for alcalase and flavorzyme (2 and 20 h, respectively) to ensure that extensive hydrolysis of the proteins sources occurred. Overall, the hydrolysates generally contained amino acids and small peptides (<800 Da residues) (Supplemental Figure 1), a composition assumed to be similar to that present in the small intestine after consumption of diets containing the equivalent intact proteins.

It can be argued that, because basal EIAA flows represent the endogenous amino acids at the terminal ileum in response to the presence of dietary DM in the gastrointestinal tract and because foods generally contain protein, the methods used for determining basal EIAA flows should be based on alimentation with diets that contain proteins. With that consideration in mind, several methods have been developed to determine basal EIAA flows that involve either protein or peptide alimentation. A relatively straightforward approach is the EHP/UF method (21, 23), which permits the determination of basal EIAA flows following peptide alimentation. The latter approach involves feeding a protein (related to proteins not containing fiber or ANFs) hydrolysate-based diet to a test animal, collecting ileal digesta, and separating the undigestible dietary peptides from the larger endogenous proteins in the ileal digesta using ultrafiltration. This approach

### Table 2

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Gelatin</th>
<th>BM</th>
<th>Casein</th>
<th>SPI</th>
<th>Lactalbumin</th>
<th>Overall SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>88.5</td>
<td>88.1</td>
<td>88.2</td>
<td>87.8</td>
<td>87.5</td>
<td>0.42</td>
<td>NS</td>
</tr>
<tr>
<td>Thr</td>
<td>84.1</td>
<td>85.0</td>
<td>84.5</td>
<td>83.8</td>
<td>82.8</td>
<td>0.55</td>
<td>NS</td>
</tr>
<tr>
<td>Ser</td>
<td>90.9</td>
<td>90.4</td>
<td>91.4</td>
<td>90.8</td>
<td>90.3</td>
<td>0.36</td>
<td>NS</td>
</tr>
<tr>
<td>Glu</td>
<td>94.5</td>
<td>93.8</td>
<td>94.1</td>
<td>93.9</td>
<td>93.5</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Ala</td>
<td>92.8</td>
<td>92.0</td>
<td>91.2</td>
<td>91.0</td>
<td>90.5</td>
<td>2.26</td>
<td>NS</td>
</tr>
<tr>
<td>Val</td>
<td>90.1$^{a,b}$</td>
<td>91.3$^a$</td>
<td>90.1$^{b}$</td>
<td>89.9$^b$</td>
<td>89.2$^b$</td>
<td>0.43</td>
<td>*</td>
</tr>
<tr>
<td>Met</td>
<td>97.4$^a$</td>
<td>96.3$^b$</td>
<td>96.3$^b$</td>
<td>96.6$^b$</td>
<td>95.8$^b$</td>
<td>0.22</td>
<td>***</td>
</tr>
<tr>
<td>Ile</td>
<td>92.1$^{a,b}$</td>
<td>92.9$^a$</td>
<td>92.3$^{b}$</td>
<td>92.0$^b$</td>
<td>91.4$^b$</td>
<td>0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Leu</td>
<td>91.2</td>
<td>91.4</td>
<td>90.9</td>
<td>90.8</td>
<td>90.3</td>
<td>0.91</td>
<td>NS</td>
</tr>
<tr>
<td>Tyr</td>
<td>96.5$^b$</td>
<td>97.3$^b$</td>
<td>96.3$^{a,b}$</td>
<td>96.0$^b$</td>
<td>95.7$^b$</td>
<td>0.25</td>
<td>**</td>
</tr>
<tr>
<td>Phe</td>
<td>94.0</td>
<td>94.6</td>
<td>93.8</td>
<td>93.9</td>
<td>93.5</td>
<td>0.26</td>
<td>NS</td>
</tr>
<tr>
<td>His</td>
<td>95.4$^a$</td>
<td>94.9$^{a,b}$</td>
<td>95.0$^b$</td>
<td>94.5$^b$</td>
<td>94.1$^b$</td>
<td>0.31</td>
<td>*</td>
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<tr>
<td>Lys</td>
<td>99.1</td>
<td>99.1</td>
<td>98.6</td>
<td>98.2</td>
<td>98.3</td>
<td>1.27</td>
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<tr>
<td>Arg</td>
<td>97.8$^a$</td>
<td>97.5$^{a,b}$</td>
<td>97.2$^b$</td>
<td>97.0$^b$</td>
<td>97.0$^{a,b}$</td>
<td>0.19</td>
<td>*</td>
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<tr>
<td>Trp</td>
<td>89.5$^b$</td>
<td>93.5$^a$</td>
<td>93.4$^a$</td>
<td>91.9$^{b}$</td>
<td>90.1$^{b,c}$</td>
<td>0.55</td>
<td>***</td>
</tr>
</tbody>
</table>

1. Values are means, $n = 8$. Labeled means in a row without a common letter differ, $P < 0.05$. BM, beef meal; EHP/UF, enzyme-hydrolyzed protein/ultrafiltration; EIAA, endogenous ileal amino acid; SPI, soy protein isolate; TIAAD, true ileal amino acid digestibility.
2. Hydrolysate used to determine the basal EIAA flows for correcting the apparent ileal amino acid digestibility values to true values.
3. NS, $P ≥ 0.05$; *0.05 > $P > 0.01$; **0.01 > $P > 0.001$; ***$P < 0.001$.
permits the determination of the basal EIAA flows for all of the amino acids at the same time (21, 23). Ideally, the most appropriate protein hydrolysate is 1 that is derived from the protein source being tested. However, for convenience, a casein hydrolysate, prepared from an enzymatic digest of intact casein, is used as a model protein hydrolysate, and it is assumed that the basal EIAA flows determined based on alimentation with the casein hydrolysate are similar to basal EIAA flows determined after alimentation with hydrolysates derived from protein sources (not containing fiber or ANFs) other than casein. The latter assumption may be challenged because it is possible that there are bioactive peptides present in the casein hydrolysate that may lead to increased or decreased secretion of endogenous proteins into the gastrointestinal tract and that any bioactive peptides present may differ for different hydrolysates (40).

In the current study, the source of protein used to prepare the protein hydrolysates had some effect on the basal EIAA flows for most of the amino acids, although for many of the amino acids the effect was not large in the context of the variability for published EIAA flow data in human ileostomates (10), rats (22), and pigs (41, 42). The main differences in basal EIAA flows across hydrolysates were observed between BM and lactalbumin hydrolysates. In addition, basal EIAA flows determined using the casein hydrolysate were not different from those determined for any of the other protein hydrolysates for any of the amino acids examined with the exception of Trp. Consequently, it would appear that a casein hydrolysate may be a suitable model protein hydrolysate for determining basal EIAA flows when using the EHP/UF method.

When a model protein hydrolysate, such as enzyme-hydrolyzed casein, is used to determine basal EIAA flows for correcting apparent digestibility to true digestibility, then an important question in practice is whether the differences in basal EIAA flows translate to differences in TIAAD. Overall, the results of the currently reported study suggest that the source of protein used to prepare the hydrolysates for determining basal EIAA flows had little effect on the TIAAD values. Moreover, the question as to the suitability of casein as a model protein hydrolysate for determining the TIAAD of protein sources in general is illustrated in Figure 1. Figure 1 shows the TIAAD of SPI, BM, gelatin, and lactalbumin determined using basal EIAA flows that were derived using either a casein hydrolysate or an hydrolysate from the corresponding protein source being tested. It is notable that, generally, the differences observed for TIAAD values across protein sources far outweigh the differences in TIAAD values within each protein source but determined using either a casein hydrolysate or a hydrolysate derived from the intact protein being evaluated.

When the TIAAD of the protein sources were compared with equivalent published values (true ileal digestibility of nitrogen or amino acids for similar protein sources determined using the EHP/UF method determined in the growing rat), there was generally a good agreement between the published data and those data presented here. For example, the true ileal nitrogen digestibility for casein (16) and the overall TIAAD of SPI (43), lactalbumin (43), and gelatin (11) were reported to be of 93%, 90%, and 92%, respectively, with the overall TIAAD of 92%, 93%, 86%, and 88% for casein, SPI, lactalbumin, and gelatin, respectively, determined in the currently reported study.

Overall, the protein source (purified protein not containing fiber or ANFs) from which different protein hydrolysates were prepared did affect the basal EIAA flows determined using the EHP/UF method for some amino acids and some protein sources. This is an important new finding and could be related to the release of different amounts and types of bioactive peptides during the digestion of different protein sources. However, the differences were generally relatively small and did not translate into differences in TIAAD. Thus, in practice and for convenience when using the EHP/UF method for determining TIAAD, it appears that it is acceptable to use a casein hydrolysate as a model protein hydrolysate for determining basal EIAA flows for protein sources other than casein.

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References


