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ORIGINAL ARTICLE

Composition of amino acids in feed ingredients for animal diets

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Abstract Dietary amino acids (AA) are crucial for animal growth, development, reproduction, lactation, and health. However, there is a scarcity of information regarding complete composition of "nutritionally nonessential AA" (NEAA; those AA which can be synthesized by animals) in diets. To provide a much-needed database, we quantified NEAA (including glutamate, glutamine, aspartate, and asparagine) in feed ingredients for comparison with "nutritionally essential AA" (EAA; those AA whose carbon skeletons cannot be formed by animals). Except for gelatin and feather meal, animal and plant ingredients contained high percentages of glutamate plus glutamine, branched-chain AA, and aspartate plus asparagine, which were 10–32, 15–25, and 8–14% of total protein, respectively. In particular, leucine and glutamine were most abundant in blood meal and casein (13% of total protein), respectively. Notably, gelatin, feather meal, fish meal, meat and bone meal, and poultry byproduct had high percentages of glycine, proline plus hydroxyproline, and arginine, which were 10–35, 9.6–35, and 7.2–7.9% of total protein, respectively. Among plant products, arginine was most abundant in peanut meal and cottonseed meal (14–16% of total protein), whereas corn and sorghum had low percentages of cysteine, lysine, methionine, and tryptophan (0.9–3% of total protein). Overall, feed ingredients of animal origin (except for gelatin) are excellent sources of NEAA and EAA for livestock, avian, and aquatic species, whereas gelatin provides highest amounts of arginine, glycine, and proline plus hydroxyproline. Because casein, corn, soybean, peanut, fish, and gelatin are consumed by children and adults, our findings also have important implications for human nutrition.

Keywords Nonessential amino acids · Composition · Food · Animals

Abbreviations

AA	Amino acids
BCAA	Branched-chain amino acids
CP	Crude protein
CSM	Cottonseed meal
DM	Dry matter
EAA	Essential amino acids
MBM	Meat and bone meal
NEAA	Nonessential amino acids
NRC	National Research Council
PBM	Poultry byproduct meal
SBM	Soybean meal

Introduction

Amino acids (AA) have been traditionally classified as nutritionally essential or nonessential (Table 1) on the basis of the growth or nitrogen balance of organisms (Baker 2009; Chen et al. 2009; Le Floc'h and Seve 2007; Stipanuk et al. 2009; Suryawan et al. 2009). Thus, research on protein nutrition has long focused on dietary composition of essential AA (EAA) whose carbon skeletons are not synthesized in animal cells (Elango et al. 2009; Ji et al. 2005; Li et al. 2009a; NRC 1998). In contrast, little attention has

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Table 1 Traditional classification of amino acids as nutritionally essential or nonessential for monogastric mammals

EAA	NEAA
Histidine	Alanine
Isoleucine	Arginine[a]
Leucine	Asparagine
Lysine	Aspartate
Methionine	Cysteine
Phenylalanine	Glutamate
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Serine
	Tyrosine

EAA amino acids whose carbon skeletons are not synthesized by animal cells, *NEAA* amino acids which are synthesized by animals through interorgan metabolism of AA and glucose

[a] Arginine is not synthesized by carnivores and is not adequately synthesized by the fetus, neonate, or gestating dam

been paid to dietary composition of complete nonessential AA (NEAA) which can be formed de novo in the body (Wu 2009; Wu et al. 2010a; Yin et al. 2009). However, emerging evidence shows that NEAA play important and versatile roles in tissue-specific or whole-body metabolism and functions (Burrin and Reeds 1997; Blachier et al. 2009; Haynes et al. 2009; Li et al. 2007, 2009b; Wu et al. 2008). For mammals, NEAA include alanine, arginine (except carnivores, ferrets, minks, and young animals), asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine (Elango et al. 2009; Wu 2009). Due to the inadequacy or lack of endogenous synthesis, arginine, glycine, and proline are EAA for avian species (Baker 2009) and fish (Li et al. 2009d).

The objective of the present study was to determine complete composition of NEAA (including glutamate, glutamine, aspartate, and asparagine) in feed ingredients of animal diets for comparison with EAA content. This provides a much-needed database to formulate the next generation of improved diets for livestock, avian, and aquatic species. Such information also has important implications for human nutrition as some of these foods (e.g., casein, corn, soybean, peanut, fish, and gelatin) are consumed by children and adults.

Materials and methods

Materials

Casein and gelatin were purchased from US Biochemical Corp (Cleveland, OH, USA). Blood meal, cookie meal, hydrolyzed feather meal, meat and bone meal (MBM), and poultry by-product meal (PBM) were obtained from Asia Regional Office (Causeway Bay, Hong Kong, China) of National Renderers Association (Alexandria, VA, USA). Menhaden fish meal was procured from Omega Protein Corp (Houston, TX, USA). Soybean meal (SBM) and dehulled SBM were products of Food & Protein Center of Texas A&M University (College Station, TX, USA). Cottonseed meal (CSM), peanut meal, corn grain, and sorghum grain were obtained from Producers Cooperative Association (Bryan, TX, USA). HPLC-grade water and methanol were purchased from Fisher Scientific (Houston, TX, USA). Other materials, including HPLC columns and AA standards, were products of Sigma Chemicals (St. Louis, MO, USA).

Chemical analyses

All ingredients were finely ground to 0.5 mm in size before use for analysis. Dry matter (DM) content was determined by drying approximately 100 mg samples to constant mass in a 105°C oven. Total nitrogen was analyzed in approximately 1.0 g samples using LECO Model FP-528 Analyzer (St. Joseph, MI, USA), and crude protein (CP) was calculated as total nitrogen multiplied by 6.25 on the basis of the assumption that protein contains 16% N (Wu 2009). For determining AA (except tryptophan) in protein, approximately 100 mg samples were hydrolyzed in 10 mL of 6 N HCl at 110°C for 24 h under N_2 (Wu et al. 1997). For tryptophan analysis, approximately 100 mg samples were hydrolyzed at 110°C for 20 h in 10 mL of 4.2 M NaOH plus 0.1 mL of 25% thiodiglycol (an antioxidant), as previously described (Wu et al. 1999). Rates of AA recoveries during acid or base hydrolysis were determined using purified bovine insulin with known AA composition (Wu and Knabe 1994) and tryptophan standard. Glutamine, glutamate, asparagine, and aspartate in protein were determined using porcine digestive enzymes, as we described (Wu et al. 2010a). Total glutathione and free AA were extracted from feed ingredients with HPLC-grade water (0.2 g sample/10 mL). For analysis, AA and total glutathione were determined in triplicate using HPLC methods involving precolumn derivatization with *o*-phthaldialdehyde (Jobgen et al. 2009; Wu et al. 2007; Wu and Meininger 2008). AA and glutathione in samples were quantified on the basis of known amounts of standards (Sigma Chemicals, St. Louis, MO, USA) using Millenium-32 Software (Waters, Milford, MA, USA).

Statistical analysis

Values are expressed as mean ± SEM. Sample size ($n = 6$/ingredient) was chosen on the basis of known

variation of AA among feed ingredients (Li et al. 2010; Mateo et al. 2007; Wu et al. 2010a, b) and statistical power calculation (Fu et al. 2010). Log transformation of variables was performed when variance of data was not homogenous among treatment groups, as assessed by the Levene's test (Lassala et al. 2010). Data on AA composition were analyzed by one-way analysis of variance using SAS (SAS Institute, Cary, NC, USA). $P < 0.05$ was taken to indicate statistical significance.

Results

Content of DM, CP, and true protein in feed ingredients

Percentages of DM and CP in ingredients varied from 88 to 97% and from 9 to 100%, respectively, depending on type (Table 2). Ingredients of animal origin (blood meal, casein, feather meal, fish meal, gelatin, MBM, and PBM) had much higher ($P < 0.05$) content of CP than ingredients of plant origin (cookie meal, CSM, peanut meal, and SBM). The content of true protein (the sum of AA in protein) was approximately 80–100% of crude protein in ingredients depending on type (Table 2). Differences between CP and true protein were smaller ($P < 0.05$) for blood meal, casein, feather meal, fish meal, gelatin, MBM, PBM, and SBM, compared with cookie meal, CSM, peanut meal, and dehulled SBM. With the exception of gelatin and SBM, the percentages of EAA were much higher ($P < 0.05$) in animal than in plant ingredients.

Content of total AA in feed ingredients

Expressed on an as-fed basis (g/100 g sample), the percentages of total AA (protein-bound plus free) in ingredients varied greatly with type (Table 3). Gelatin had the highest content of arginine (7.7%), followed by feather meal (5.7%). Among plant ingredients, arginine was the most abundant AA in peanut meal (5.7%) and CSM (4.5%), a predominant AA in SBM (4.6%) and low in corn and sorghum grains (0.38–0.41%). Alanine content was higher ($P < 0.05$) in animal products (2.8–7.8%) than in plant ingredients (0.71–2.0%). Except for blood meal, feather meal, and PBM which contained 1.1–4.2% cysteine, all other ingredients had low content of cysteine (0.05–0.7%). In contrast, animal and plant ingredients contained relatively high percentages of glutamate plus glutamine, which were 6.9–21 and 1.7–8.6%, respectively (Table 4). Interestingly, the content of glutamate plus glutamine in blood meal was <50% of that in casein ($P < 0.05$). Except for blood meal, the content of glutamate plus glutamine was much higher ($P < 0.05$) than that of aspartate plus asparagine. Glycine and proline were most

Table 2 Content of dry matter and crude protein in food ingredients (%, as-fed basis)

	Blood meal	Casein	Cookie meal	Corn grain	CSM	Feather meal	Fish meal	Gelatin	MBM	Peanut meal	PBM	SBM	SBM (DH)	Sorghum grain
DM	$91.8^{c} \pm 0.03$	$91.7^{c} \pm 0.04$	$90.8^{d} \pm 0.04$	$89.0^{f} \pm 0.05$	$90.0^{e} \pm 0.03$	$95.1^{b} \pm 0.04$	$91.8^{c} \pm 0.04$	$89.0^{f} \pm 0.03$	$96.1^{a} \pm 0.04$	$91.8^{c} \pm 0.02$	$96.5^{a} \pm 0.02$	$89.0^{f} \pm 0.03$	$96.4^{a} \pm 0.04$	$89.1^{f} \pm 0.03$
CP[A]	$89.6^{b} \pm 0.14$	$88.0^{b} \pm 0.09$	$12.3^{h} \pm 0.12$	$9.3^{i} \pm 0.09$	$40.3^{g} \pm 0.22$	$82.1^{c} \pm 0.15$	$63.4^{d} \pm 0.33$	$100.1^{a} \pm 0.04$	$52.0^{e} \pm 0.28$	$43.9^{f} \pm 0.18$	$64.3^{d} \pm 0.40$	$43.6^{f} \pm 0.12$	$51.8^{e} \pm 0.10$	$10.1^{i} \pm 0.10$
TP[B]	$88.3^{b} \pm 0.76$	$86.2^{b} \pm 0.84$	$10.5^{h} \pm 0.20$	$8.2^{i} \pm 0.16$	$32.5^{g} \pm 0.73$	$81.0^{c} \pm 0.95$	$63.7^{d} \pm 0.60$	$97.4^{a} \pm 0.81$	$50.7^{e} \pm 0.66$	$35.1^{g} \pm 0.92$	$60.4^{d} \pm 0.63$	$38.2^{fg} \pm 0.45$	$41.6^{f} \pm 0.57$	$8.8^{i} \pm 0.38$
EA	$41.9^{a} \pm 0.38$	$37.2^{b} \pm 0.52$	$3.3^{g} \pm 0.12$	$3.0^{g} \pm 0.10$	$10.7^{f} \pm 0.49$	$24.9^{c} \pm 0.52$	$24.8^{c} \pm 0.34$	$14.4^{e} \pm 0.56$	$15.4^{e} \pm 0.42$	$10.8^{f} \pm 0.49$	$18.4^{d} \pm 0.40$	$14.5^{e} \pm 0.36$	$15.5^{e} \pm 0.41$	$3.2^{g} \pm 0.22$
NA	$46.4^{d} \pm 0.51$	$49.0^{c} \pm 0.43$	$7.2^{j} \pm 0.15$	$5.2^{k} \pm 0.13$	$21.8^{i} \pm 0.56$	$56.1^{b} \pm 0.72$	$38.9^{f} \pm 0.58$	$83.0^{a} \pm 0.63$	$35.3^{g} \pm 0.71$	$24.3^{h} \pm 0.63$	$42.0^{e} \pm 0.46$	$24.3^{h} \pm 0.52$	$26.2^{h} \pm 0.49$	$5.6^{k} \pm 0.30$

Values are mean ± SEM ($n = 6$)

CP crude protein, *CSM* cottonseed meal, *DH* dehulled, *DM* dry matter, *MBM* meat and bone meal, *PBM* poultry byproduct meal, *SBM* soybean meal, *EA* total amounts of essential AA, *NA* total amounts of nonessential AA

[a–k] Means with different superscripts within a row differ ($P < 0.05$)

[A] Crude protein = N% × 6.25

[B] True protein is the sum of AA in protein, which was calculated on the basis of molecular weights of AA residues in protein

Table 3 Composition of total AA in food ingredients (%, as-fed basis)

AA	Blood meal	Casein	Cookie meal	Corn grain	CSM	Feather meal	Fish meal	Gelatin	MBM	Peanut meal	PBM	SBM	SBM (DH)	Sorghum grain
Ala	$7.82^{b} \pm 0.10$	$2.77^{e} \pm 0.06$	$0.52^{j} \pm 0.01$	$0.71^{i} \pm 0.01$	$1.42^{g} \pm 0.03$	$4.18^{d} \pm 0.06$	$5.07^{c} \pm 0.04$	$9.01^{a} \pm 0.15$	$4.78^{c} \pm 0.06$	$1.86^{f} \pm 0.03$	$4.91^{c} \pm 0.07$	$1.95^{f} \pm 0.03$	$2.08^{f} \pm 0.05$	$0.96^{h} \pm 0.01$
Arg	$4.91^{c} \pm 0.05$	$3.40^{e,f} \pm 0.03$	$0.58^{g} \pm 0.01$	$0.38^{h} \pm 0.01$	$4.54^{d} \pm 0.05$	$5.74^{b} \pm 0.06$	$4.85^{c,d} \pm 0.05$	$7.68^{a} \pm 0.07$	$3.67^{e} \pm 0.04$	$5.68^{b} \pm 0.07$	$4.63^{d} \pm 0.04$	$3.18^{f} \pm 0.03$	$3.12^{f} \pm 0.05$	$0.41^{h} \pm 0.01$
Asn	$4.67^{a} \pm 0.06$	$2.56^{c} \pm 0.04$	$0.40^{g} \pm 0.02$	$0.35^{g} \pm 0.01$	$1.57^{f} \pm 0.06$	$1.67^{e,f} \pm 0.05$	$2.92^{b} \pm 0.04$	$1.42^{f} \pm 0.04$	$2.21^{d} \pm 0.03$	$1.80^{e} \pm 0.04$	$2.73^{b,c} \pm 0.06$	$2.10^{d} \pm 0.03$	$2.42^{c} \pm 0.04$	$0.31^{g} \pm 0.01$
Asp	$6.20^{a} \pm 0.05$	$3.88^{c} \pm 0.03$	$0.45^{i} \pm 0.02$	$0.43^{i} \pm 0.01$	$1.94^{h} \pm 0.05$	$2.92^{e,f} \pm 0.06$	$4.34^{b} \pm 0.05$	$2.87^{f} \pm 0.05$	$3.07^{e,f} \pm 0.04$	$2.52^{g} \pm 0.05$	$4.10^{b,c} \pm 0.08$	$3.14^{e} \pm 0.05$	$3.40^{d} \pm 0.06$	$0.36^{j} \pm 0.01$
Cys	$1.92^{b} \pm 0.07$	$0.43^{f} \pm 0.03$	$0.18^{g} \pm 0.01$	$0.20^{g} \pm 0.01$	$0.70^{d} \pm 0.04$	$4.16^{a} \pm 0.16$	$0.67^{d} \pm 0.02$	$0.05^{h} \pm 0.00$	$0.49^{e} \pm 0.04$	$0.65^{d} \pm 0.02$	$1.05^{c} \pm 0.05$	$0.70^{d} \pm 0.02$	$0.69^{d} \pm 0.03$	$0.19^{g} \pm 0.01$
Gln	$4.32^{b} \pm 0.09$	$11.2^{a} \pm 0.16$	$1.44^{h} \pm 0.03$	$1.02^{i} \pm 0.05$	$3.81^{d} \pm 0.08$	$2.86^{f,g} \pm 0.10$	$3.94^{c,d} \pm 0.06$	$3.03^{f} \pm 0.08$	$2.81^{f,g} \pm 0.06$	$2.66^{g} \pm 0.04$	$3.54^{e} \pm 0.05$	$3.80^{d} \pm 0.07$	$4.11^{b,c} \pm 0.09$	$0.85^{i} \pm 0.02$
Glu	$6.38^{b} \pm 0.11$	$9.38^{a} \pm 0.12$	$1.92^{g} \pm 0.04$	$0.64^{i} \pm 0.03$	$4.39^{e,f} \pm 0.10$	$4.81^{c,d} \pm 0.13$	$6.01^{b} \pm 0.11$	$5.26^{c} \pm 0.13$	$4.05^{f} \pm 0.06$	$4.18^{f} \pm 0.08$	$4.89^{c,d} \pm 0.11$	$4.17^{f} \pm 0.12$	$4.53^{d,e} \pm 0.14$	$1.18^{h} \pm 0.03$
Gly	$3.86^{e} \pm 0.05$	$1.86^{i} \pm 0.04$	$0.78^{j} \pm 0.02$	$0.40^{k} \pm 0.01$	$2.12^{h,i} \pm 0.04$	$8.95^{c} \pm 0.11$	$6.58^{d} \pm 0.07$	$33.6^{a} \pm 0.32$	$8.67^{c} \pm 0.12$	$3.17^{f} \pm 0.06$	$9.42^{b} \pm 0.07$	$2.30^{h} \pm 0.03$	$2.72^{g} \pm 0.05$	$0.39^{k} \pm 0.01$
His	$5.57^{a} \pm 0.08$	$2.78^{b} \pm 0.05$	$0.22^{j} \pm 0.01$	$0.23^{j} \pm 0.01$	$1.08^{g} \pm 0.03$	$0.88^{h} \pm 0.02$	$1.51^{c} \pm 0.02$	$0.74^{i} \pm 0.02$	$1.19^{e,f} \pm 0.02$	$0.95^{h} \pm 0.02$	$1.30^{d,e} \pm 0.02$	$1.13^{f,g} \pm 0.02$	$1.15^{f,g} \pm 0.03$	$0.23^{j} \pm 0.01$
Hyp	$0.51^{f} \pm 0.01$	$0.14^{g} \pm 0.01$	0.00	0.00	$0.05^{h} \pm 0.00$	$4.95^{b} \pm 0.10$	$1.86^{e} \pm 0.05$	$12.8^{a} \pm 0.24$	$2.88^{d} \pm 0.06$	$0.07^{h} \pm 0.00$	$3.31^{c} \pm 0.08$	$0.08^{h} \pm 0.01$	$0.07^{h} \pm 0.00$	0.00
Ile	$2.54^{d} \pm 0.05$	$4.91^{a} \pm 0.06$	$0.51^{h} \pm 0.01$	$0.34^{i} \pm 0.01$	$1.19^{g} \pm 0.02$	$3.79^{b} \pm 0.04$	$3.26^{c} \pm 0.04$	$1.17^{g} \pm 0.04$	$1.92^{e} \pm 0.05$	$1.41^{f} \pm 0.04$	$2.32^{d} \pm 0.03$	$2.03^{e} \pm 0.04$	$2.10^{e} \pm 0.05$	$0.38^{i} \pm 0.01$
Leu	$11.4^{a} \pm 0.13$	$8.82^{b} \pm 0.05$	$0.88^{j} \pm 0.02$	$1.13^{i} \pm 0.02$	$2.26^{h} \pm 0.03$	$6.75^{c} \pm 0.06$	$5.24^{d} \pm 0.05$	$2.61^{g} \pm 0.04$	$3.56^{f} \pm 0.04$	$2.48^{g,h} \pm 0.04$	$4.21^{e} \pm 0.05$	$3.44^{f} \pm 0.05$	$3.70^{f} \pm 0.07$	$1.21^{i} \pm 0.02$
Lys	$8.25^{a} \pm 0.07$	$7.49^{b} \pm 0.09$	$0.41^{k} \pm 0.01$	$0.25^{l} \pm 0.01$	$1.66^{i} \pm 0.03$	$2.16^{h} \pm 0.05$	$5.29^{c} \pm 0.04$	$3.75^{d} \pm 0.08$	$3.16^{f} \pm 0.04$	$1.37^{j} \pm 0.01$	$3.44^{e,f} \pm 0.05$	$2.80^{g} \pm 0.03$	$2.87^{g} \pm 0.04$	$0.22^{l} \pm 0.01$
Met	$1.16^{d} \pm 0.03$	$2.64^{a} \pm 0.05$	$0.19^{i} \pm 0.01$	$0.21^{i} \pm 0.01$	$0.66^{f} \pm 0.01$	$0.75^{e} \pm 0.02$	$2.02^{b} \pm 0.02$	$1.03^{d} \pm 0.03$	$1.10^{d} \pm 0.02$	$0.47^{h} \pm 0.01$	$1.39^{c} \pm 0.03$	$0.60^{g} \pm 0.01$	$0.64^{g} \pm 0.02$	$0.20^{i} \pm 0.01$
Phe	$5.83^{a} \pm 0.07$	$4.87^{b} \pm 0.04$	$0.50^{h} \pm 0.01$	$0.46^{h} \pm 0.01$	$2.02^{f} \pm 0.04$	$3.95^{c} \pm 0.05$	$2.78^{d,e} \pm 0.03$	$1.67^{g} \pm 0.05$	$1.85^{f,g} \pm 0.04$	$1.93^{f} \pm 0.03$	$2.36^{e} \pm 0.04$	$2.21^{e} \pm 0.03$	$2.44^{e} \pm 0.03$	$0.51^{h} \pm 0.01$
Pro	$6.29^{e} \pm 0.12$	$10.8^{c} \pm 0.14$	$0.98^{k} \pm 0.03$	$1.06^{k} \pm 0.02$	$1.89^{j} \pm 0.06$	$11.8^{b} \pm 0.15$	$4.25^{g} \pm 0.03$	$20.6^{a} \pm 0.30$	$5.86^{f} \pm 0.13$	$2.30^{i} \pm 0.04$	$6.72^{d} \pm 0.12$	$3.05^{h} \pm 0.05$	$3.18^{h} \pm 0.08$	$0.96^{k} \pm 0.03$
Ser	$4.49^{c} \pm 0.10$	$5.08^{b} \pm 0.04$	$0.56^{i} \pm 0.02$	$0.45^{j} \pm 0.01$	$1.72^{h} \pm 0.04$	$8.80^{a} \pm 0.14$	$2.80^{e} \pm 0.04$	$3.44^{d} \pm 0.04$	$2.08^{g} \pm 0.05$	$2.03^{g} \pm 0.03$	$2.67^{e} \pm 0.05$	$2.12^{g} \pm 0.04$	$2.35^{f} \pm 0.03$	$0.46^{j} \pm 0.01$
Trp	$1.30^{a} \pm 0.02$	$1.24^{a} \pm 0.03$	$0.15^{h} \pm 0.01$	$0.07^{i} \pm 0.00$	$0.44^{d,e} \pm 0.01$	$0.80^{b} \pm 0.02$	$0.70^{c} \pm 0.02$	$0.22^{g} \pm 0.01$	$0.39^{f} \pm 0.01$	$0.38^{f} \pm 0.01$	$0.49^{e} \pm 0.01$	$0.62^{d} \pm 0.01$	$0.63^{d} \pm 0.01$	$0.10^{i} \pm 0.00$
Thr	$3.95^{a} \pm 0.04$	$4.10^{a} \pm 0.08$	$0.42^{h} \pm 0.01$	$0.31^{i} \pm 0.01$	$1.25^{g} \pm 0.03$	$3.97^{a} \pm 0.05$	$4.11^{a} \pm 0.04$	$3.45^{b} \pm 0.04$	$2.42^{d} \pm 0.04$	$1.67^{f} \pm 0.02$	$2.85^{c} \pm 0.05$	$1.76^{f} \pm 0.02$	$2.03^{e} \pm 0.04$	$0.32^{i} \pm 0.01$
Tyr	$2.86^{b} \pm 0.05$	$5.06^{a} \pm 0.05$	$0.55^{j} \pm 0.01$	$0.43^{k} \pm 0.01$	$1.10^{h} \pm 0.03$	$2.04^{d} \pm 0.04$	$2.36^{c} \pm 0.03$	$0.93^{i} \pm 0.02$	$1.45^{g} \pm 0.03$	$1.39^{g} \pm 0.03$	$1.84^{e} \pm 0.03$	$1.66^{f} \pm 0.03$	$1.72^{e,f} \pm 0.04$	$0.45^{k} \pm 0.01$
Val	$8.21^{a} \pm 0.06$	$6.03^{b} \pm 0.08$	$0.53^{i} \pm 0.02$	$0.44^{j} \pm 0.01$	$1.69^{h} \pm 0.02$	$5.76^{c} \pm 0.07$	$3.80^{d} \pm 0.03$	$1.96^{g} \pm 0.04$	$2.23^{f} \pm 0.03$	$1.70^{h} \pm 0.04$	$2.89^{e} \pm 0.05$	$2.09^{f,g} \pm 0.04$	$2.25^{f} \pm 0.04$	$0.50^{i} \pm 0.01$

Values are mean ± SEM ($n = 6$). Molecular weights of intact AA were used to calculate the content of peptide-bound AA in feed ingredients

CSM cottonseed meal, *DH* dehulled, *Hyp* hydroxyproline, *MBM* meat and bone meal, *PBM* poultry byproduct meal, *SBM* soybean meal

[a–l] Means with different superscripts within a row differ ($P < 0.05$)

abundant in fish meal, gelatin, MBM, and PBM (6.1–21%), while these ingredients had relatively high percentages of alanine (4.2–9.0%) and aspartate plus asparagine (4.3–7.3%). Thus, proline content was highest ($P < 0.05$) in gelatin (21%), followed by feather meal (12%), PBM (6.7%), and MBM (5.9%). Gelatin, feather meal, PBM, MBM, and fish meal also had large amounts of hydroxyproline (1.9–12.8%), whereas other ingredients contained very low or little quantity of this AA (0.0–0.20%). It is noteworthy that glycine content was relatively lower ($P < 0.05$) in blood meal (3.9%) and casein (1.9%), compared with most of other AA. Tyrosine content was generally much higher ($P < 0.05$) in animal ingredients (0.93–5.1%) than in plant products (0.43–1.7%), with the highest and lowest percentages in casein (5.1%) and corn grain (0.43%), respectively.

On an as-fed basis (g/100 g sample), animal ingredients contained higher ($P < 0.05$) content of EAA than plant products (Table 4). For example, BCAA content (g/100 g sample) in blood meal and casein was 22 and 20%, respectively, but only approximately 2% in cookie meal, corn grain, and sorghum grain. Of particular note, in some ingredients (casein, corn grain, SBM, and sorghum), BCAA content was similar to that of glutamate plus glutamine. Additionally, lysine content was high in blood meal (8.3%), casein (7.5%), and fish meal (5.3%), low in corn and sorghum grains (0.22–0.25%), and intermediate in other ingredients (1.4–3.4%). Histidine content was intermediate in blood meal (5.6%), casein (2.8%), fish meal, PBM, and MBM (1.2–1.5%), but was low in corn and sorghum (0.23%). Of particular interest, all plant products had low percentages of methionine (0.19–0.66%) and tryptophan (0.07–0.63%), whereas the content of these two EAA was much higher ($P < 0.05$) in animal ingredients (1.0–2.6%) than in plant products (0.49–1.3%). Threonine content was intermediate in animal ingredients (2.4–4.1%) but much lower in plant products (0.31–2.0%).

Content of predominant AA and sulfur AA in total proteins of feed ingredients

Gelatin protein contained only 6% BCAA (Table 5). However, BCAA accounted for 15–25% of total protein in plant products and other animal ingredients. Except for feather meal and gelatin, which contained low content of glutamate plus glutamine (9.6 and 8.4% of total protein, respectively), the content of these two AA in proteins of all ingredients was higher ($P < 0.05$) than 12%, reaching as much as 32% in cookie meal, 26% in CSM, 24% in casein, 21% in SBM, and 20% in corn grain. In particular, leucine and glutamine were most predominant AA in blood meal (13% of total protein). In most of the tested ingredients, the percentages of aspartate plus asparagine (g/100 g protein)

Table 4 Content of abundant AA and sulfur AA in food ingredients

AA	Blood meal	Casein	Cookie meal	Corn grain	CSM	Feather meal	Fish meal	Gelatin	MBM	Peanut meal	PBM	SBM	SBM (DH)	Sorghumr grain
Content (g/100 g of ingredient)														
BA	$22.1^{a} \pm 0.19$	$19.8^{b} \pm 0.12$	$1.92^{i} \pm 0.05$	$1.91^{i} \pm 0.04$	$5.14^{h} \pm 0.06$	$16.3^{c} \pm 0.10$	$12.3^{d} \pm 0.09$	$5.74^{h} \pm 0.08$	$7.71^{f,g} \pm 0.12$	$5.58^{h} \pm 0.10$	$9.43^{e} \pm 0.17$	$7.56^{g} \pm 0.11$	$8.05^{f} \pm 0.15$	$2.09^{i} \pm 0.06$
GG	$10.7^{b} \pm 0.22$	$20.6^{a} \pm 0.29$	$3.36^{g} \pm 0.04$	$1.66^{i} \pm 0.05$	$8.19^{c,d} \pm 0.12$	$7.67^{e} \pm 0.13$	$9.95^{b} \pm 0.07$	$8.29^{c} \pm 0.20$	$6.86^{f} \pm 0.08$	$6.84^{f} \pm 0.09$	$8.43^{c} \pm 0.13$	$7.97^{d,e} \pm 0.12$	$8.64^{c} \pm 0.15$	$2.03^{h} \pm 0.04$
AE	$10.9^{a} \pm 0.09$	$6.44^{d} \pm 0.05$	$0.85^{i} \pm 0.03$	$0.78^{i,j} \pm 0.02$	$3.51^{h} \pm 0.04$	$4.59^{g} \pm 0.05$	$7.26^{b} \pm 0.06$	$4.28^{g} \pm 0.08$	$5.29^{f} \pm 0.07$	$4.32^{g} \pm 0.05$	$6.83^{c} \pm 0.07$	$5.24^{f} \pm 0.05$	$5.81^{e} \pm 0.06$	$0.67^{j} \pm 0.01$
PH	$6.80^{e} \pm 0.08$	$11.0^{c} \pm 0.14$	$0.98^{j} \pm 0.03$	$1.06^{j} \pm 0.02$	$1.94^{i} \pm 0.06$	$16.8^{b} \pm 0.18$	$6.12^{f} \pm 0.06$	$33.5^{a} \pm 0.41$	$8.75^{d} \pm 0.15$	$2.37^{h} \pm 0.04$	$10.1^{c} \pm 0.16$	$3.14^{g} \pm 0.05$	$3.25^{g} \pm 0.08$	$0.96^{j} \pm 0.03$
CM	$3.08^{b} \pm 0.09$	$3.07^{b} \pm 0.06$	$0.37^{h} \pm 0.02$	$0.41^{h} \pm 0.02$	$1.35^{f} \pm 0.07$	$4.91^{a} \pm 0.11$	$2.69^{c} \pm 0.04$	$1.08^{g} \pm 0.04$	$1.59^{e} \pm 0.05$	$1.12^{g} \pm 0.04$	$2.44^{d} \pm 0.07$	$1.31^{f} \pm 0.04$	$1.33^{f} \pm 0.05$	$0.39^{h} \pm 0.02$
Percent of total protein (%)														
BA	$25.4^{a} \pm 0.42$	$23.1^{b} \pm 0.38$	$18.2^{d} \pm 0.33$	$23.5^{b} \pm 0.36$	$16.2^{g} \pm 0.27$	$20.3^{c} \pm 0.30$	$19.8^{c} \pm 0.32$	$5.94^{f} \pm 0.11$	$15.3^{g} \pm 0.24$	$16.0^{g} \pm 0.21$	$15.8^{g} \pm 0.25$	$20.1^{c} \pm 0.38$	$19.7^{c} \pm 0.34$	$23.6^{b} \pm 0.31$
GG	$12.4^{f} \pm 0.26$	$24.0^{b,c} \pm 0.47$	$32.1^{a} \pm 0.43$	$20.3^{d} \pm 0.37$	$25.6^{b} \pm 0.30$	$9.55^{g} \pm 0.14$	$15.7^{e} \pm 0.25$	$8.40^{g} \pm 0.17$	$13.7^{e,f} \pm 0.22$	$19.7^{d} \pm 0.33$	$14.2^{e} \pm 0.26$	$20.9^{d} \pm 0.38$	$20.8^{d} \pm 0.34$	$23.2^{c} \pm 0.36$
AE	$12.5^{b,c} \pm 0.24$	$7.52^{g} \pm 0.09$	$8.16^{f} \pm 0.13$	$9.64^{e} \pm 0.16$	$11.0^{c,d} \pm 0.20$	$5.61^{h} \pm 0.12$	$11.4^{c,d} \pm 0.18$	$4.31^{i} \pm 0.09$	$10.6^{d} \pm 0.19$	$12.4^{b,c} \pm 0.26$	$11.5^{c,d} \pm 0.18$	$13.7^{a,b} \pm 0.23$	$14.2^{a} \pm 0.32$	$7.55^{g} \pm 0.12$
PH	$7.75^{g} \pm 0.18$	$13.0^{d} \pm 0.32$	$9.36^{f} \pm 0.25$	$13.1^{d} \pm 0.28$	$6.03^{h} \pm 0.15$	$21.0^{b} \pm 0.36$	$9.64^{f} \pm 0.28$	$34.5^{a} \pm 0.69$	$17.5^{c} \pm 0.37$	$6.80^{h} \pm 0.22$	$16.9^{c} \pm 0.40$	$8.01^{g} \pm 0.14$	$7.92^{g} \pm 0.16$	$11.0^{e} \pm 0.28$
CM	$3.46^{e,f} \pm 0.07$	$3.61^{e} \pm 0.09$	$3.55^{e} \pm 0.11$	$5.07^{b} \pm 0.13$	$4.28^{c,d} \pm 0.10$	$6.09^{a} \pm 0.15$	$4.27^{c,d} \pm 0.08$	$1.14^{g} \pm 0.04$	$3.25^{f} \pm 0.07$	$3.16^{f} \pm 0.08$	$4.08^{d} \pm 0.12$	$3.46^{e} \pm 0.10$	$3.13^{f} \pm 0.08$	$4.48^{c} \pm 0.15$

Values (mean ± SEM, $n = 6$) are expressed as mg/g sample for free AA (except cysteine) or μg/g sample for glutathione and cysteine

AE aspartate plus asparagine, *BA* branched-chain AA (isoleucine + leucine + valine), *DH* dehulled, *CM* cysteine plus methionine, *GG* glutamate plus glutamine, *MBM* meat and bone meal, *PBM* poultry byproduct meal, *PH* proline plus hydroxyproline, *SBM* soybean meal

[a–j] Means with different superscripts within a row differ ($P < 0.05$)

Table 5 Content of total free AA and glutathione in food ingredients (as-fed basis)

AA	Blood meal	Casein	Cookie meal	Corn grain	CSM	Feather meal	Fish meal	Gelatin	MBM	Peanut meal	PBM	SBM	SBM (DH)	Sorghumr grain
TFA	$7.56^{h} \pm 0.15$	$3.89^{d} \pm 0.06$	$2.66^{e} \pm 0.08$	$0.23^{i} \pm 0.01$	$0.80^{h} \pm 0.02$	$1.03^{g} \pm 0.02$	$14.3^{a} \pm 0.13$	$0.71^{h} \pm 0.02$	$1.32^{f} \pm 0.04$	$4.50^{c} \pm 0.09$	$1.52^{f} \pm 0.05$	$1.02^{g} \pm 0.03$	$0.99^{g} \pm 0.04$	$1.36^{f} \pm 0.07$
Ala	$0.76^{a} \pm 0.03$	$0.23^{c} \pm 0.01$	$0.13^{d} \pm 0.01$	$0.01^{f} \pm 0.00$	$0.05^{e} \pm 0.00$	$0.05^{e} \pm 0.00$	$0.58^{b} \pm 0.03$	$0.06^{e} \pm 0.00$	$0.06^{e} \pm 0.00$	$0.65^{b} \pm 0.04$	$0.07^{e} \pm 0.00$	$0.07^{e} \pm 0.00$	$0.07^{e} \pm 0.00$	$0.25^{c} \pm 0.01$
βAla	$0.02^{f,g} \pm 0.00$	$0.04^{d,e} \pm 0.00$	$0.04^{d,e} \pm 0.00$	$0.01^{g} \pm 0.00$	$0.04^{d,e} \pm 0.00$	$0.02^{f,g} \pm 0.00$	$0.07^{b} \pm 0.00$	0.00	$0.09^{a} \pm 0.01$	$0.03^{e,f} \pm 0.00$	$0.05^{c,d} \pm 0.00$	$0.06^{b,c} \pm 0.00$	$0.05^{c,d} \pm 0.00$	$0.03^{e,f} \pm 0.00$
Arg	$0.15^{e} \pm 0.01$	$0.96^{b} \pm 0.02$	$0.57^{c} \pm 0.01$	$0.01^{g} \pm 0.00$	$0.03^{g} \pm 0.00$	$0.02^{g} \pm 0.01$	$7.37^{a} \pm 0.16$	$0.03^{g} \pm 0.00$	$0.20^{d} \pm 0.01$	$0.20^{d} \pm 0.01$	$0.21^{d} \pm 0.01$	$0.10^{f} \pm 0.00$	$0.03^{g} \pm 0.00$	$0.03^{g} \pm 0.00$
Asp	$0.23^{e} \pm 0.01$	$0.60^{b} \pm 0.02$	$0.50^{c} \pm 0.02$	$0.01^{h} \pm 0.00$	$0.02^{h} \pm 0.00$	$0.15^{f} \pm 0.01$	$0.86^{a} \pm 0.04$	$0.13^{f} \pm 0.01$	$0.34^{d} \pm 0.02$	$0.36^{d} \pm 0.02$	$0.24^{e} \pm 0.02$	$0.13^{f} \pm 0.01$	$0.04^{h} \pm 0.00$	$0.09^{g} \pm 0.01$
Asn	$0.03^{g} \pm 0.00$	$0.21^{d} \pm 0.01$	$0.20^{d} \pm 0.01$	$0.01^{g} \pm 0.00$	$0.01^{g} \pm 0.00$	$0.01^{g} \pm 0.00$	$2.09^{a} \pm 0.08$	$0.14^{e} \pm 0.01$	$0.35^{b} \pm 0.01$	$0.05^{f} \pm 0.00$	$0.25^{c} \pm 0.01$	$0.21^{d} \pm 0.01$	$0.02^{g} \pm 0.00$	$0.12^{e} \pm 0.01$
Cit	$0.11^{b} \pm 0.01$	$0.48^{a} \pm 0.03$	$0.01^{e} \pm 0.00$	0.00	0.00	0.00	$0.01^{e} \pm 0.00$	0.00 ± 0.00	$0.01^{e} \pm 0.00$	$0.03^{e} \pm 0.00$	0.00	$0.02^{e} \pm 0.00$	$0.01^{e} \pm 0.00$	$0.02^{e} \pm 0.00$
Glu	$0.39^{d} \pm 0.01$	$0.68^{b} \pm 0.02$	$0.49^{e} \pm 0.02$	$0.01^{j} \pm 0.00$	$0.03^{i-j} \pm 0.00$	$0.04^{i} \pm 0.00$	$1.31^{a} \pm 0.06$	$0.09^{h} \pm 0.01$	$0.11^{h} \pm 0.01$	$0.51^{c} \pm 0.03$	$0.33^{e} \pm 0.01$	$0.18^{g} \pm 0.01$	$0.10^{h} \pm 0.01$	$0.24^{f} \pm 0.01$
Gln	$0.15^{a} \pm 0.01$	$0.01^{d} \pm 0.00$	0.00	0.00	0.00	0.00	$0.06^{b} \pm 0.00$	$0.04^{c} \pm 0.00$	0.00	0.00	0.00	$0.02^{d} \pm 0.00$	0.00	0.00
Gly	$0.62^{a} \pm 0.03$	$0.07^{g} \pm 0.00$	$0.07^{g} \pm 0.00$	$0.11^{f} \pm 0.01$	$0.43^{b} \pm 0.03$	$0.20^{d} \pm 0.01$	$0.15^{e} \pm 0.01$	$0.03^{h} \pm 0.00$	$0.04^{h} \pm 0.00$	$0.46^{b} \pm 0.03$	$0.07^{g} \pm 0.00$	$0.03^{h} \pm 0.00$	$0.33^{c} \pm 0.02$	$0.17^{d,e} \pm 0.01$
Leu	$0.72^{a} \pm 0.05$	$0.03^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.02^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.04^{d} \pm 0.01$	$0.08^{c} \pm 0.01$	$0.02^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.33^{b} \pm 0.02$	$0.03^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.04^{d} \pm 0.00$	$0.08^{c} \pm 0.01$
Lys	$0.22^{a} \pm 0.01$	$0.05^{c,d} \pm 0.00$	$0.03^{d,e} \pm 0.00$	$0.01^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.03^{d,e} \pm 0.00$	$0.20^{a} \pm 0.01$	0.00 ± 0.00	$0.02^{e} \pm 0.00$	$0.15^{b} \pm 0.01$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$
Orn	$0.08^{a} \pm 0.00$	$0.02^{c} \pm 0.00$	$0.03^{c} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.05^{b} \pm 0.00$	$0.05^{b} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.05^{b} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$	$0.02^{e} \pm 0.00$
Pro	$0.71^{a} \pm 0.01$	$0.07^{d} \pm 0.00$	$0.04^{e,f} \pm 0.00$	$0.02^{f} \pm 0.00$	$0.02^{f} \pm 0.00$	$0.25^{b} \pm 0.01$	$0.13^{c} \pm 0.01$	$0.05^{d,e} \pm 0.00$	$0.06^{d,e} \pm 0.00$	$0.28^{b} \pm 0.01$	$0.12^{c} \pm 0.01$	$0.05^{d,e} \pm 0.00$	$0.05^{d,e} \pm 0.00$	$0.06^{d,e} \pm 0.00$
Ser	$0.08^{e} \pm 0.01$	$0.03^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.02^{d} \pm 0.00$	$0.02^{d} \pm 0.00$	$0.02^{d} \pm 0.00$	$0.45^{a} \pm 0.03$	$0.02^{d} \pm 0.00$	$0.04^{d} \pm 0.00$	$0.16^{b} \pm 0.01$	$0.04^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.04^{d} \pm 0.00$
Tau	$1.52^{a} \pm 0.06$	$0.03^{b} \pm 0.00$	0.00	0.00	0.00	$0.03^{b} \pm 0.00$	$0.02^{b} \pm 0.00$	0.00	$0.02^{b} \pm 0.00$	0.00	$0.02^{b} \pm 0.00$	0.00	0.00	0.00
Cys	$0.48^{j} \pm 0.02$	$0.30^{k} \pm 0.01$	$3.72^{e} \pm 0.15$	$6.94^{d} \pm 0.32$	$2.06^{f} \pm 0.13$	$0.69^{j} \pm 0.03$	$1.40^{g} \pm 0.08$	$16.8^{b} \pm 1.0$	$1.52^{g} \pm 0.09$	$55.7^{a} \pm 2.0$	$1.05^{h} \pm 0.06$	$17.0^{b} \pm 0.72$	$10.7^{c} \pm 0.76$	$1.50^{g} \pm 0.07$
GSH	$13.5^{f} \pm 0.8$	$6.22^{g} \pm 0.3$	$64.7^{d} \pm 1.4$	$115^{c} \pm 3$	$119^{c} \pm 4$	$0.70^{j} \pm 0.04$	$6.63^{g} \pm 0.42$	$1.41^{h} \pm 0.11$	$6.60^{g} \pm 0.3$	$634^{a} \pm 15$	$67.7^{d} \pm 1.5$	$169^{b} \pm 5$	$122^{c} \pm 3$	$44.8^{e} \pm 2.5$

Values (mean ± SEM, $n = 6$) are expressed as mg/g sample for free AA (except cysteine) or μg/g sample for glutathione and cysteine

βAla β-alanine, *Cit* citrulline, *Cys* free cysteine + free cystine, *DH* dehulled, *GSH* total glutathione, *MBM* meat and bone meal, *Orn* ornithine, *PBM* poultry byproduct meal, *SBM* soybean meal, *Tau* taurine, *TFA* total free amino acids

$^{a-k}$ Means with different superscripts within a row differ ($P < 0.05$)

ranged from 10 to 14% in protein, whereas aspartate alone represented 3.6–7 and 4.6–8.2% of total protein, respectively, in animal products (except for gelatin) and plant ingredients. Notably, proline plus hydroxyproline accounted for 35 and 21% of total protein in gelatin and feather meal, respectively. The content of methionine plus cysteine in protein was highest ($P < 0.05$) in feather meal (6.1%), intermediate in casein, cookie meal, CSM, fish meal, MBM, peanut meal, PBM, SBM, and dehulled SBM (3.1–5.1%), and lowest ($P < 0.05$) in gelatin (1.1%).

Content of glutathione and free AA in feed ingredients

Except for fish meal which contained 1.4% free AA (g/100 g sample), total free AA accounted for <1% of total AA in other ingredients (Table 5). Thus, data on total AA content in Table 4 represent essentially true protein in the ingredients. Among the analyzed ingredients, blood meal and casein contained higher ($P < 0.05$) content of free AA than other products. Taurine was relatively abundant in blood meal (0.15%), present at 0.02–0.03% in other animal ingredients (casein, feather meal, and fish meal), and absent in gelatin and all plant products. Many of free AA were not detected in plant ingredients (a detection limit 0.3 nmol/mL). Interestingly, the content of glutathione was highest ($P < 0.05$) in peanut meal (634 ppm), followed by SBM (169 ppm), CSM (119 ppm), and animal products (6–68 ppm). Similarly, the content of free cysteine was highest ($P < 0.05$) in peanut meal (56 ppm), followed by SBM (17 ppm). Among the analyzed samples, the content of free citrulline content was highest in casein (0.05%) but negligible in other products. Likewise, ornithine and β-alanine were negligible in all of the tested ingredients.

Discussion

The traditional classification of NEAA has underestimated their important functions in nutrition, physiology, and health (Frank et al. 2007; Jobgen et al. 2006; Wu and Morris 1998; Wu 2009). Thus, information about NEAA composition in feed ingredients is missing from the current National Research Council (NRC)-recommended nutrient requirements for animals (NRC 1994, 1998). Compelling evidence from recent studies shows that NEAA play versatile roles in regulating nitrogen metabolism, gene expression, cell signaling, and whole-body homeostasis (Avruch et al. 2009; Curi et al. 2005; Escobar et al. 2006; Fu et al. 2005; Palii et al. 2009; Rhoads and Wu 2009; Tan et al. 2010). Unfortunately, information about complete composition of NEAA (including glutamate, glutamine, aspartate, and asparagine) in diets is currently not available and has often been ignored by researchers (e.g., Deng et al.

2009; Han et al. 2009; Kong et al. 2009; Ma et al. 2010; NRC 1998). Additionally, glutamate plus glutamine and aspartate plus asparagine in dietary proteins are generally presented in the published literature as glutamate and aspartate, respectively, because standard conditions of acid hydrolysis convert glutamine into glutamate and asparagine into aspartate (Wu 2009). To our knowledge, this is the first report of complete NEAA in select food ingredients. Such a database is important for formulating balanced diets for young, growing, lactating, and gestating animals which cannot synthesize adequately at least some NEAA, including arginine (He et al. 2009; Ma et al. 2010; Mateo et al. 2007; Wu et al. 2007), glutamine (Haynes et al. 2009; Wu et al. 1996, 2010a), glutamate (Hou et al. 2010; Liu et al. 2002; Zimmerman 1975), and proline (Ball et al. 1986; Kirchgessner et al. 1995; Wu 1997).

Glutamine and glutamate were the most abundant and third most abundant AA in casein (a major protein in milk), respectively (Table 5). For example, glutamine alone represented 13% of total protein in casein. This explains why the milk of all species is rich in protein-bound glutamate plus glutamine (Davis et al. 1994; Wu and Knabe 1994). Interestingly, glutamine and glutamate were the second and most abundant AA in some plant proteins, respectively, or among the most abundant AA in other ingredients (Table 3). Based on gene sequences of some known proteins in select foods, Lenders et al. (2009) estimated that glutamine accounted for 1% (kidney bean) to 33% (whole grain bread) of total protein in common foods. Our findings that glutamine and glutamate are particularly abundant in proteins of animal and plant ingredients underscore the importance of these two NEAA in animal growth and development (Blachier et al. 2010; Burrin and Reeds 1997; Flynn et al. 2009; Wang et al. 2009). For example, both glutamate and glutamate are major energy substrates in the mammalian small intestine to maintain its mucosal integrity and function (Stoll and Burrin 2006; Wu et al. 1995). Additionally, glutamine upregulates expression of antioxidative genes (Brasse-Lagnel et al. 2009; Wang et al. 2008), inhibits expression of inflammatory cytokines (Curi et al. 2005), and prevents endotoxin- or oxidant-induced apoptosis (Haynes et al. 2009) in intestinal cells. Thus, casein or blood meal would be an excellent source of glutamine for weanling neonates to ameliorate gut inflammation, injury, and atrophy (Tan et al. 2009a, b).

Aspartate and alanine were the fourth most abundant AA in SBM and other ingredients (blood meal, corn grain, fish meal, gelatin, PBM, and sorghum grain), respectively (Table 3). In addition, both aspartate and alanine were among the most abundant AA in other ingredients of animal and plant origin. This is consistent with important roles for these two NEAA in nutrition (Wu 2009). For example, alanine is a major glucogenic substrate in all mammals, and glucose is essential for the functions of brain and red blood cells (Jobgen et al. 2006). Additionally, like glutamate, 95% of dietary aspartate is degraded by the small intestine in first pass as an important oxidative fuel (Reeds and Burrin 2001; Stoll and Burrin 2006). In all ingredients tested, the content of asparagine was much lower than that of aspartate (Table 3). Thus, aspartate was the major constituent of the aspartate plus asparagine components reported for anima diets (Wu et al. 2010a, b) Unlike aspartate, approximately 85% of dietary asparagine enters the portal circulation to support whole-body protein synthesis (Wu et al. 2010b). Thus, it can be surmised that endogenous synthesis of aspartate is much greater than that of asparagine in animals.

Proline plus hydroxyproline constitute one-third of AA in collagens on molar basis (Phang et al. 2008), and this is also true for glycine (Wu et al. 1999). Thus, ingredients of animal origin (hydrolyzed feather meal, fish meal, gelatin, MBM, and PBM) containing connective tissue are excellent sources of these three AA (Table 3) that play important roles in nutrition and physiology (Wu and Morris 1998; Wu 2009; Wu et al. 2009). Interestingly, glycine content was relatively high in CSM, peanut meal, and cookie meal (Table 3). In contrast, expressed as the percentage of total protein, glycine was among the least abundant AA in casein and blood meal, while being intermediate in SBM and sorghum grain. This explains why the milk of mammals (including humans, cows, and pigs) is remarkably deficient in glycine (Davis et al. 1994; Wu and Knabe 1994), and why dietary glycine is inadequate for maximum protein synthesis in both suckling and postweaning mammals (Wu et al. 2010b) as well as fish (Li et al. 2009d). Such knowledge will lead to the development of new formulations for feeding (1) breast-fed or formula milk-fed neonates whose foods have low glycine content (Kim and Wu 2004; Wu et al. 2010b); (2) poultry which cannot adequately synthesize proline or glycine relative to their needs (Austic 1976; Baker 2009); and (3) wounded animals whose tissue repair requires large amounts of proline and glycine (Wu et al. 2009). While hydroxyproline was traditionally considered to have little nutritional value (Maynard et al. 1979), this unique AA is now recognized as (1) a quantitatively important substrate for the synthesis of glycine, pyruvate, and glucose; (2) a scavenger of oxidants; and (3) a regulator of the cellular redox state (Phang et al. 2008; Wu et al. 2010).

Arginine content was relatively low in casein (Table 3), explaining why the milk of most species (including humans, cows, and pigs) is remarkably deficient in this AA (Davis et al. 1994; Wu and Knabe 1994). However, arginine was the most abundant AA in CSM and peanut meal, and among the most abundant AA in hydrolyzed feather meal, fish meal, gelatin, MBM, PBM, and SBM (Table 3).

Expressed per gram of ingredient, the predominant source of arginine among the tested ingredients was gelatin, followed by hydrolyzed feather meal, peanut meal, blood meal, PBM, and CSM. Such an abundance of arginine in gelatin has not previously been recognized and could be taken into consideration in the formulation of diets for mammalian, avian, and aquatic species. Because arginine plays important roles in enhancing embryonic/fetal survival and growth (Mateo et al. 2007; Wu et al. 2006; Zeng et al. 2008), lactation performance (Kim and Wu 2009; Mateo et al. 2008), tissue protein synthesis (Wu et al. 2010d; Yao et al. 2008), postnatal growth and development (Wu et al. 2004), spermatogenesis (Wu et al. 2009), cardiovascular function, and immune response (Li et al. 2007; Tan et al. 2009a, b), arginine-rich ingredients could be used to improve animal health and the efficiency of nutrient utilization.

Appropriate combinations of corn and SBM in diets could provide adequate levels of all EAA (with exception of lysine for growing animals) to meet the current NRC-recommended dietary requirements of livestock species and poultry (NRC 1994, 1998). However, if a diet for postweaning pigs contains a high level (e.g., 37%) of SBM, it supplies 40–60% greater amounts of most EAA than needed as substrates for tissue protein accretion (Wu et al. 2010b). Excess EAA must be degraded via interorgan cooperation by both the small intestine and extra-intestinal tissues because free AA are not stored in the body (Wu 2009). This constitutes a significant waste of dietary protein, which is the most expensive component of any diet for animals (Baker 2009; Kim and Wu 2004, 2009). Although NRC (1998) currently does not recommend dietary requirements of NEAA (Table 1) for swine (except arginine for the young pig), there is evidence that some of them are not adequately synthesized by animals (Wu et al. 2010a, b). For example, the typical corn- and SBM-based diet does not provide adequate amounts of arginine, proline, aspartate, glutamate, glutamine, or glycine for postweaning growing pigs (Wu et al. 2010b). In this regard, it is noteworthy that these six AA were particularly abundant in blood meal, hydrolyzed feather meal, fish meal, MBM, and PBM (Table 3). Additionally, while all ingredients provide various amounts of glutathione (a major antioxidant), only animal products contain taurine (Table 5), which is a major antioxidant, a key regulator of cellular redox state, and a necessary osmolyte in the body (Wu 2009). Thus, through complementary effects (Baker 2009; Wu 2009), appropriate inclusion of blood meal, hydrolyzed feather meal, fish meal, MBM, and PBM in plant protein-based diets plays an crucial role in formulating balanced diets for nursery, growing, gestating, and lactating livestock species (Li et al. 2009c; Mateo et al. 2008; Wu et al. 2007), as well as poultry (Baker 2009; NRC 1994) and fish (Li et al. 2009c). This will help substantially to improve their production performance, efficiency of nutrient utilization, and health.

As noted previously, it has long been assumed tactically, without much evidence, that animals or humans could synthesize sufficient amounts of all NEAA and did not need them in diets for optimal nutrition or health (NRC 1994, 1998). At present, little is known about quantitative requirements of NEAA by mammals (Wu et al. 2009), birds (Baker 2009), or fish (Li et al. 2009d). However, results of recent studies indicate that sow milk supplies at most only 40% of arginine for protein accretion in 7–21-day-old suckling pigs and that inadequate synthesis of arginine is a major factor limiting their maximum growth (Wu et al. 2004). Likewise, endogenous synthesis provides at least 0.88 g glutamine/kg bodyweight per day in milk-fed young pigs (Wu et al. 2010b) but still cannot support their maximum growth (Haynes et al. 2009). There is also evidence that de novo synthesis of arginine (Tan et al. 2009a; Wu et al. 2010d), glutamine (Wang et al. 2008; Wu et al. 1996), or proline (Wu et al. 2010c) is insufficient to maintain intestinal-mucosal morphology or maximum growth of the gut and the whole body in postweaning pigs.

Dietary requirements (DR) of NEAA by animals and humans for growth, reproduction, wool production, or lactation can be estimated as follows:

$$\mathrm{DR} = (\mathrm{MN_{SI}} + \mathrm{MN_{EIT}} - \mathrm{ES_{SI}} - \mathrm{ES_{EIT}})/\mathrm{RE},$$

where $\mathrm{MN_{SI}}$ and $\mathrm{MN_{EIT}}$ are the metabolic needs of the small intestine (SI) and extra-intestinal tissues (EIT), respectively; $\mathrm{ES_{SI}}$ and $\mathrm{ES_{EIT}}$ are endogenous syntheses in the small intestine and extra-intestinal tissues, respectively; and RE is protein digestibility (rate of the entry of dietary protein-bound AA into the portal vein). Metabolic needs of NEAA include (1) oxidation as energy sources, (2) protein synthesis, (3) generation of biologically active substances, and (4) formation of tissue- or sex-specific products (e.g., mucin, egg, spermatozoa, embryo/fetus, wool, and milk). Calculated values of dietary NEAA requirements should be verified by feeding trials, nitrogen-balance experiments, or studies of functional outcome (e.g., fertility, health, and survival).

In summary, this is the first report of the content of glutamate, glutamine, aspartate, and asparagine in feedstuffs. The study also provides complete composition of NEAA in feed ingredients for animal diets. Except for gelatin, both animal and plant ingredients contained high content of glutamate plus glutamine, BCAA, proline plus hydroxyproline, and aspartate plus asparagine. While glutamine and glutamate were among the most abundant AA in casein and plant proteins, glycine and proline plus hydroxyproline were distinctly predominant in hydrolyzed feather meal, fish meal, gelatin, MBM, and PBM. Among

the tested ingredients, gelatin contained the highest amounts of arginine, glycine, proline, and hydroxyproline. Thus, animal products are excellent sources of both EAA and NEAA to support maximal growth and production performance of animals. Because casein, corn, soybean, peanut, fish, and gelatin are consumed by children and adults, our findings also have important implications for human nutrition (Elango et al. 2009; McKnight et al. 2010).

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