Composition of free and peptide-bound amino acids in beef chuck, loin, and round cuts[1,2]

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ABSTRACT: Meat is a food for humans. However, beef consumption in the United States has steadily declined by >14% over the past decade due to a variety of factors, including insufficient knowledge of animal protein. This study quantified all proteinogenic AA as well as nutritionally and physiologically significant nonproteinogenic AA and small peptides in beef cuts from 3 subprimals (chuck, round, and loin). Beef carcasses (n = 10) were selected at 3 commercial packing plants in the United States. Retail-cut samples were analyzed for the nitrogenous substances after acid, alkaline, or enzymatic hydrolysis and after deproteinization. In these chuck, round, and loin cuts, total amounts of glutamate (free plus peptide bound) were the highest (69–75 mg/g dry weight) followed by lysine, leucine, arginine, and glutamine in descending order. This is the first study to determine aspartate, asparagine, glutamate, and glutamine in meat proteins of any animal species. In all the beef samples evaluated, glutamine was the most abundant free AA (4.0–5.7 mg/g dry weight) followed by taurine, alanine, glutamate, and β-alanine. Additionally, samples from all beef cuts had high concentrations of anserine, carnosine, and glutathione, which were 2.8 to 3.7, 15.2 to 24.2, and 0.68 to 0.79 mg/g dry weight, respectively. Beef top loin steaks appear to provide higher protein nutrition values than top round steaks and under blade roasts, but all are excellent sources of proteinogenic AA as well as antioxidant AA and peptides to improve human growth, development, and health. Our findings may help guide future decisions regarding human and animal nutrition.

Key words: amino acids, antioxidants, beef, peptides, protein

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INTRODUCTION

Lean meat is a food for human consumption, but beef consumption per capita in the United States has steadily declined by >14% over the past decade (USDA, 2012). This reduction may result, in part, from a lack of understanding of meat as an important source of AA and antioxidant peptides in human diets as well as other factors (e.g., economics and consumer perceptions about fat content). Adequate provision of AA enhances muscle protein synthesis and alleviates sarcopenia in elderly subjects (Riddle et al., 2016), and arginine reduces obesity and ameliorates cardiovascular dysfunction (McKnight et al., 2010). Certain free AA (e.g., glutamate, glycine, and β-alanine) provide "meaty flavor" to improve appetite and gastrointestinal function (San Gabriel and Uneyama, 2013). In addition, dietary taurine is essential for children and conditionally essential for adults to maintain retinal and cardiac functions (Wu, 2013). Furthermore, carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-1-methylhistidine), and glutathione (γ-Glu-Cys-Gly) are potent antioxidants in cells (Hipkiss et al., 1997).

Although there are reports of free and peptide-bound AA in beef cuts (e.g., Greenwood et al., 1951; Ma et al., 1961; Franco et al., 2010), the data are incomplete, as neither glutamate, glutamine, asparagines, and aspartate in meat proteins of any species nor free β-alanine and glutathione were measured by

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Table 1. Composition of nutrients in beef cuts[1]

Type of cut[2]	Water, g/100 g fresh weight	DM, g/100 g fresh weight	CP, g/100 g DM	Crude fat, g/100 g DM	Carbohydrates + other OM, g/100 g DM	Minerals, g/100 g DM
Chuck	70.3 (0.19)[b]	29.7 (0.19)[b]	68.0 (0.94)[b]	20.3 (1.10)[a]	7.96 (0.78)[b]	3.74 (0.09)
Round	71.0 (0.24)[a]	29.0 (0.24)[c]	72.1 (0.35)[a]	14.2 (1.03)[b]	9.94 (0.90)[a]	3.76 (0.12)
Loin	69.2 (0.22)[c]	30.8 (0.22)[a]	73.4 (0.51)[a]	20.0 (1.39)[a]	3.14 (0.37)[c]	3.46 (0.13)

[a–c]Within a column, means sharing different superscript letters differ ($P < 0.05$).

[1]Values are means (SEM); $n = 10$.

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

the authors. Values for many free AA and carnosine in beef meat are highly inconsistent and differ by 10 to 50 fold (e.g., Imanari et al., 2010; Iwamoto et al., 2010; Szterk and Roszko, 2014). Detailed knowledge of free and protein-bound AA in meat will not only provide precise information on meat composition but will also help explain the nutritional value of beef. The objective of this study was to quantify and compare all proteinogenic AA as well as nutritionally and physiologically significant nonproteinogenic AA and small peptides in selected beef cuts from the chuck, loin, and round.

MATERIALS AND METHODS

Reagents

The sources of HPLC-grade water, HPLC-grade methanol, and other chemicals as well as HPLC columns were the same as previously described (Dai et al., 2014). Polypropylene tubes (17 by 120 mm; Fisher Scientific, Houston, TX; catalog number 14-959-49B) were used to prepare AA standards and store extracts of beef samples.

Beef Cuts

Ten beef carcasses provided 3 types of beef cuts per carcass for this study. Beef carcasses (295 to 400 kg) were selected at 3 commercial packing plants in the United States (West et al., 2014; Acheson et al., 2015). Carcass selection criteria were assigned based on contribution to a larger, multiyear, collaborative study that was designed to accurately represent the attributes of the U.S. beef supply identified by the 2005 National Beef Quality Audit (Garcia et al., 2008; A-maturity, 67% USDA Choice, 33% USDA Select, 50% Yield Grade 2, 50% Yield Grade 3, 67% steers, 33% heifers, 11.1% dairy, and 88.9% nondairy). Actual carcasses used for the present study were A-maturity, 70% USDA Choice, 30% USDA Select, 54% Yield Grade 2, 46% Yield Grade 3, 77% steers, 23% heifers, 54% dairy, and 46% nondairy. Chuck, round, and loin subprimals were collected from identified carcasses and fabricated into retail cuts. As described by West et al. (2014), Beef Chuck, Under Blade Roasts were fabricated by squaring up the Beef Chuck, Under Blade Roast (which consists of the serratus ventralis, rhomboideus, and splenius muscles) and removing one 5.08-cm-thick portion from each end of the subprimal. Beef Round, Top Round Steaks were produced as described by Acheson et al. (2015), by removing the cap muscle (gracilis) and the soft side (pectineus, adductor, and sartorius muscles) from the top round (semimembranosus muscle). All exterior fat was trimmed to 0 cm and the anterior surface was faced before steak cutting. Starting from the anterior end of the top round, 4 top round steaks were cut at a thickness of 1.91 cm each. Beef Loin, Top Loin Steaks also were cut as described by Acheson et al. (2015). Briefly, boneless top loin steaks (longissimus lumborum muscle) were cut 2.54 cm in thickness from the anterior to posterior end of each loin subprimal, and all exterior fat was trimmed to 0 cm. As outlined in both West et al. (2014) and Acheson et al. (2015), all beef cuts were dissected to separate fat, lean, and refuse based on standardized protocols. Following the dissection, samples were placed in a stainless steel strainer and submerged in liquid nitrogen until completely frozen. The frozen samples were then transferred into a 6.62 L Robot Coupe (BLIXER 6V; Robot Coupe USA, Inc., Ridgeland, MS) batch processor and blended until a fine, homogenous powder was formed (Acheson et al., 2015). The beef homogenates were then stored at −80°C until analyzed.

Chemical Analyses

Frozen beef samples, which were the same as the stored homogenates previously described, were finely reground to 0.5 mm in size before use for analysis. Dry matter, CP, fats, minerals, and carbohydrates in the samples were determined in triplicate as we previously described (Li et al., 2011b). Crude protein content in beef cuts was calculated as nitrogen content × 6.25 (Alexander and Elvehjem, 1956; Schönfeldt et al., 2010).

Analyses of AA and small peptides were performed in triplicate using our established HPLC methods (Dai et al., 2014). Briefly, for determining total AA (including proline and 4-hydroxyproline but not tryptophan) in samples, approximately 100-mg samples were hydrolyzed in 10 mL of 6 M HCl at 110°C for 24 h under nitrogen (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 a tryptophan standard. Glutamine, glutamate, asparagine, and aspartate in protein were determined using porcine digestive enzymes, as we previously described (Dai et al., 2014). Collagen content in beef cuts was calculated as hydroxyproline content × 7.5 (Berge et al., 1993). To determine concentrations of free AA and small peptides (carnosine, anserine, and glutathione) in beef cuts, each sample (approximately 200 mg) was homogenized in 2 mL of 1.5 $M\ HClO_4$, and the acidified solution was neutralized with 1 mL of 2 $M\ K_2CO_3$. The extracts were analyzed for free AA, carnosine, anserine, and glutathione (Wu and Meininger, 2008; Wang et al., 2014). Amino acids and small peptides in samples were quantified on the basis of known amounts of standards (Sigma Chemicals, St. Louis, MO) using the Millenium-32 Software (Waters Corp., Milford, MA). Except for glycine, taurine and β-alanine, all AA analyzed in the present study are L-isomers. Results were compared among the 3 beef cuts.

Statistical Analysis

Values are expressed as mean ± SEM. 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). Log transformations were undertaken for β-alanine and carbohydrates. Data were analyzed by 1-way ANOVA, as described by Assaad et al. (2014). Probability values ≤ 0.05 were taken to indicate statistical significance.

RESULTS

Nutrient Composition in Beef Cuts

The composition of major nutrient classes in beef cuts is summarized in Table 1. The under blade roast had 2.4% higher ($P < 0.05$) DM content than the top round steak but 3.6% lower ($P < 0.05$) DM content than the top loin steak. On a DM basis, the under blade roast had 5.7 and 7.4% lower ($P < 0.05$) CP content than the top round steak and top loin steak, respectively. In contrast, the content of carbohydrates plus other non-fat and non-CP organic matter (OM) in the under blade roast was lower ($P < 0.05$) than that in the top round steak but was much higher ($P < 0.05$) than that in the top loin steak. Fat content in the under blade roast did not differ ($P > 0.05$) from that in the top loin steak but was higher ($P < 0.05$) than that in the top round steak. Mineral content did not differ ($P > 0.05$) among the 3 types of beef cuts.

Table 2. Total amounts of proteinogenic AA in beef cuts[1]

	Type of beef cuts[2]		
	Chuck	Round	Loin
Amino acids	mg/g dry weight		
Alanine	42.2 (0.26)[b]	44.5 (0.34)[a]	45.4 (0.29)[a]
Arginine	47.9 (0.35)[b]	51.0 (0.39)[a]	52.4 (0.38)[a]
Asparagine	30.3 (0.25)[b]	32.9 (0.30)[a]	33.4 (0.36)[a]
Aspartate	37.7 (0.34)[b]	40.3 (0.32)[a]	41.1 (0.37)[a]
Cysteine[3]	10.1 (0.07)[b]	10.8 (0.08)[a]	11.2 (0.09)[a]
Glutamate	68.9 (0.35)[c]	73.8 (0.45)[b]	75.1 (0.37)[a]
Glutamine	46.8 (0.29)[c]	48.5 (0.30)[b]	49.9 (0.33)[a]
Glycine	31.0 (0.32)[b]	33.3 (0.36)[a]	33.7 (0.28)[a]
Histidine	29.4 (0.23)[b]	31.0 (0.20)[a]	31.7 (0.26)[a]
4-Hydroxyproline	1.73 (0.04)	1.74 (0.05)	1.77 (0.05)
Isoleucine	38.4 (0.40)[b]	40.5 (0.32)[a]	41.1 (0.40)[a]
Leucine	61.8 (0.30)[b]	65.1 (0.28)[a]	66.7 (0.34)[a]
Lysine	66.6 (0.36)[b]	70.4 (0.57)[a]	72.0 (0.49)[a]
Methionine	23.7 (0.24)[b]	24.8 (0.21)[a]	25.3 (0.26)[a]
Phenylalanine	30.9 (0.29)[b]	33.1 (0.30)[a]	33.5 (0.28)[a]
Proline	30.0 (0.27)[b]	31.5 (0.35)[a]	32.9 (0.38)[a]
Serine	32.0 (0.27)[b]	34.2 (0.29)[a]	35.4 (0.34)[a]
Threonine	34.3 (0.22)[b]	35.8 (0.43)[a]	37.0 (0.30)[a]
Tryptophan	9.34 (0.07)[b]	9.77 (0.06)[a]	10.0 (0.08)[a]
Tyrosine	27.1 (0.24)[b]	28.9 (0.37)[a]	30.1 (0.51)[a]
Valine	44.8 (0.37)[b]	46.9 (0.33)[a]	47.4 (0.29)[a]
Total[4]	745 (2.3)[c]	789 (2.8)[b]	807 (2.6)[a]

[a–c]Within a row, means sharing different superscript letters differ ($P < 0.05$).

[1]Values are means (SEM); $n = 10$. Amino acids are peptide-bound AA (those present in proteins, large peptides, and small peptides) and free AA. Calculations were based on the molecular weights of intact AA.

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

[3]Including cysteine plus one-half cystine.

[4]Including AA listed in this table.

Total Amounts of Proteinogenic AA in Beef Cuts

Total amounts of proteinogenic (protein-forming) AA in beef cuts included peptide-bound AA (present in proteins, large peptides, and small peptides) and free AA. These AA are precursors for peptide synthesis, and the data are shown in Table 2. Except for 4-hydroxyproline, total amounts of proteinogenic AA in the under blade roasts were lower ($P < 0.05$) than those in top round steaks and top loin steaks. Total amounts of 4-hydroxyproline did not differ ($P > 0.05$) among the 3 types of beef cuts. Total amounts of glutamate and glutamine were higher ($P < 0.05$) in top loin steaks than in top round steaks, but total amounts of other proteinogenic AA did not differ ($P > 0.05$) between these 2 cuts of beef. Total amounts of proteinogenic AA in top loin steaks were 8.3 and 2.3%

Table 3. Amounts of polypeptide-bound AA in beef cuts[1]

	Type of beef cuts[2]		
	Chuck	Round	Loin
Amino acids	mg/g dry weight		
Alanine	41.3 (0.25)	43.5 (0.33)	44.1 (0.28)
Arginine	47.8 (0.35)^b	50.9 (0.39)^a	52.2 (0.38)^a
Asparagine	30.2 (0.25)^b	32.7 (0.30)^a	33.2 (0.36)^a
Aspartate	37.6 (0.34)^b	40.2 (0.32)^a	41.0 (0.37)^a
Cysteine[3]	9.50 (0.06)^b	10.2 (0.07)^a	10.7 (0.08)^a
Glutamate	68.0 (0.34)^b	72.8 (0.44)^a	74.0 (0.36)^a
Glutamine	41.1 (0.26)^b	44.5 (0.28)^a	44.8 (0.29)^a
Glycine	30.5 (0.30)^b	32.7 (0.35)^a	33.2 (0.26)^a
Histidine	29.2 (0.22)^b	30.7 (0.19)^a	31.4 (0.25)^a
4-Hydroxyproline	1.67 (0.04)	1.68 (0.05)	1.70 (0.05)
Isoleucine	38.3 (0.39)^b	40.3 (0.31)^a	40.9 (0.39)^a
Leucine	61.5 (0.30)^b	64.8 (0.27)^a	66.2 (0.33)^a
Lysine	66.1 (0.35)^b	70.1 (0.56)^a	71.7 (0.48)^a
Methionine	23.5 (0.23)^b	24.7 (0.20)^a	25.2 (0.25)^a
Phenylalanine	30.6 (0.28)^b	32.7 (0.29)^a	33.2 (0.27)^a
Proline	29.6 (0.26)^b	31.0 (0.34)^a	32.4 (0.37)^a
Serine	31.7 (0.26)^b	33.9 (0.28)^a	35.0 (0.33)^a
Threonine	34.0 (0.21)^b	35.5 (0.42)^a	36.5 (0.29)^a
Tryptophan	9.29 (0.07)^b	9.71 (0.06)^a	9.95 (0.08)^a
Tyrosine	26.9 (0.23)^b	28.6 (0.37)^a	29.8 (0.50)^a
Valine	44.6 (0.36)^b	46.7 (0.32)^a	47.2 (0.28)^a
Total	735 (2.3)^c	778 (2.7)^b	795 (2.5)^a

$^{a–c}$Within a row, means sharing different superscript letters differ ($P < 0.05$).

[1]Values are means (SEM); $n = 10$. Polypeptide-bound AA, which were calculated as the difference between total AA (minus di- and tripeptides) and free AA, are those AA present in proteins and oligopeptides (excluding anserine, carnosine, and glutathione). Calculations were based on the molecular weights of intact AA.

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

[3]Including cysteine plus one-half cystine.

Table 4. Amounts of true protein (TP) in beef cuts[1]

	Type of beef cuts[2]		
Variable	Chuck	Round	Loin
True protein,[3] mg/g dry weight	626 (2.0)^c	665 (2.2)^b	679 (1.9)^a
Collagen, mg/g dry weight	12.5 (0.32)	12.6 (0.36)	12.8 (0.38)
Ratio of TP to CP	0.921 (0.008)	0.923 (0.009)	0.925 (0.009)

$^{a–c}$Within a row, means sharing different superscript letters differ ($P < 0.05$).

[1]Values are means (SEM); $n = 10$.

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

[3]Amounts of true protein were calculated according to the molecular weights of AA residues in polypeptides. Collagen was included as part of polypeptides and calculated as hydroxyproline content × 7.5.

higher ($P < 0.05$) than those in under blade roasts and top round steaks, respectively.

Amounts of Polypeptide-Bound AA in Beef Cuts

Polypeptide-bound AA were calculated as the difference between total AA (minus di- and tripeptides) and free AA. Therefore, AA in polypeptides were those AA present in proteins and large polypeptides (excluding anserine, carnosine, and glutathione), and the data are shown in Table 3. Except for alanine and 4-hydroxyproline, amounts of polypeptide-bound AA in under blade roasts were lower ($P < 0.05$) than those in top round steaks and top loin steaks. Amounts of alanine and 4-hydroxyproline in polypeptides did not differ ($P > 0.05$) among the 3 types of beef cuts. Amounts of polypeptide-bound AA in top loin steaks were 8.2 and 2.2% higher ($P < 0.05$) than those in under blade roasts and top round steaks, respectively. Similar results were obtained when amounts of polypeptides (taken as true protein) were calculated on the basis of the molecular weights of AA residues (Table 4). As part of protein, amounts of collagen did not differ ($P > 0.05$) among the 3 types of beef cuts. The ratio of true protein to CP was approximately 0.92:1.00 in beef (Table 4). For proteins from these chuck, round, and loin cuts, the percentage of glutamate was the highest ($P < 0.05$) followed by lysine, leucine, arginine, and glutamine in descending order (Table 5). The content of total AA in protein differed ($P < 0.05$) among the 3 types of beef cuts (Table 3).

Amounts of Free Proteinogenic AA in Beef Cuts

Free AA were those AA that were present in beef in the free, nonpeptide form. Data on free proteinogenic AA in beef cuts are summarized in Table 6. Glutamine was the most abundant free AA in all beef cuts, followed by alanine, glutamate, praline, and glycine in descending order. Except for free aspartate, cysteine, glutamine, 4-hydroxyproline, and proline, amounts of free AA were highest ($P < 0.05$) in top loin steaks, intermediate ($P < 0.05$) in top round steaks, and lowest ($P < 0.05$) in under blade roasts. Amounts of free aspartate, cysteine, and proline did not differ ($P > 0.05$) between top round steaks and top loin steaks, and their amounts were higher ($P < 0.05$) in top round and top loin steaks than in under blade roasts. In contrast, amounts of free glutamine were highest ($P < 0.05$) in under blade roasts, intermediate ($P < 0.05$) in top loin steaks, and lowest ($P < 0.05$) in top round steaks. Amounts of free 4-hydroxyproline did not differ ($P > 0.05$) among the 3 types of beef cuts.

Amounts of Free Nonproteinogenic AA in Beef Cuts

Data on amounts of free nonproteinogenic AA in beef cuts are shown in Table 7. Taurine was the most abundant nonproteinogenic AA in all beef cuts, followed by β-alanine, ornithine, and citrulline in descending order. Amounts of taurine were lower ($P <$

Table 5. Percentages of AA residues in proteins of beef cuts[1]

	Type of beef cuts[2]		
	Chuck	Round	Loin
Amino acids	g/100 g protein		
Alanine	5.24 (0.04)	5.18 (0.04)	5.16 (0.04)
Arginine	6.80 (0.05)	6.82 (0.06)	6.87 (0.05)
Asparagine	4.14 (0.04)	4.23 (0.04)	4.20 (0.05)
Aspartate	5.17 (0.05)	5.21 (0.04)	5.20 (0.05)
Cysteine[3]	1.42 (0.02)	1.43 (0.01)	1.46 (0.02)
Glutamate	9.52 (0.05)	9.59 (0.06)	9.56 (0.05)
Glutamine	5.72 (0.04)	5.82 (0.05)	5.76 (0.04)
Glycine	3.69 (0.05)	3.73 (0.04)	3.71 (0.04)
Histidine	3.20 (0.04)	3.16 (0.06)	3.18 (0.05)
4-Hydroxyproline	0.23 (0.01)	0.22 (0.01)	0.22 (0.01)
Isoleucine	5.24 (0.06)	5.20 (0.05)	5.18 (0.06)
Leucine	8.43 (0.05)	8.35 (0.04)	8.38 (0.05)
Lysine	9.21 (0.06)	9.19 (0.07)	9.23 (0.06)
Methionine	3.25 (0.05)	3.21 (0.04)	3.17 (0.04)
Phenylalanine	4.32 (0.05)	4.35 (0.04)	4.34 (0.04)
Proline	3.85 (0.04)	3.79 (0.05)	3.83 (0.05)
Serine	4.17 (0.04)	4.20 (0.04)	4.25 (0.05)
Threonine	4.59 (0.03)	4.50 (0.05)	4.55 (0.04)
Tryptophan	1.35 (0.01)	1.32 (0.01)	1.33 (0.02)
Tyrosine	3.85 (0.04)	3.88 (0.05)	3.94 (0.06)
Valine	5.99 (0.06)	5.90 (0.05)	5.85 (0.04)

[1]Values are means (SEM); $n = 10$. Calculations were based on the molecular weights of AA residues in protein. Within each row, means do not differ among the 3 beef cuts ($P > 0.05$).

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

[3]Including cysteine plus one-half cystine.

Table 6. Amounts of free proteinogenic AA in beef cuts[1]

	Type of beef cuts[2]		
	Chuck	Round	Loin
Amino acids	μg/g dry weight		
Alanine	860 (30)[c]	1,031 (44)[b]	1,248 (49)[a]
Arginine	110 (3.8)[c]	142 (7.2)[b]	170 (8.0)[a]
Asparagine	153 (5.8)[c]	183 (5.2)[b]	206 (6.3)[a]
Aspartate	50 (2.1)[b]	51 (2.5)[b]	65 (2.7)[a]
Cysteine[3]	309 (8.0)[b]	365 (11)[a]	362 (13)[a]
Glutamate	615 (17)[c]	723 (35)[b]	801 (20)[a]
Glutamine	5,661(163)[a]	4,028 (95)[c]	5,069 (103)[b]
Glycine	335 (12)[c]	405 (15)[b]	456 (16)[a]
Histidine	267 (10)[c]	301 (8.2)[b]	338 (11)[a]
4-Hydroxyproline	72 (1.4)	72 (1.6)	73 (1.7)
Isoleucine	172 (8.7)[c]	203 (7.4)[b]	236 (8.0)[a]
Leucine	302 (12)[c]	357 (11)[b]	398 (12)[a]
Lysine	289 (11)[c]	318 (8.5)[b]	349 (10)[a]
Methionine	128 (4.6)[c]	159 (6.5)[b]	180 (5.3)[a]
Phenylalanine	297 (7.8)[b]	346 (8.3)[a]	352 (9.0)[a]
Proline	485 (12)[b]	543 (10)[a]	535 (13)[a]
Serine	288 (9.2)[c]	325 (9.5)[b]	365 (10)[a]
Threonine	302 (10)[b]	348 (8.0)[b]	389 (11)[a]
Tryptophan	49 (1.2)[b]	65 (1.3)[a]	67 (2.3)[a]
Tyrosine	205 (8.8)[b]	237 (10)[a]	244 (8.4)[a]
Valine	218 (7.5)[c]	243 (6.5)[b]	278 (8.0)[a]
Total	11,669 (207)[a]	10,445 (187)[b]	12,198 (230)[a]

[a–c]Within a row, means sharing different superscript letters differ ($P < 0.05$).

[1]Values are means (SEM); $n = 10$. Calculations were based on the molecular weights of intact AA.

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

[3]Including cysteine plus one-half cystine.

0.05) in under blade roasts than those in top round and top loin steaks but did not differ ($P > 0.05$) between top round and top loin steaks. Amounts of β-alanine and ornithine were highest ($P < 0.05$) in top loin steaks, intermediate ($P < 0.05$) in top rounds steaks, and lowest ($P < 0.05$) in under blade roasts. Amounts of citrulline did not differ ($P > 0.05$) among the 3 types of cuts.

Amounts of Small Peptides in Beef Cuts

Data on amounts of small peptides (anserine, carnosine, and glutathione) are summarized in Table 7. Carnosine was the most abundant small peptide, followed by anserine and glutathione in descending order. Amounts of glutathione in under blade roasts were lower ($P < 0.05$) than those in top round and top loin steaks, but glutathione content did not differ ($P > 0.05$) between top round and top loin steaks. Amounts of anserine and carnosine were the highest ($P < 0.05$) in top loin steaks, intermediate ($P < 0.05$) in top round steaks, and lowest ($P < 0.05$) in under blade roasts. The ratios of carnosine to anserine were 5.45, 6.58, and 6.61 (g/g) in under blade roasts, top round steaks, and top loin steaks, respectively.

DISCUSSION

Lean beef is an important source of dietary AA for children and adults to sustain adequate protein nutrition and health (Ma et al., 1961; Holden et al., 1986; Wu et al., 2014a). Therefore, sufficient knowledge about the composition of free and protein-bound AA

Table 7. Amounts of small peptides and free nonproteinogenic AA in beef cuts[1]

	Type of beef cuts[2]		
Amino acids	Chuck	Round	Loin
β-Alanine, μg/g dry weight	453 (10)[c]	615 (12)[b]	712 (18)[a]
Citrulline, μg/g dry weight	60 (2.8)	61 (2.9)	63 (2.2)
Ornithine, μg/g dry weight	137 (6.1)[c]	168 (5.3)[b]	195 (7.0)[a]
Taurine, mg/g dry weight	2.34 (0.06)[b]	2.78 (0.08)[a]	2.92 (0.09)[a]
Anserine, mg/g dry weight	2.79 (0.09)[c]	3.25 (0.10)[b]	3.66 (0.12)[a]
Carnosine, mg/g dry weight	15.2 (0.59)[c]	21.4 (0.86)[b]	24.2 (0.82)[a]
Glutathione, μg/g dry weight	675 (17)[b]	773 (20)[a]	792 (18)[a]

[a–c]Within a row, means sharing different superscript letters differ ($P < 0.05$).

[1]Values are means (SEM); $n = 10$. Calculations were based on the molecular weights of intact AA or intact small peptides.

[2]Chuck = under blade roasts; Loin = top loin steaks; Round = top round steaks.

Table 8. Comparison of published data on the composition of free AA and small peptides in beef meat

	Ala	Asp	Cys[1]	Glu	Gln	His	Tau[2]	β-Ala[3]	Car[4]	Ans[5]
Published studies	mg/100 g wet weight									
Franco et al. (2010)	20.1	2.91	5.42	14.9	–[6]	42.2	58.4	–	–	–
Imanari et al. (2010)	23.7	2.17	–	4.48	55.6	–	53.6	–	219	61.8
Iwamoto et al. (2010)	19.3	0.43	0.04	3.42	20.2	1.39	10.1	–	174	46.8
Mateescu et al. (2012)	–	–	–	–	–	–	–	–	372	67
Purchas et al. (2004)	–	–	–	–	–	–	38.6	–	453	–
Szterk and Roszko (2014)	21.5	0.23	3.59	–	35.2	1.83	31.7	–	14.5	–
Thornton et al. (2015)	–	–	–	–	–	9.5	–	–	8.9	8.5
Alexander and Elvehjem (1956)	–	–	–	–	–	–	80	80	–	–
Clifford (1922)	–	–	–	–	–	–	–	–	1,000	–
Present study	25.8 to 38.5	1.50 to 2.00	9.27 to 11.3	18.5 to 24.7	124 to 170	8.00 to 10.4	70.1 to 90.0	13.6 to 21.9	457 to 746	83.6 to 113

[1]Including cysteine plus one-half cystine.

[2]Tau = taurine.

[3]β-Ala = β-alanine.

[4]Car =carnosine.

[5]Ans = anserine.

[6]"–" denotes the lack of data.

as well as small peptides is essential to promote human consumption of this healthy food. However, despite studies of beef meat, data on nitrogenous nutrients in this animal product are incomplete and highly inconsistent (e.g., Gbeenhut et al., 1948; Purchas et al., 2004; Hall and Schönfeldt, 2013; Jensen et al., 2014; Tables 8 and 9). Given the exceedingly large variations in the reported values of AA, small peptides, and protein in beef, accurate data are necessary for nutritionists and medical professionals to make quantitative recommendation for its consumption by humans. To our knowledge, this is the first study to determine all proteinogenic AA as well as nutritionally and physiologically significant nonproteinogenic AA and small peptides in beef meat. The content of CP in our 3 beef cuts was similar to that reported by Underwood et al. (2010) and West et al. (2014) for lean beef.

Free AA (e.g., glutamate, β-alanine, and glycine) provide humans with "meaty" flavor and are important nutrients (San Gabriel and Uneyama, 2013). However, as previously noted, their significance has been grossly underestimated. For example, amounts of free cystine/cysteine in 100 g wet beef meat have been reported to be 0.04 (Iwamoto et al., 2010) or 8.6 mg (Franco et al., 2010) and amounts of free histidine in 100 g wet beef meat have been reported to be 1.6 (Iwamoto et al., 2010) or 38 mg (Franco et al., 2010). In the absence of data on glutamine, Franco et al. (2010) concluded that histidine was the most abundant free AA in beef muscle, followed by alanine and glutamate in descending order. In contrast, we found that concentrations of free glutamine, alanine, and glutamate in beef cuts were 10 to 20, 3.2 to 3.7, and 2.3 to 2.4 times those for free histidine (Table 6). Qualitatively similar results were obtained by Imanari et al. (2010). Based on wet weight (Table 1), concentrations of glutamine and glutamate in beef cuts are approximately 15 and 0.5 m*M*, respectively (Table 6). Such a high abundance of free glutamine plus glutamate in beef meat has not previously been recognized (e.g., Imanari et al., 2010; Iwamoto et al., 2010; Jensen et al., 2014; Szterk and Roszko, 2014). Whether the predominance of free glutamine in meat is unique to beef or whether the previously reported values of free glutamine in beef muscle were underestimated owing to possible analytical problems is not known at present. Nonetheless, high amounts of free glutamine plus glutamate in beef are consistent with their vital roles as major metabolic fuels, stimulators of protein synthesis, modulators of immune response, and regulators of epithelial integrity and function in the small intestine (Wu, 2009; Jiao et al., 2015; Wang et al., 2015).

A significant observation of this study is that taurine is the most abundant β-AA in beef meat, ranging from 2.3 to 2.9 mg/g dry weight (Table 7). These values are similar to those reported for bovine semitendinosus muscle (2.0 mg/g dry weight; Franco et al., 2010) and lean meat (2.7 and 2.9 mg/g dry weight; Alexander and Elvehjem, 1956; Imanari et al., 2010). Taurine plays essential roles in physiology, including serving as 1) a major antioxidant in the body, 2) a regulator of fluid homeostasis in cells and retinal photoreceptor activity, 3) a key component of the nerve conduction network, and 4) a modulator of the digestion and absorption of dietary fats and lipid-soluble vitamins (Jong et al., 2012). Because humans have a low ability to synthesize taurine at any stage of the life

Table 9. Comparison of published data on the percentages of proteinogenic AA in beef meat protein

	Published data by various authors					
	Franco et al. (2010)	Holló et al. (2001)	Nguyen and Zarkadas (1989)	Greenwood et al. (1951)	Alexander and Elvehjem (1956)	Present study[1]
Amino acids	g/100 g protein					
Alanine	6.55	6.68	5.23	–[2]	6.2	5.16–5.24
Arginine	7.10	6.07	6.58	6.72	6.7	6.80–6.87
Asp + Asn	10.5	8.91	9.33	8.80	10.0	9.31–9.44
Aspartate	–	–	–	–	–	5.17–5.20
Asparagine	–	–	–	–	–	4.14–4.23
Cysteine	1.02	1.01	0.99	1.40	0.9	1.42–1.46
Glu + Gln	16.7	16.6	15.5	14.9	15.8	15.2–15.4
Glutamate	–	–	–	–	–	9.52–9.59
Glutamine	–	–	–	–	–	5.72–5.82
Glycine	4.82	6.31	3.67	7.34	4.6	3.69–3.73
Histidine	1.70	4.06	4.04	3.16	3.5	3.05–3.09
4-Hydroxyproline	–	–	0.29	–	0.7	0.22–0.23
Isoleucine	6.96	4.30	5.28	5.24	5.7	5.18–5.24
Leucine	7.45	8.21	8.45	8.35	7.7	8.35–8.43
Lysine	9.67	9.07	9.38	8.52	8.7	9.19–9.23
Methionine	3.19	1.68	3.29	2.29	2.4	3.23–3.28
Phenylalanine	5.12	–	4.22	4.14	4.3	4.32–4.35
Proline	4.02	5.00	3.88	5.59	3.8	3.91–4.01
Serine	3.78	3.93	3.68	4.04	4.4	4.17–4.25
Threonine	4.83	4.71	4.66	4.02	4.4	4.50–4.59
Tyrosine	4.08	3.66	3.88	3.33	3.4	3.85–3.94
Valine	6.06	4.60	5.69	5.91	5.2	5.85–5.99

[1]Calculations were based on the molecular weights of AA residues in protein.

[2]"–" denotes the lack of data.

cycle, children and adults fed taurine-free diets are deficient in this AA (Laidlaw et al., 1988). Under stress or diseased conditions (e.g., heat stress, infection, obesity, diabetes, and cancer), taurine synthesis in the body is impaired due to hepatic dysfunction and the reduced availability of methionine. At present, dietary requirements of taurine have not been established for healthy adults and are probably approximately 75 mg/d based on its urinary excretion of 72 mg/d (Laidlaw et al., 1988). Based on our results (Table 7), 28.35 g (1 ounce) dried beef can provide 65 to 80 mg taurine, which can meet 87 to 107% of daily taurine requirement by adults. Note that taurine has antiobesity, antidiabetes, and anticancer effects (Schaffer et al., 2014) but is absent from plant-based foods (Jong et al., 2012). This further underscores the significance of beef as a major source of taurine for humans.

Beef contains antioxidant dipeptides (carnosine and anserine), their common precursor AA (β-alanine), and glutathione (Williams, 2007). However, there is considerate confusion about concentrations of these small peptides in meat. For example, amounts of carnosine in 100 g wet beef meat have been reported to be 14 mg (Szterk and Roszko, 2014) or 1 g (Clifford, 1922) and amounts of anserine in 100 g wet beef meat have been reported to be 8.5 (Thornton et al., 2015) or 67 mg (Mateescu et al., 2012). These large variations in nutrient composition may result, in part, from differences in either beef breeds or analytical techniques. To our knowledge, this is the first study to simultaneously quantify small peptides and β-alanine in beef cuts. The findings provide much-needed information on the content of these substances in meat. Specifically, we found that beef cuts contained high amounts of carnosine, anserine, glutathione, and β-alanine (Table 7). The ratios of carnosine to anserine (g/g) in our beef cuts were 5.4 to 6.6, which are similar to the value of 5.6 reported by Mateescu et al. (2012) but higher than other values of 3.6 (Imanari et al., 2010) and 3.7 (Iwamoto et al., 2010). Of particular interest, beef meat contained high amounts of β-alanine (453–712 μg/g dry weight; Table 7), which was not determined in most previous studies (e.g., Franco et al., 2010; Mateescu et al., 2012; Thornton et al., 2015) and was reported to be 2.7 mg/g dry weight (Alexander and Elvehjem, 1956). Carnosine acts as an intracellular proton buffer to mediate diverse physiological processes and on the hypothalamic suprachiasmatic nucleus to regulate the circadian clock (Nagai et al., 2012). Additionally, carnosine affects sympathetic and parasympathetic nerves innervating the adrenal glands, brown adipose tissue, kidneys, liver, pancreas, stomach, and white adipose tissue, therefore improving appetite, digestion, absorption, thermogenesis, and metabolic profiles (Hipkiss et al., 1997; Sale et al., 2013; Trexler et al., 2015). Likewise, anserine and glutathione function to scavenge oxidants, prevent protein glycation, and ameliorate oxidative stress (Wu et al., 2004; Hipkiss and Gaunitz, 2014). Importantly, these peptides can inhibit the growth of tumor cells through redox signaling while providing patients with nutritionally essential AA (Nagai et al., 2012; Hipkiss and Gaunitz, 2014).

Meat contains high amounts of protein and balanced proportions of all proteinogenic AA relative to human requirements (Dillon, 2013). For example, protein content in beef cuts ranges from 63 to 68% on a DM basis (Table 4). In contrast, most staple foods of plant origin (except for legumes) have a protein content of <12% (DM basis) and are deficient in most AA, including lysine, methionine, cysteine, tryptophan, threonine, and glycine (Young and Pellett, 1994; Wang et al., 2013). Disappointingly, concentrations of some individual AA in polypeptides (e.g., histidine and methionine) in animal-source foods, including beef meat (Table 9), previously have been underestimated, and the total amount of lysine in general meat was cited to be 4.89 g/100 g DM (Wu et al., 2014a). This value is 27 to 32% lower than that found for beef cuts (Table 2). Therefore, data from the present work support the dietary significance of red meat as an abundant source of all nutritionally essential AA and functional AA for humans (Wu, 2013).

The ratios of polypeptide-bound AA in our 3 types of beef cuts are generally similar to those reported by Alexander and Elvehjem (1956), Greenwood et al. (1951), and Nguyen and Zarkadas (1989) with the exception of histidine, methionine, cysteine, glycine, and hydroxyproline (Table 8). First, the high content of histidine in beef protein (4% [Nguyen and Zarkadas, 1989] and 4.1% [Holló et al., 2001]) may be explained by inclusion of carnosine-derived histidine in the calculations. Consistent with this suggestion, we obtained a similar value of 4.1% histidine in beef protein when carnosine was included as part of total AA. In contrast, the low content of histidine (1.7%) in beef protein reported by Franco et al. (2010) cannot be explained at present. It is unknown whether the recovery of histidine from protein hydrolysis was determined in the previous studies. Second, the higher content of glycine and proline in beef protein (7.3 and 5.6%, respectively; Greenwood et al., 1951) than our and others' values (Table 9) may result from a high percentage of collagen in their beef cuts. Third, amounts of polypeptide-bound methionine in protein (1.91%) reported by Holló et al. (2001) is only approximately 50% of the value from Nguyen and Zarkadas (1989) and the present study (Table 5). Fourth, our value of 4-hydroxyproline content in meat protein is similar to that reported by Nguyen and Zarkadas (1989) but was only approximately 32% of the value published by Alexander and Elvehjem (1956). Of note, all the previous studies failed to distinguish glutamate from glutamine or aspartate from asparagine in meat protein (Table 9). To our knowledge, this is the first study to quantify aspartate, asparagine, glutamate, and glutamine in meat proteins of any animal species (Table 9). Interestingly, glutamate and glutamine were the most and the fifth most abundant AA in proteins of beef cuts, respectively (Table 5). These data assume nutritional and physiological importance, as these 2 AA are now known to promote intestinal health, enhance muscle protein synthesis, and improve immunity in humans (Rhoads and Wu, 2009; Brosnan and Brosnan, 2013; Hou et al., 2015).

Based on the composition of nutritionally essential AA in beef (Table 2) vs. food grains (Millward, 1999), consumption of meat can substantially reduce the need for plant-based foods in human diets to satisfy AA requirements (Wu et al., 2014a,b). For example, to meet the Institute of Medicine–recommended dietary allowance of methionine plus cysteine by the 70-kg adult human (19 mg/kg BW per day; IOM, 2005), daily intake of meat, wheat flour, and white rice would be 39, 390, and 700 g DM, respectively (Wu et al., 2014a). The excessive amount of carbohydrates that would be consumed in the wheat flour or white rice can be converted into fat in the body, therefore possibly contributing to the development of obesity, dyslipidemia, and other metabolic disorders (Jobgen et al., 2006; Wu, 2013). Conversely, intake of lean meat, which contains <20.5% fat on a DM basis (Table 1), plays a key role in promoting protein synthesis and sustaining skeletal-muscle mass and function (including physical strength) while improving insulin sensitivity, ameliorating ageing-associated sarcopenia, and reducing white fat accretion (McKnight et al., 2010; Dillon, 2013; Riddle et al., 2016).

It is worth mentioning that under blade roasts, top round steaks, and top loin steaks had different composition of free and polypeptide-bound AA (Table 2) as well as nonproteinogenic AA and antioxidants peptides (Table 7). Specifically, amounts of total AA, β-alanine, anserine, and carnosine were highest in top loin steaks, intermediate in top round steaks, and lowest in under blade roasts. Likewise, differences in physical and chemical properties as well as total nitrogen and protein concentrations among these cuts have been reported by other researchers due to different types of muscle fibers in these meat cuts (Von Seggern et al., 2004; Franco et al., 2010; Schönfeldt et al., 2010; Hall and Schönfeldt, 2013). Overall, top loin steaks appear to provide higher protein-nutrition values than under blade roasts and top round steaks. Nonetheless, given the important role of animal protein intake on optimal human health (Wu, 2016), all beef cuts are excellent sources of AA and antioxidant peptides to meet nutritional and physiological needs of children and adults. For example, high amounts of leucine in beef meat can activate the mechanistic target of rapamycin signaling pathway to stimulate protein synthesis in human skeletal muscle and, therefore, ameliorate sarcopenia (Li et al., 2011a; Riddle et al., 2016). Additionally, an abundant supply of all AA from beef meat for syntheses of low-molecular-weight metabolites (e.g., neurotransmitters, vasodilators, signaling molecules, and mediators of immune response) plays a crucial role in maintaining physiological homeostasis in humans (Wu, 2013). Therefore, our findings are expected to renew interest in human consumption of lean beef for promoting optimal growth, development, health, and well-being in children and adults.

In conclusion, we determined, for the first time to our knowledge, the composition of all free proteinogenic and polypeptide-bound AA in beef meat. In under blade roasts, top round steaks, and top loin steaks, total amounts of glutamate (free plus peptide-bound) were the highest among all AA followed by lysine, leucine, arginine, and glutamine in descending order. Similar results were obtained for AA composition in meat protein. In all 3 beef cuts, glutamine was the most abundant free AA, followed by taurine, alanine, glutamate, and β-alanine. All the beef cuts had high concentrations of antioxidant peptides (anserine, carnosine, and glutathione). Although the 3 types of beef cuts moderately differ in nutrient composition, consumption of these foods by humans is expected to improve growth, development, and health. Our findings may have important implications for guiding the practice of human and animal nutrition.

LITERATURE CITED

Acheson, R. J., D. R. Woerner, J. N. Martin, K. E. Belk, T. E. Engle, T. R. Brown, J. C. Brooks, A. M. Luna, L. D. Thompson, H. L. Grimes, A. N. Arnold, J. W. Savell, K. B. Gehring, L. W. Douglass, J. C. Howe, K. Y. Patterson, J. M. Roseland, J. R. Williams, A. Cifelli, J. M. Leheska, and S. H. McNeill. 2015. Nutrient database improvement project: Separable components and proximate composition of raw and cooked retail cuts from the beef loin and round. Meat Sci. 110:236–244. doi:10.1016/j.meatsci.2015.06.001

Alexander, J. C., and C. A. Elvehjem. 1956. Isolation and identification of nitrogenous components in meat. J. Agric. Food Chem. 4:708–711. doi:10.1021/jf60066a008

Assaad, H., L. Zhou, R. J. Carroll, and G. Wu. 2014. Rapid publication-ready MS-Word tables for one-way ANOVA. SpringerPlus 3:474. doi:10.1186/2193-1801-3-474

Berge, P., J. Culioli, M. Renerre, C. Touraille, D. Micol, and Y. Geay. 1993. Effect of feed protein on carcass composition and meat quality in steers. Meat Sci. 35:79–92. doi:10.1016/0309-1740(93)90071-O

Brosnan, J. T., and M. E. Brosnan. 2013. Glutamate: A truly functional amino acid. Amino Acids 45:413–418. doi:10.1007/s00726-012-1280-4

Clifford, W. M. 1922. The effect of cold storage on the carnosine content of muscle. Biochem. J. 16:341–343. doi:10.1042/bj0160341

Dai, Z. L., Z. L. Wu, S. C. Jia, and G. Wu. 2014. Analysis of amino acid composition in proteins of animal tissues and foods as pre-column *o*-phthaldialdehyde derivatives by HPLC with fluorescence detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 964:116–127. doi:10.1016/j.jchromb.2014.03.025

Dillon, E. L. 2013. Nutritionally essential amino acids and metabolic signaling in aging. Amino Acids 45:431–441. doi:10.1007/s00726-012-1438-0

Franco, D., L. González, E. Bispo, P. Rodriguez, J. I. I. Garabal, and T. Moreno. 2010. Study of hydrolyzed protein composition, free amino acid, and taurine content in different muscles of Galician blonde beef. J. Muscle Foods 21:769–784. doi:10.1111/j.1745-4573.2010.00218.x

Garcia, L. G., K. L. Nicholson, T. W. Hoffman, T. E. Lawrence, D. S. Hale, D. B. Griffin, J. W. Savell, D. L. VanOverbeke, J. B. Morgan, K. E. Belk, T. G. Field, J. A. Scanga, J. D. Tatum, and G. C. Smith. 2008. National Beef Quality Audit -2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. J. Anim. Sci. 86:3533-3543. doi:10.2527/jas.2007-0782

Gbeenhut, I. T., E. J. Sibny, and C. A. Elvehjem. 1948. The lysine, methionine and threonine content of meats. J. Nutr. 35:689–701.

Greenwood, D. A., H. R. Kraybill, and B. S. Schweigert. 1951. Amino acid composition of fresh and cooked beef cuts. J. Biol. Chem. 193:23–28.

Hall, N. G., and H. C. Schönfeldt. 2013. Total nitrogen vs. amino-acid profile as indicator of protein content of beef. Food Chem. 140:608–612. doi:10.1016/j.foodchem.2012.08.046

Hipkiss, A. R., and F. Gaunitz. 2014. Inhibition of tumour cell growth by carnosine: Some possible mechanisms. Amino Acids 46:327–337. doi:10.1007/s00726-013-1627-5

Hipkiss, A. R., J. E. Preston, D. T. Himswoth, V. C. Worthington, and N. J. Abbot. 1997. Protective effects of carnosine against malondialdehyde-induced toxicity towards cultured rat brain endothelial cells. Neurosci. Lett. 238:135–138. doi:10.1016/S0304-3940(97)00873-2

Holden, J. M., E. Lanza and W. R. Wolf. 1986. Nutrient composition of retail ground beef. J. Agric. Food Chem. 34:302–308. doi:10.1021/jf00068a039

Holló, G., J. Csapó, E. Szűcs, J. Tőzsér, I. Repa, and I. Holló. 2001. Influence of breed, slaughter weight and gender on chemical composition of beef. Part 1. Amino acid profile and biological value of proteins. Asian-Australas. J. Anim. Sci. 14:1555–1559.

Hou, Y. Q., Y. L. Yin, and G. Wu. 2015. Dietary essentiality of "nutritionally nonessential amino acids" for animals and humans. Exp. Biol. Med. 240:997–1007. doi:10.1177/1535370215587913

Imanari, M., M. Higuchi, N. Shiba, and A. Watanabe. 2010. Accurate analysis of taurine, anserine, carnosine and free amino acids in a cattle muscle biopsy sample. Anim. Sci. J. 81:369–376. doi:10.1111/j.1740-0929.2010.00751.x

Institute of Medicine (IOM). 2005. Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Proteins, and Amino Acids, The National Academies Press, Washington, D.C. doi:10.17226/10490

Iwamoto, E., F. Iwaki, and A. Oka. 2010. Effects of dietary protein level in the early fattening period on free amino acids and dipeptides in the blood and Longissimus thoracis muscle in Japanese Black steers. Anim. Sci. J. 81:338–344. doi:10.1111/j.1740-0929.2010.00746.x

Jensen, I. J., J. Dort, and K. E. Eilertsen. 2014. Proximate composition, antihypertensive and antioxidative properties of the semimembranosus muscle from pork and beef after cooking and in vitro digestion. Meat Sci. 96:916–921. doi:10.1016/j.meatsci.2013.10.014

Jiao, N., Z. L. Wu, Y. Ji, B. Wang, Z. L. Dai, and G. Wu. 2015. L-Glutamate enhances barrier and anti-oxidative functions in intestinal porcine epithelial cells. J. Nutr. 145:2258–2264. doi:10.3945/jn.115.217661

Jobgen, W. S., S. K. Fried, W. J. Fu, C. J. Meininger, and G. Wu. 2006. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. J. Nutr. Biochem. 17:571-588. doi:10.1016/j.jnutbio.2005.12.001

Jong, C. J., J. Azuma, and S. Schaffer. 2012. Mechanisms underlying the antioxidant activity of taurine: Prevention of mitochondrial oxidant production. Amino Acids 42:2223–2232. doi:10.1007/s00726-011-0962-7

Laidlaw, S. A., T. D. Shultz, J. T. Cecchino, and J. D. Kopple. 1988. Plasma and urine taurine levels in vegans. Am. J. Clin. Nutr. 47:660–663.

Lassala, A., F. W. Bazer, T. A. Cudd, S. Datta, D. H. Keisler, M. C. Satterfield, T. E. Spencer, and G. Wu. 2010. Parenteral administration of L-arginine prevents fetal growth restriction in undernourished ewes. J. Nutr. 140:1242–1248. doi:10.3945/jn.110.125658

Li, F. N., Y. L. Yin, B. E. Tan, X. F. Kong, and G. Wu. 2011a. Leucine nutrition in animals and humans: MTOR signaling and beyond. Amino Acids 41:1185–1193. doi:10.1007/s00726-011-0983-2

Li, X. L., R. Rezaei, P. Li, and G. Wu. 2011b. Composition of amino acids in feed ingredients for animal diets. Amino Acids 40:1159–1168. doi:10.1007/s00726-010-0740-y

Ma, R. M., M. B. Matlack, and R. L. Hinder. 1961. A study of the free amino acids in bovine muscles. J. Food Sci. 26:485–491. doi:10.1111/j.1365-2621.1961.tb00394.x

Mateescu, R. G., A. J. Garmyn, M. A. O'Neil, R. G. Tait Jr., A. Abuzaid, M. S. Mayes, D. J. Garrick, A. L. Van Eenennaam, D. L. VanOverbeke, G. G. Hilton, D. C. Beitz, and J. M. Reecy. 2012. Genetic parameters for carnitine, creatine, creatinine, carnosine, and anserine concentration in longissimus muscle and their association with palatability traits in Angus cattle. J. Anim. Sci. 90:4248–4255. doi:10.2527/jas.2011-5077

McKnight, J. R., M. C. Satterfield, W. S. Jobgen, S. B. Smith, T. E. Spencer, C. J. Meininger, C. J. McNeal, and G. Wu. 2010. Beneficial effects of L-arginine on reducing obesity: Potential mechanisms and important implications for human health. Amino Acids 39:349–357. doi:10.1007/s00726-010-0598-z

Millward, D. J. 1999. The nutritional value of plant-based diets in relation to human amino acid and protein requirements. Proc. Nutr. Soc. 58:249–260. doi:10.1017/S0029665199000348

Nagai, K., M. Tanida, A. Niijima, N. Tsuruoka, Y. Kiso, Y. Horii, J. Shen, and N. Okumura. 2012. Role of L-carnosine in the control of blood glucose, blood pressure, thermogenesis, and lipolysis by autonomic nerves in rats: Involvement of the circadian clock and histamine. Amino Acids 43:97–109. doi:10.1007/s00726-012-1251-9

Nguyen, Q., and C. G. Zarkadas. 1989. Comparison of the amino acid composition and connective tissue protein contents of selected bovine skeletal muscles. J. Agric. Food Chem. 37:1279–1286. doi:10.1021/jf00089a017

Purchas, R. W., S. M. Rutherfurd, P. D. Pearce, R. Vather, and B. H. P. Wilkinson. 2004. Concentrations in beef and lamb of taurine, carnosine, coenzyme Q_{10}, and creatine. Meat Sci. 66:629–637. doi:10.1016/S0309-1740(03)00181-5

Rhoads, J. M., and G. Wu. 2009. Glutamine, arginine, and leucine signaling in the intestine. Amino Acids 37:111–122. doi:10.1007/s00726-008-0225-4

Riddle, E. S., M. H. Stipanuk, and A. E. Thalacker-Mercer. 2016. Amino acids in healthy aging skeletal muscle. Front. Biosci. E8:326–350.

Sale, C., G. G. Artioli, B. Gualano, B. Saunders, R. M. Hobson, and R. C. Harris. 2013. Carnosine: From exercise performance to health. Amino Acids 44:1477–1491. doi:10.1007/s00726-013-1476-2

San Gabriel, A., and H. Uneyama. 2013. Amino acid sensing in the gastrointestinal tract. Amino Acids 45:451–461. doi:10.1007/s00726-012-1371-2

Schaffer, S. W., T. Ito, and J. Azuma. 2014. Clinical significance of taurine. Amino Acids 46:1–5. doi:10.1007/s00726-013-1632-8

Schönfeldt, H. C., R. T. Naudé, and E. Boshoff. 2010. Effect of age and cut on the nutritional content of South African beef. Meat Sci. 86:674–683. doi:10.1016/j.meatsci.2010.06.004

Szterk, A., and M. Roszko. 2014. Simultaneous determination of free amino acids, L-carnosinem purine, pyrimidine, and nucleosides in meat by liquid chromatography/single quadrupole mass spectrometry. J. Liquid Chromatogr. Relat. Technol. 37:664–680. do i:10.1080/10826076.2012.758136

Thornton, K. J., R. P. Richard, M. J. Colle, M. E. Doumit, M. J. de Veth, C. W. Hunt, and G. K. Murdoch. 2015. Effects of dietary potato by-product and rumen-protected histidine on growth, carcass characteristics and quality attributes of beef. Meat Sci. 107:64–74. doi:10.1016/j.meatsci.2015.04.009

Trexler, E. T., A. E. Smith-Ryan, J. R. Stout, J. R. Hoffman, C. D. Wilborn, C. Sale, R. B. Kreider, R. Jäger, C. P. Earnest, L. Bannock, B. Campbell, D. Kalman, T. N. Ziegenfuss, and J. Antonio. 2015. International society of sports nutrition position stand: Beta-alanine. J. Int. Soc. Sports Nutr. 13:30. doi:10.1186/s12970-015-0090-y

Underwood, K. R., J. F. Tong, P. L. Price, A. J. Roberts, E. E. Grings, B. W. Hess, W. J. Means, and M. Du. 2010. Nutrition during mid to late gestation affects growth, adipose tissue deposition, and tenderness in cross-bred beef steers. Meat Sci. 86:588–593. doi:10.1016/j.meatsci.2010.04.008

USDA. 2012, Livestock, dairy, and poultry outlook (multiple reports from 2006 to 2012). Economic Research Service, United States Department of Agriculture, Washington, DC.

Von Seggern, D. D., C. R. Calkins, D. D. Johnson, J. E. Brickler, and B. L. Gwartney. 2004. Muscle profiling: Characterizing the muscles of the beef chuck and round. Meat Sci. 66:629–637.

Wang, B., G. Wu, Z. G. Zhou, Z. L. Dai, Y. L. Sun, C. Zhang, N. Jiao, Y. Ji, W. Li, W. W. Wang, and Z. L. Wu. 2015. Glutamine and intestinal barrier function. Amino Acids 47:2143–2154. doi:10.1007/s00726-014-1773-4

Wang, W. W., Z. L. Dai, Z. L. Wu, G. Lin, S. C. Jia, S. D. Hu, S. Dahanayaka, and G. Wu. 2014. Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs. Amino Acids 46:2037–2045. doi:10.1007/s00726-014-1758-3

Wang, W. W., Z. L. Wu, Z. L. Dai, Y. Yang, J. J. Wang, and G. Wu. 2013. Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids 45:463-477. doi:10.1007/s00726-013-1493-1

West, S. E., K. B. Harris, A. N. Haneklaus, J. W. Savell, L. D. Thompson, J. C. Brooks, J. K. Pool, A. M. Luna, T. E. Engle, J. S. Schutz, D. R. Woerner, S. L. Arcibeque, K. E. Belk, L. Douglass, J. M. Leheska, S. McNeill, J. C. Howe, J. M. Holden, M. Duvall, and K. Patterson. 2014. Nutrient database improvement project: The influence of USDA quality and yield grade on the separable components and proximate composition of raw and cooked retail cuts from the beef chuck. Meat Sci. 97:558–567. doi:10.1016/j.meatsci.2014.01.018

Williams, P. G. 2007. Nutritional composition of red meat. Nutr. Diet. 64(Suppl. 4):S113–S119. doi:10.1111/j.1747-0080.2007.00197.x

Wu, G. 2009. Amino acids: Metabolism, functions, and nutrition. Amino Acids 37:1–17. doi:10.1007/s00726-009-0269-0

Wu, G. 2013. Amino acids: Biochemistry and nutrition, CRC Press, Boca Raton, FL. doi:10.1201/b14661

Wu, G. 2016. Dietary protein intake and human health. Food Funct. 7:1251–1265. doi:10.1039/C5FO01530H

Wu, G., F. W. Bazer, and H. R. Cross. 2014a. Land-based production of animal protein: Impacts, efficiency, and sustainability. Ann. N. Y. Acad. Sci. 1328:18–28. doi:10.1111/nyas.12566

Wu, G., P. K. Davis, N. E. Flynn, D. A. Knabe, and J. T. Davidson. 1997. Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. J. Nutr. 127:2342–2349.

Wu, G., Y. Z. Fang, S. Yang, J. R. Lupton, and N. D. Turner. 2004. Glutathione metabolism and its implications for health. J. Nutr. 134:489-492.

Wu, G., J. Fanzo, D. D. Miller, P. Pingali, M. Post, J. L. Steiner, and A. E. Thalacker-Mercer. 2014b. Production and supply of high-quality food protein for human consumption: Sustainability, challenges and innovations. Ann. N. Y. Acad. Sci. 1321:1–19. doi:10.1111/nyas.12500

Wu, G., and D. A. Knabe. 1994. Free and protein-bound amino acids in sow's colostrum and milk. J. Nutr. 124:415–424.

Wu, G., and C. J. Meininger. 2008. Analysis of citrulline, arginine, and methylarginines using high-performance liquid chromatography. Methods Enzymol. 440:177–189. doi:10.1016/S0076-6879(07)00810-5

Wu, G., T. L. Ott, D. A. Knabe, and F. W. Bazer. 1999. Amino acid composition of the fetal pig. J. Nutr. 129:1031–1038.

Young, V. R., and P. L. Pellett. 1994. Plant proteins in relation to human protein and amino acid nutrition. Am. J. Clin. Nutr. 59(Suppl. 5):1203S–1212S.