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In vitro digestibility of commercial whey protein supplements

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ABSTRACT

Amongst the sport dietary supplements, those manufactured with whey protein (WP) represent an important amino acid source. However, as a result of product lack of uniformity, the nutritional quality of this type of product is uncertain. Thus, the aim of this study was to investigate the protein quality of WP supplements produced by U.S. (WP-USA), and Brazilian companies (WP-BRA), evaluating the *in vitro* protein digestibility, and the essential amino acid (EEA) composition. In addition, the amino acid (AAS) and protein digestibility-corrected amino acid (PDCAAS) scores were calculated. Although WP-USA supplement exhibited greater ($P < 0.05$) digestibility than WP-BRA counterparts, both WP supplements exhibited greater ($P < 0.05$) digestibility than soy and caseinate isolate supplements, which were used as reference. Considering the WHO/FAO/UNU protein standard for non-athletic adult, the WP-USA and WP-BRA supplements scored high AAS. In addition, the PDCAAS values on both supplement groups were >1.0, with exception of threonine and valine in WP-USA, and isoleucine and leucine in WP-BRA. However, when the calculated AAS and PDCAAS based on the suggestion for adult athletes were considered, both supplements exhibited suboptimal score values for several EAA. In addition, both WP-USA and WP-BRA supplements were unable to supply the suggested adult athlete EAA requirement.

1. Introduction

Protein supplements are one of the most widely consumed supplements by athletes and physically active individuals (Phillips, 2012). Amongst the ingredients used to manufacture this type of product (i.e. caseinates, whey, egg, soy, and wheat proteins), whey protein (WP) is the most commercialized in the sports nutrition market due to its high nutritional value when compared to other proteins sources (Ha & Zemel, 2003; Khanam, Kumkum, & Swamylingappa, 2013). WP represents 20–30% of the proteins present in bovine milk; it is a complex mixture of globular protein molecules consisting mostly of α-lactalbumin (α-La), β-lactoglobulin (β-Lg) (Urista, Fernández, Rodriguez, Cuenca, & Téllez, 2011). The protein fractions α-La and β-Lg (variants A and B) represent almost 70% of the proteins present in whey (Walstra, Wouters, & Geurts, 2006).

Differences in the physical–chemical composition of WP supplements potentially influence its nutritional effect on the human body (Manninen, 2009). The nutritional quality of WP supplement depends on amino acid composition, bioavailability of essential amino acids, protein digestibility, and physiological utilization of specific amino acids after digestion and absorption (Lemon, Berardi, & Noreen, 2002). Whey protein is considered an important source of essential amino acids (EAA), of which the branched-chain amino acids (BCAA) leucine, isoleucine and valine have been associated with increased stimulus of skeletal muscle protein synthesis (Rankin & Darragh, 2006).

Moreover, protein digestibility is an important factor to estimate the protein availability for intestinal absorption after digestion reflecting on the efficiency of protein utilization on diet (FAO/WHO/UNU, 2007). The *in vitro* protein digestibility (IVPD) assay is a widely used method to determine the digestibility parameter. The IVPD mimics conditions simulated by the digestive processes occurring in the human gastrointestinal tract through proteolytic enzymes (i.e. pepsin-pancreatin enzyme system or papain system), measuring the percentage of proteins which is hydrolyzed by such enzymes (Hur, Jin, Lim, Decker, & Julian, 2011). This method is

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faster, more affordable, and equally effective than *in vivo* assays (Pires, Vieira, José, & Neuza, 2006).

The AAS does not consider whether the protein is digestible or not (Mokrane et al., 2010). Therefore, protein digestibility-corrected amino acid score (PDCAAS) is a recognized and approved method for evaluating protein quality taking into account the AAS and the digestibility parameter of the food matrix. This parameter derives from the AAS, and is corrected based on the digestibility assay of the protein (FAO/WHO/UNU, 2007).

Several reports investigated the technological properties of whey protein as ingredients into a wide variety of products (Bhushan & Etzel, 2009; Urista et al., 2011; Youn-Ho & Lawrence, 2002) such as, infant formula (Jost, Maire, Maynard, & Secretin, 1999; Lönnerdal, 2014; Shuang, Zhang, Ming-Ming, Huang, & Yi-Ping, 2014); in addition, studies were already undertaken to correlate the intake of whey protein supplements with physical performance (Hoffman, Tranchina, Rashti, Kang, & Faigenbaum, 2008; Hulmi, Christopher, & Jeffrey, 2010; Uchida, Bacurau, Aoki, & Bacurau, 2008). However, there is limited information regarding the WP supplement protein quality. In this context, the purpose of this study was to investigate the protein quality of commercial WP supplements produced by U.S. and Brazilian companies based on *in vitro* protein digestibility (IVPD) assay, EAA, AAS and PDCAAS.

2. Material and methods

2.1. Sampling

All samples (WP, soy protein, and caseinate isolate powder) used in the present study were acquired from a commercial retailer specialized on nutritional supplements. Fifteen WP supplements manufactured at different countries were investigated, eight from USA companies (WP-USA), and seven from Brazilian companies (WP-BRA). In addition, supplements manufactured with soy protein and caseinate isolate powder were used as references.

2.2. In vitro protein digestibility (IVPD) assay

The *in vitro* protein digestibility was evaluated based on method described by Akeson and Stahmann (1964), with modifications. Briefly, aliquots of 250 mg of each sample or 250 μL of deionized water (for the blank) were suspended in 15 mL of 0.1 mol equi/L HCl containing 1.5 mg/mL pepsin (Sigma®, St. Louis, MO, USA), and incubated for 3 h at 37 °C in a water bath. The pepsin hydrolysis ceased after neutralization with the addition of 7.5 mL of 0.5 mol equi/L of NaOH. Then, the pancreatic digestion initiated with the addition of 10 mL of 0.2 mol/L phosphate buffer (pH 8.0) containing 10 mg of pancreatin (Sigma®, St. Louis, MO, USA) with 1 mL of 0.005 mol/L sodium azide to prevent microbial growth, and were incubated at 37 °C overnight. After the pancreatic hydrolysis, 1 mL of 10 g/100 mL of trichloroacetic acid was added, followed centrifugation at 503 × *g* for 20 min. The supernatant was collected, and the total protein content was estimated based on the nitrogen content using Kjeldahl AOAC method 930.29 (AOAC, 2012), For comparative purpose, supplements manufactured with soy protein and caseinate isolate powder were used as references. The IVPD values were calculated according to the equation:

$$\%\ \text{Digestibility} = (\text{Ns} - \text{Nb})/\text{Ns} \times 100$$

where Ns and Nb represent the nitrogen content in the sample and in the blank, respectively.

2.3. Determination of essential amino acids (EAA) content

The essential amino acids (histidine, threonine, methionine, valine, phenylalanine, isoleucine, leucine and lysine) content were analyzed by high-performance liquid chromatography, using method described by Alvares et al. (2012). Briefly, 50 μL of sample previously diluted according to the manufacturer's recommendation, was mixed with 50 μL of 1.5 mol/L perchloric acid (v/v). After 2 min at room temperature, 1.125 mL of ultrapure water and 25 μL of 2 mol/L potassium carbonate were added. The tubes were centrifuged at 10,000 × g for 1 min, and then, 100 μL of the supernatant was diluted with 100 μL of 1.2 g/100 mL benzoic acid and 1.4 mL of ultrapure water. The amino acids were identified using a pre-column derivation with o-ophthaldialdehyde (Sigma®, St. Louis, MO, USA). The HPLC instrument was equipped with a quaternary pump (LC-20AD, Shimadzu Corporation, Japan), a 5 μm reverse-phase C18 column (4.6 mm ID × 150 mm from Supelco®, Bellefonte, PA, USA) guarded by a 5 μm reverse-phase C18 guard column Ascentis® (4.0 mm ID × 20 mm from Sigma®, Bellefonte, PA, USA), and a fluorescence detector (RF-10AXL, Shimadzu Corporation, Japan) monitoring excitation and emission wavelengths at 340 nm and 455 nm, respectively. The samples were separated by mobile phase gradient using 0.1 mol/L sodium acetate (pH 7.2), and methanol at 1.1 mL/min flow. The total running time per sample was 49 min and the column temperature was kept at room temperature.

2.4. Amino acid score (AAS) and protein digestibility-corrected amino acid score (PDCAAS)

The AAS was calculated by dividing each individual amino acid content by their respective reference value, considering the daily amino acid requirement for non-athletic individuals (Joint WHO/FAO/UNU Expert Consultation, 2007) and physically active adults (Jeukendrup & Gleeson, 2009). The PDCAAS were calculated by multiplying the most limiting AAS value of each essential amino acid by the protein digestibility.

2.5. Statistical analysis

Eight different WP supplements manufactured in the USA (WP-USA), and seven different ones manufactured in Brazil (WP-BRA) were used in this study. Seven distinct samples were used for each manufacturer ($n = 7$). Differences on EAA, AAS and PDCAAS values between WP-USA and WP-BRA were evaluated using Student's *t* test. In addition, the difference on IVPD amongst WP supplements

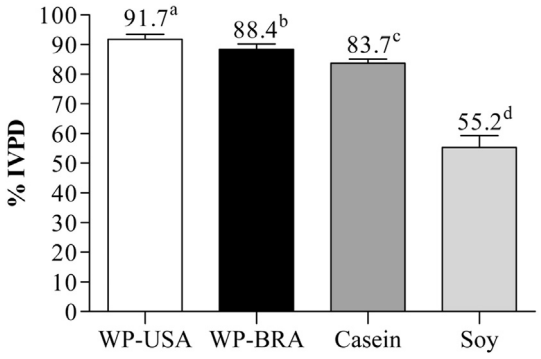

Fig. 1. The *in vitro* protein digestibility values (%) of WP-USA, WP-BRA, soy isolate and caseinate isolate supplements. [a–d] Different letters denote difference at 95% of confidence level ($P < 0.05$) (n = 7). WP-USA = whey protein supplements produced by USA companies; WP-BRA = whey protein supplements produced by Brazilian companies.

(USA and BRA) and supplements manufactured with soy protein and caseinate isolate powders were investigated using ANOVA followed by Tukey's test. All statistical analyses were performed using Graphpad 5 Prism (La Jolla, CA, USA) considering 95% of confidence level. The results were expressed as mean ± standard error.

3. Results and discussion

WP-USA supplements exhibited greater IVPD ($P < 0.05$) than WP-BRA supplements (Fig. 1). To the best of our knowledge there is lack of information regarding the digestibility of WP supplements. Nevertheless, this parameter is an important factor to determine the nutritional quality of these products (FAO/WHO/UNU, 2007). The variation on IVPD values is potentially explained by differences on the overall composition of such supplements, particularly with respect to the protein quality (Eriksen et al., 2010; Sindayikengera & Wen-shui, 2006). In addition, differences on protein quality is possibly related to processing conditions, including farm practices, period of lactation, whey extraction method, method of purification (membrane filtration vs ion exchange), and thermal processing (Onwulata, Konstance, & Tomasula, 2004; Walstra et al., 2006). In this study the total protein values ranged from 61.2 ± 2.41 g/100 g to 79.5 ± 2.03 g/100 g (WP-USA) and 48.1 ± 1.77 g/100 g to 75.2 ± 2.43 g/100 g (WP-BRA). There was a significant difference in protein content between the WP-USA and WP-BRA supplements (72.83 ± 5.8 g/100 g vs 63.36 ± 8.4 g/100 g). This difference may be related to the supply of amino acids offered by these products.

Protein modification plays an important role on food quality and its attributes. Modifications such as amino acid side chain oxidation; protein–protein cross-linking and backbone cleavage can negatively influence product properties including a decrease in nutritional value, digestibility, functionality, and health claims (Kerwin & Remmele, 2007).

Amongst the WP supplements available the most widely consumed ones are formulated using whey protein concentrate (WPC), whey protein isolate (WPI), or a blend of concentrate and isolate. The difference between WPC and WPI depends on the processing condition during the protein purification step (Maughan, 2013; Urista et al., 2011), which affects the protein content; WPC and WPI usually contain 35–80 g/100 g, and ≥90 g/100 g of protein content, respectively (Carunchia, Croissant, & Drake, 2005).

During the manufacture of WPC and WPI, the thermal processing potentially negatively affect protein quality, biological availability of amino acids, and digestibility (Rufián-Henares, Andrade, Jiménez-Pérez, & Morales, 2007). Thus, according to Lacroix et al. (2008) the greater the processing temperature the greater is the negative impact on protein digestibility.

Furthermore, the IVPD value of soy protein powders (55.2 ± 4.0) and caseinate isolate powder (83.73 ± 1.3) were lower ($P < 0.05$; Fig. 1) than WP-USA and WP-BRA supplements. Different proteins sources promote distinct positive effects for both protein supplementation, and athletic performance (Hoffman & Falvo, 2004). The consumption of different proteins sources stimulates different anabolic responses depending on the tissue type (Phillips, 2011; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). Tang et al. (2009) investigated the influence of isonitrogenous quantities of soy, casein, and whey protein on the stimulation of muscle protein synthesis, and concluded that the ingestion of whey protein promoted greater increase on blood essential and branched-chain amino acids content than either casein or soy. This effect may be related to how quickly whey proteins are digested (Hall, Millward, Long, & Morgan, 2003).

The IVPD values reported on this study are in agreement with those documented by Mokrane et al. (2010) and Sindayikengera and Wen-shui (2006). Furthermore, Pires et al. (2006) evaluated the influence of protein source on the protein digestibility, and reported that soy protein exhibited the lowest protein digestibility when compared to casein and whey proteins. This observation may be attributed to the fact that vegetable sources contain anti-nutritional factors that form a more complex protein structure, which may decrease protein digestibility (Butts, Monro, & Moughan, 2012; Lowery, Edel, & McBride, 2012; Pires et al., 2006). In terms of casein proteins, although they exhibit high biological value, the caseins undergo less digested and absorbed than whey proteins (Sindayikengera et al., 2006; Wilson & Wilson, 2006) because of coagulation of caseins at the acidic pH of the digestive tract (Marcus et al., 2010).

Essential amino acid content, AAS and PDCAAS of WP-USA and WP-BRA, calculated based on the reference values suggested by the Joint WHO/FAO/UNU Expert Consultation (2007), are exhibited on Table 1. In terms of individual essential amino acid contents, isoleucine, lysine, and valine exhibited similar ($P > 0.05$) values between WP-USA and WP-BRA. Leucine and phenylalanine were greater ($P < 0.05$) in WP-USA than their counterparts while, histidine, methionine and threonine were greater ($P < 0.05$) in WP-BRA than in WP-USA. Supplements manufactured by both USA and Brazilian companies received high scores for most of the essential amino acids investigated. However, WP-BRA supplements demonstrated leucine values lower than 1.0; essential amino acids exhibiting AAS below 1.0 were regarded as a limiting amino acid (Joint WHO/FAO/UNU Expert Consultation, 2007). Regarding the AAS values, both WP-USA and WP-BRA exhibited similar ($P > 0.05$) scores for isoleucine, lysine, and valine. In addition, leucine and phenylalanine were greater ($P < 0.05$) in WP-USA than their Brazilian counterparts, while histidine, methionine and threonine were greater ($P < 0.05$) in WP-BRA than in WP-USA. Based on the corrected digestibility calculation it was observed that the PDCAAS values were numerically lower than 1.0 for threonine and valine in WP-USA, and for isoleucine and leucine in WP-BRA. In addition, WP-BRA exhibited the lowest values for PDCAAS when compared to WP-USA due to the greater *in vitro* protein digestibility observed on WP-USA than WP-BRA. Therefore, WP-USA PDCAAS values demonstrated a smaller decrease when compared to their respective AAS values. This observation corroborates the usefulness of PDCAAS to better investigate the amino acidic quality of food products as the food matrix digestibility is considered (Mokrane et al., 2010).

International health agencies established the daily protein intake requirement based on non-athletic individuals as a recommendation for the general population (Joint WHO/FAO/UNU Expert Consultation, 2007). At cellular level, the increased requirement for protein input on strength-trained athletes reflects the biological adaptation to support muscle protein accretion stimulated by increased protein synthesis rather than protein catabolism; thus, adult athletes potentially require greater protein intake (up to 125% accretion) than non-athletic individuals (Lemon, 1997; Phillips, 2014). Therefore, to investigate if WP supplements meet suggested adult athlete requirements (Jeukendrup & Gleeson, 2009), a 2-fold adjustment was applied to the recommended values of the Joint WHO/FAO/UNU Expert Consultation (2007).

The AAS and PDCAAS values for WP-USA and WP-BRA were calculated based on the recommendation for adult athletes as exhibited in Table 2. Both supplements demonstrated AAS values lower than 1.0 for the majority of the essential amino acids evaluated. However, histidine, lysine, and phenylalanine values on WP-USA, and histidine, lysine, methionine, and phenylalanine values on WP-BRA were above the reference value. In terms of AAS values,

Table 1
Essential amino acids (EAA) content, amino acid score (AAS) and the protein digestibility-corrected amino acid score (PDCAAS) values calculated for WP-USA supplements and WP-BRA supplements.

EAA	Reference[a]	EAA content (mg kg^{-1})		AAS		PDCAAS	
		WP-USA	WP-BRA	WP-USA	WP-BRA	WP-USA	WP-BRA
His	10	17.4 ± 1.9*	24.1 ± 3.9	2.1 ± 0.4*	3.2 ± 0.7	1.9 ± 0.4*	2.7 ± 0.6
Ile	20	22.2 ± 3.0	20.7 ± 3.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
Leu	39	58.0 ± 5.0*	30.9 ± 6.3	1.6 ± 0.7*	0.9 ± 0.1	1.4 ± 0.7*	0.7 ± 0.1
Lys	30	119.4 ± 22.6	146.4 ± 44.2	4.3 ± 0.8	5.9 ± 1.0	3.9 ± 0.7	5.3 ± 0.9
Met	10	11.5 ± 0.9*	16.5 ± 2.2	1.2 ± 0.1*	1.9 ± 0.2	1.1 ± 0.09*	1.7 ± 0.2
Phe	25	53.4 ± 10.4*	41.6 ± 8.6	2.3 ± 0.4*	1.9 ± 0.3	2.1 ± 0.4*	1.6 ± 0.3
Thr	15	17.5 ± 1.7*	19.3 ± 1.4	1.0 ± 0.2*	1.7 ± 0.2	0.9 ± 0.3*	1.5 ± 0.3
Val	26	25.0 ± 5.2	25.7 ± 3.7	1.0 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	1.0 ± 0.1

The values are mean ± standard error ($n = 7$).
*Denotes difference between WP-USA a4nd WP-BRA ($P < 0.05$).
EAA = essential amino acid; AAS = amino acid score; PDCAAS = protein digestibility-corrected amino acid score; WP-USA = whey protein supplements produced by USA companies; WP-BRA = whey protein supplements produced by Brazilian companies.
[a] Reference values (mg kg^{-1}) for daily intake of amino acids for non-athletic adult (Joint WHO/FAO/UNU Expert Consultation, 2007).

leucine and phenylalanine values were greater ($P < 0.05$) in WP-USA than in WP-BRA, whereas histidine, methionine and threonine values were greater ($P < 0.05$) in WP-BRA than in WP-USA. As for PDCAAS values, only lysine and phenylalanine in WP-USA and histidine and lysine on WP-BRA demonstrated scores above the suggested values for adult athlete. Furthermore, WP-USA and WP-BRA supplements exhibited similar numeric values for isoleucine, lysine, and valine in both AAS and PDCAAS parameters.

The AAS is a ratio between the actual content of individual amino acids in food/diet and the recommended value of such amino acid (Joint WHO/FAO/UNU Expert Consultation, 2007). This parameter does not consider whether the protein is digestible or not (Mokrane et al., 2010); thus, PDCAAS was developed to adjust the AAS according to the food protein digestibility, and has been widely used to evaluate the protein quality of food products (Joint WHO/FAO/UNU Expert Consultation, 2007). According to Boye, Wijesinha-Bettoni, and Burlingame (2012), PDCAAS values greater than 1.0, should be considered equal to 1.0 as they fully meet the daily requirement. In the present study, the essential amino acids with PDCAAS values below 1.0, considering the suggested reference for non-athletic adult, were threonine and valine, and isoleucine and leucine, on WP-USA and on WP-BRA supplements, respectively. However, when the recommended PDCAAS values for adult athletes are considered, only lysine and threonine in WP-USA, and histidine and lysine on WP-BRA exhibited values higher than the recommended values. According to Phillips (2011), the high-quality protein products (PDCAAS > 1.0) contain increased levels of branched-chain amino acids. Several authors previously reported that the high content of BCAA, particularly leucine, are important stimulating factors for protein synthesis (Hoffman et al. 2008; Hulmi et al., 2010; Uchida et al., 2008). WP-USA supplements contained PDCAAS values of BCAA greater than the recommended for non-athletic adults, while on WP-BRA supplements only one BCAAs (valine) score was greater than 1.0. Nevertheless, considering the recommended values for adult athletes, neither WP-USA nor WP-BRA supplements reached the value. According to Judy and Ira (2001), the addition of specific amino acids such as BCAA, are frequently used to fortify protein supplements, which is used as an alternative to nullify or even reverse the negative effects of product processing on these essential amino acids contents.

Table 2
Amino acid (AAS) and protein digestibility-corrected amino acid scores (PDCAAS) scores of WP-USA and WP-BRA supplements calculated for adult athletes.

EAA	Reference[a]	AAS		PDCAAS	
		WP-USA	WP-BRA	WP-USA	WP-BRA
His	20	1.1 ± 0.2*	1.6 ± 0.3	0.9 ± 0.2*	1.4 ± 0.3
Ile	40	0.6 ± 0.07	0.6 ± 0.07	0.5 ± 0.06	0.4 ± 0.06
Leu	78	0.9 ± 0.3*	0.5 ± 0.08	0.8 ± 0.3*	0.4 ± 0.08
Lys	60	2.1 ± 0.4	3.4 ± 0.9	1.9 ± 0.3	3.0 ± 0.8
Met	20	0.6 ± 0.05*	1.0 ± 0.1	0.5 ± 0.04*	0.8 ± 0.1
Phe	50	1.2 ± 0.2*	1.0 ± 0.1	1.0 ± 0.2*	0.8 ± 0.1
Thr	30	0.6 ± 0.1*	0.8 ± 0.1	0.5 ± 0.1*	0.7 ± 0.1
Val	52	0.6 ± 0.1	0.6 ± 0.08	0.5 ± 0.1	0.5 ± 0.07

The values are mean ± standard error ($n = 7$).
*Denotes difference between WP-USA and WP-BRA ($P < 0.05$).
AAS = amino acid score; PDCAAS = protein digestibility-corrected amino acid; WP-USA = whey protein supplements manufactured by USA companies; WP-BRA = whey protein supplements manufactured by Brazilian companies.
[a] Reference values (mg kg^{-1}) for adult athlete suggested by Jeukendrup and Gleeson (2009) based on daily intake of amino acids for non-athletic adult (Joint WHO/FAO/UNU Expert Consultation, 2007).

4. Conclusion

In conclusion, WP-USA supplements exhibited better nutritional quality, based on *in vitro* digestibility than the WP-BRA counterparts. Considering the WHO/FAO/UNU protein standard for non-athletic adults, the WP-USA and WP-BRA supplements scored high AAS values. In addition, the PDCAAS values on both supplement groups were >1.0, with exception of threonine and valine in WP-USA, and isoleucine and leucine in WP-BRA. Nevertheless, when the AAS and PDCAAS were calculated based on the suggestion for adult athletes, both supplement groups exhibited suboptimal score values for several EAA. Based on these findings, whey protein manufactures should revise their processing techniques in order to optimize the protein quality of WP products.

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