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Research Article

Chemical score of different protein sources to four *Macrobrachium* species

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ABSTRACT. Food production for aquaculture requires finding other protein sources or ingredients as potential alternatives in the formulation of aquaculture feeds, due to the shortage and high price of protein sources that are most commonly used. The aim of this analysis was to evaluate the relationship between the essential amino acids in 13 types of proteins available in the market with the essential amino acids found in the muscle of four of the most important farmed prawn species of the genus *Macrobrachium* (*M. amazonicum*, *M. rosenbergii*, *M. americanum* and *M. tenellum*). The results obtained showed the limiting amino acids of each ingredient for each species, thereby allowing for formulation of commercial foods that meet the nutritional needs to support optimal growth of these prawns in culture. In conclusion, there is a difference in the amino acids most often present as first limiting between sources of animal and plant origin. Thereby, it is possible evaluate complementarities between these sources to achieve an amino acids profile close to that of *Macrobrachium* species.

Keywords: *Macrobrachium*, prawns, crustacean, amino acid, protein, nutrition.

Índice de aminoácidos esenciales de diferentes fuentes proteicas para cuatro especies de *Macrobrachium*

RESUMEN. La producción de alimentos para la acuicultura requiere la búsqueda de otras fuentes o ingredientes proteínicos como alternativas potenciales en la formulación de piensos para la acuicultura, debido a la escasez y alto precio de las fuentes de proteínas utilizadas con mayor frecuencia. El objetivo de este análisis fue evaluar la relación entre los aminoácidos esenciales de 13 tipos de proteínas disponibles en el mercado con aminoácidos esenciales presentes en el músculo de cuatro de las más importantes especies de langostinos cultivadas del género *Macrobrachium* (*M. amazonicum*, *M. rosenbergii*, *M. americanum* y *M. tenellum*). Los resultados obtenidos muestran los aminoácidos limitantes de cada ingrediente para cada especie, lo que permite la formulación de alimentos comerciales que satisfagan las necesidades nutricionales para apoyar el crecimiento óptimo de estos langostinos en cultivo. En conclusión, existe una diferencia entre los aminoácidos que con más frecuencia se presentan como primer limitante entre las fuentes de origen animal y vegetal. De este modo, es posible evaluar complementaciones entre estas fuentes para lograr un perfil de aminoácidos cercano al de las especies de *Macrobrachium*.

Palabras clave: *Macrobrachium,* langostinos, crustáceos, aminoácidos, proteínas, nutrición.

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INTRODUCTION

In recent decades, there has been an emphasis on using native species in aquaculture (Portella *et al*., 2013); however the commercial production of freshwater prawns remains an opportunity that has not been exploited in most countries of Latin America. Within native American species with aquaculture potential, the highlight is the genus *Macrobrachium*.

Feeding is vital for profitable prawn farming, as this may constitute 40 to 60% of production costs. The protein level in commercial feeds is one of the most important nutritional parameters (Vega-Villasante *et al*., 2011), and its optimal use allows proper feed utilization by cultured species and minimizes nutrient losses to the environment. Good handling of feeds prevents them from becoming a source of contaminants with adverse effects on the aquatic environment (Terrazas *et al*., 2010).

In nutrition, the biological value of a protein depends mainly on its composition of essential amino acids (EAA) (Espinosa-Chaurand *et al.*, 2013), knowing this composition, it is possible to predict, within certain limits, its performance in the animal organism (Suárez *et al*., 2006). Several studies have been performed using the amino acid profile of the muscle from animals to predict the animal's protein requirements (Guilherme *et al*., 2007; Yamasaki, 2012; Portella *et al*., 2013).

Studies have shown that crustaceans generally require the same 10 EAA: arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp) and valine (Val). However, virtually no research has been conducted on the essential amino acid requirements of prawn species of the genus *Macrobrachium*, except for *M. rosenbergii* (Roustaian *et al*., 2000). As a result, diets designed for native species of the entire genus *Macrobrachium* have been adapted based on *M. rosenbergii* or penaeid shrimp diets, with little empirical evidence in place.

To date, the formulated feed industry has not thoroughly relied on advances in biological/physiological aquaculture studies, have not been adopted by the formulated feed industries; this has promoted the use of empirical formulas that lack basic requirements to meet the demands of the cultured organisms (Ramírez *et al*., 2010). The source of animal protein commonly used in the feed industry is fish meal, therefore its high demand, low availability and high cost (Glencross *et al*., 2007; Guilherme *et al*., 2007; Espinosa-Chaurand *et al*., 2013).

Alternative sources for proteins have been used widely for mammals and poultry feedstuffs, but few data are available on their use for aquaculture species, data are lacking on the inclusion levels of these ingredients in feed, their impact on cost and their biochemical quality. Consequently, researches are needed to understand and evaluate the alternative sources of protein derived from agro industrial by-products or other organisms potential that will substitute fully or partially for fish meal (García-Galano *et al*., 2007; Glencross *et al*., 2007).

It is accepted that the amino acid profile of food proteins provides an important perspective of nutritional value, but imprecise and uncertain accuracy of analytical procedures and the hydrolysis method used can give variations in amino acid data for the regulation of protein quality. Also, because the information gap regarding the nutritional requirements of the prawn species of the genus *Macrobrachium* (except for *M. rosenbergii*), this review documents used the values reported in different studies published on the amino acid composition of four species of *Macrobrachium*, of different sizes obtained with different experimental circumstances, but were not compared. Therefore, the objective of this work was to analyze the proportion the composition of amino acids in the muscle of four species of prawn of the genus *Macrobrachium* with the amino acid content of ingredients that are used in formulated feeds. The three-fold purpose was to evaluate their amino acids scores, to establish the amino acids that limit the quality of these sources in feeds, and to suggest the possibility of complementation among protein sources.

MATERIALS AND METHODS

To conduct this analysis, four species of the genus *Macrobrachium* with known information about the amino acid composition of abdominal muscle were selected. The following amino acid compositions were used: *M. amazonicum* postlarvae reported by Portella *et al*. (2013) (obtained with an ion exchange amino acid analyzer); *M. rosenbergii* larvae (Roustaian *et al*., 2000) (obtained by high resolution liquid chromatography in reverse phase); juveniles of *M. rosenbergii* (Tidwell *et al*., 1998) and *M. americanum* (Yamasaki, 2012), and adults of *M. tenellum* (Espinosa *et al*., 2013) (obtained by high resolution liquid chromatography with fluorescence detection).

Thirteen protein sources (animal, vegetable and unicellular origin) were selected that are commonly used in the feed industry, or that could be considered as partial substitutes for ingredients normally used. Data from plant-based ingredients and egg were obtained from USDA food composition tables (2014). Protein sources were: sorghum meal (nutrient key number 20648), wheat meal (nutrient key number 20080), soybean meal (nutrient key number 16419), *Spirulina* (nutrient key number 11667), baking yeast (nutrient key number 18375) and whole egg dried (nutrient key number 1173). The data of ingredients of animal origin were obtained from data sheets published by the company Proteínas Marinas y Agropecuarias, S.A. de C.V. (http://www.protmagro.com/productos.htm): squid meal, shrimp meal, fish meal (I), standard poultry meal, pork meal and hydrolyzed feather meal. The composition of fish meal (II) was obtained from García-Galano *et al.* (2007), and the composition of *Artemia* nauplii from García-Ortega *et al*. (1998).

Chemical score (CS) calculations of protein were performed according to Block & Mitchell (1946) by dividing the content of EAA of the tested protein, between the amino acid content in the reference protein, using the amino acid composition of prawns muscles as reference protein. The lower ratio indicates the limiting amino acid of the protein source, and therefore its CS. Values less than 1 show the limiting amino acids, the lowest CS value is the first amino acid limiting of protein source.

RESULTS

The EAA compositions of the used ingredients are in Table 1. The estimated CS of the protein ingredients, taken as reference to the amino acid profile of *M. amazonicum* postlarvae (Tables 2, 3) shows that tryptophan is most frequently presented as the first and second limiting amino acid in the studied sources, followed by lysine; may be considered as the limiting amino acids of the protein quality of the evaluated ingredients for feeding this species.

For ingredients evaluated for larvae of *M. rosenbergii*, the limiting essential amino acids are aromatic amino acids (Trp and Phe) and histidine (Tables 2, 4).

In juveniles of *M. rosenbergii* and *M. americanum* (Tables 2, 5, 6) it is noted that basic amino acids (Arg, His and Lys) are most frequently presented as the first or second limiting amino acids of protein from any source, followed by methionine. Same as in *M. tenellum* (Tables 2, 7) basic amino acids, threonine and methionine are also presented as a first limiting amino acid in most evaluated sources.

DISCUSSION

For crustaceans, protein is essential for growth and development as it provides energy and is needed for the production of hormones, antibodies, enzymes and tissues (Bhavan *et al*., 2010).

Whole egg, *Artemia* and *Spirulina* show a closer profile to the amino acid requirements of *M. amazonicum* postlarvae (Table 3). In animal protein sources, the highlight is shrimp meal, which is consistent with that reported by García-Galano *et al.* (2007), who mentioned that the integration of shrimp meal to food for penaeid shrimp has a positive effect on growth due to its excellent profile of amino acids, in addition to its attractant power.

Of the ingredients evaluated for larvae of *M. rosenbergii* (Table 4), the only ingredient that covers all requirements of amino acids is the whole egg, followed by *Spirulina* and *Artemia*, which support their use in larval production.

As noted earlier, massive larval rearing of aquatic organisms, particularly shrimp, still depends on live food (microalgae and *Artemia nauplii*), but the high production costs, contamination risks in farms and variations of its nutritional value of live foods motivate a search for alternative artificial foods (including the use of dried seaweed, microparticulate diets, micro-encapsulated, yeast and different species of non-pathogenic bacteria) to replace partially or totally natural food (Jaime-Ceballos, 2006). In this sense, Santos-Gutiérrez *et al*. (2011) evaluated a semi wet product as a food supplement, formulated with chicken egg and fresh squid as protein sources and found that this experimental food can be used as a partial supplement of *Artemia nauplii* in the production of *M. rosenbergii* larvae. Also, Bhavan *et al*. (2010) determined the growth yield of *M. rosenbergii* postlarvae fed with enriched *Artemia* with *Spirulina* and yeast and found that the two ingredients produced favorable results, but *Spirulina* produced higher growth than yeast; therefore, both *Spirulina* and yeast can be used as supplements in feed management practices.

For juveniles of *M. rosenbergii* (Table 5), *Spirulina*, yeast and soybean meal resulted in the best quality for the species. Considering this, Prasad *et al*. (2013) suggested that foods added with 0.5% *Saccharomyces cerevisiae* yeast are suitable to promote growth of *M. rosenbergii* postlarvae. Similarly, Parmar *et al*. (2012) demonstrated that the incorporation of 1% brewer's yeast in the diet improve the immune response and control white muscle disease in *M. rosenbergii*. Also for this species, it is observed that the amino acid profiles of soybean meal fit better than fishmeal, which coincides with Hasanuzzaman *et al*. (2009), who reported higher weights and better protein efficiency ratio and feed conversion when 80% of fish meal was replaced by soybean meal in the diet of juveniles. Regarding the squid meal that showed low values of

Table 1. Profile of essential amino acid (EAA) of protein ingredients used (g amino acids per 100 g protein). *I Proteínas Marinas y Agropecuarias, S.A. de C.V; II García-Galano *et al.* (2007); III USDA; IV García-Ortega *et al.* (1998).

EAA	Squid meal I	Shrimp meal I	Fish meal I	Fish meal II	Poultry meal I	Feather meal I	Whole egg dried III	*Artemia* IV	Sorghum meal III	Wheat meal III	Soybean meal III	*Spirulina* dried III	Yeast III
Arg	4.66	6.80	3.73	6.5	5.83	6.51	40.48	6.80	3.91	4.91	7.09	7.22	5.02
His	1.43	2.30	1.53	3.3	2.17	1.70	16.79	2.50	1.98	2.70	2.46	1.89	2.25
Ile	2.60	6.30	3.64	4.5	1.29	4.12	42.03	4.70	3.67	3.35	4.43	5.58	4.67
Leu	4.77	6.80	4.69	7.2	3.80	7.12	62.73	6.50	12.87	6.80	7.44	8.61	7.22
Lys	4.42	9.30	5.17	7.2	5.61	2.10	50.60	7.30	2.06	2.72	6.08	5.26	8.11
Met	1.80	1.70	1.72	2.7	1.13	0.53	25.60	2.30	1.72	1.73	1.23	2.00	1.46
Phe	2.37	4.70	2.68	4.1	2.07	4.30	43.45	3.90	5.23	5.16	4.77	4.83	4.33
Thr	2.63	4.30	2.49	4.3	2.25	4.15	33.81	4.30	3.70	2.78	3.97	5.17	4.92
Trp	-	0.60	0.67	1	-	0.30	9.17	1.20	1.26	1.32	1.33	1.62	1.34
Val	2.83	6.90	3.26	5.3	2.01	5.50	47.38	4.90	4.59	4.27	4.56	6.11	5.71
% PC	73-75	49	58-60	61	60-64	80	81	56	8	15	47	57	40

Table 2. First and second limiting EAA of each protein ingredients evaluated for *Macrobrachium* species. *1 first limiting EAA, 2 second limiting EAA. - not limiting EAA.

Species		Sources												
		Animal origin								Vegetal origin			Unicellular	
		Squid meal	Shrimp meal	Fish meal I	Fish meal II	Poultry meal	Feather meal	Whole egg dried	*Artemia*	Sorghum meal	Wheat meal	Soybean meal	*Spirulina* dried	Yeast
Postlarvae	1	0.47 (Lys)	0.15 (Trp)	0.16 (Trp)	0.24 (Trp)	0.28 (Ile)	0.07 (Trp)	0.28 (Trp)	0.29 (Trp)	0.22 (Lys)	0.29 (Lys)	0.32 (Trp)	0.39 (Trp)	0.33 (Trp)
M. amazonicum	2	0.57 (His. Ile. Leu)	0.82 (Leu)	0.46 (Arg)	0.76 (Lys)	0.45 (Val)	0.22 (Lys)	0.73 (Arg)	0.77 (Lys)	0.31 (Trp)	0.32 (Trp)	0.64 (Lys)	0.55 (Lys)	0.62 (Arg)
Larvae	1	0.22 (Phe)	0.40 (Trp)	0.25 (Phe)	0.39 (Phe)	0.20 (Phe)	0.20 (Trp)	-	0.37 (Phe)	0.26 (Lys)	0.35 (Lys)	0.45 (Phe)	0.46 (Phe)	0.41 (Phe)
M. rosenbergii	2	0.41 (His)	0.44 (Phe)	0.44 (His)	0.67 (Trp)	0.26 (Ile)	0.24 (Met)	-	0.71 (His)	0.49 (Phe)	0.49 (Phe)	0.56 (Met)	0.54 (His)	0.64 (His)
Juvenile	1	0.38 (His)	0.61 (His)	0.41 (His)	0.82 (Arg)	0.30 (Ile)	0.20 (Met)			0.26 (Lys)	0.35 (Lys)	0.46 (Met)	0.50 (His)	0.55 (Met)
M. rosenbergii	2	0.55 (Phe)	0.64 (Met)	0.47 (Arg)	0.88 (His)	0.42 (Met)	0.27 (Lys)			0.50 (Arg)	0.62 (Arg)	0.66 (His)	0.67 (Lys)	0.60 (His)
Juvenile	1	0.55 (Arg)	0.72 (Met)	0.44 (Arg)	0.77 (Arg)	0.33 (Ile)	0.22 (Met)			0.30 (Lys)	0.39 (Lys)	0.52 (Met)	0.75 (Lys)	0.60 (Arg)
M. americanum	2	0.63 (Lys)	0.81 (Arg)	0.73 (Leu. Met)	-	0.48 (Met)	0.3 (Lys)			0.47 (Arg)	0.58 (Arg)	0.84 (Arg)	0.85 (Met)	0.62 (Met)
Adults	1	0.44 (Thr)	0.59 (Met)	0.41 (Thr)	0.71 (Thr)	0.32 (Ile)	0.18 (Met)			0.22 (Lys)	0.28 (Lys)	0.43 (Met)	0.55 (Lys)	0.51 (Met)
M. tenellum	2	0.46 (Lys)	0.71 (Thr)	0.52 (Arg)	0.75 (Lys)	0.37 (Thr)	0.22 (Lys)			0.55 (Arg)	0.46 (Thr)	0.63 (Lys)	0.69 (Met)	0.71 (Arg)

Table 3. Protein profile EAA (gamino acid per 100 g protein) of *M. amazonicum* postlarvae muscle (Portella *et al*., 2013) and chemical score of protein ingredients. *Limiting amino acid; 1 first limiting; 2 second limiting; 3 third limiting. I Proteínas Marinas y Agropecuarias, S.A. de C.V.; II García-Galano *et al.* (2007).

Macrobrachium amazonicum		Chemical score												
		Squid meal	Shrimp meal	Fish meal I	Fish meal II	Poultry meal	Feather meal	Whole egg dried	*Artemia*	Sorghum meal	Wheat meal	Soybean meal	*Spirulina* dried	Yeast
Arg	8.10	0.58*[3]	0.84*[3]	0.46*[2]	0.80*[3]	0.72	0.80	0.73*[2]	0.84	0.48*[3]	0.61*[3]	0.87*[3]	0.89	0.62*[2]
His	2.50	0.57*[2]	0.92	0.61	1.32	0.87	0.68	1.06	1.00	0.79	1.08	0.99	0.76*[3]	0.90
Ile	4.60	0.57*[2]	1.37	0.79	0.98	0.28*[1]	0.90	1.32	1.02	0.80	0.73	0.96	1.21	1.02
Leu	8.30	0.57*[2]	0.82*[2]	0.57	0.87	0.46*[3]	0.86	1.12	0.78*[3]	1.55	0.82	0.90	1.04	0.87
Lys	9.50	0.47*[1]	0.98	0.54*[3]	0.76*[2]	0.59	0.22*[2]	0.78*[3]	0.77*[2]	0.22*[1]	0.29*[1]	0.64*[2]	0.55*[2]	0.85*[3]
Met	1.00	1.80	1.70	1.72	2.70	1.13	0.53*[3]	3.66	2.30	1.72	1.73	1.23	2.00	1.46
Phe	3.90	0.61	1.21	0.69	1.05	0.53	1.10	1.61	1.00	1.34	1.32	1.22	1.24	1.11
Thr	4.10	0.64	1.05	0.61	1.05	0.55	1.01	1.00	1.05	0.90	0.68 *	0.97	1.26	1.20
Trp	1.20		0.15*[1]	0.16*[1]	0.24*[1]		0.07*[1]	0.28*[1]	0.29*[1]	0.31*[2]	0.32*[2]	0.32*[1]	0.39*[1]	0.33*[1]
Val	4.50	0.63	1.53	0.72	1.18	0.45*[2]	1.22	1.65	1.09	1.02	0.95	1.01	1.36	1.27

Table 4. Protein profile EAA (g amino acid per 100 g protein) of *M. rosenbergii* larvae muscle (Roustaian *et al*., 2000) and chemical score of protein ingredients. *Limiting amino acid; 1 first limiting; 2 second limiting; 3 third limiting. I Proteínas Marinas y Agropecuarias, S.A. de C.V; II García-Galano *et al.* (2007).

Macrobrachium rosenbergii		Chemical score												
		Squid meal	Shrimp meal	Fish meal I	Fish meal II	Poultry meal	Feather meal	Whole egg dried	*Artemia*	Sorghum meal	Wheat meal	Soybean meal	*Spirulina* dried	Yeast
Arg	7.50	0.62	0.91	0.50	0.87	0.78	0.87	5.40	0.91	0.52*[3]	0.65	0.94	0.96	0.67
His	3.50	0.41*[2]	0.66*[3]	0.44*[2]	0.94	0.62	0.49	4.80	0.71*[2]	0.57	0.77	0.70*[3]	0.54*[2]	0.64*[2]
Ile	4.90	0.53*[3]	1.29	0.74	0.92	0.26*[2]	0.84	8.58	0.96	0.75	0.68	0.90	1.14	0.95
Leu	8.70	0.55	0.78	0.54	0.83*[3]	0.44	0.82	7.21	0.75*[3]	1.48	0.78	0.86	0.99	0.83
Lys	7.80	0.57	1.19	0.66	0.92	0.72	0.27*[3]	6.49	0.94	0.26*[1]	0.35*[1]	0.78	0.67*[3]	1.04
Met	2.20	0.82	0.77	0.78	1.23	0.51	0.24*[2]	11.63	1.05	0.78	0.78	0.56*[2]	0.91	0.66*[3]
Phe	10.60	0.22*[1]	0.44*[2]	0.25*[1]	0.39*[1]	0.20*[1]	0.41	4.10	0.37*[1]	0.49*[2]	0.49*[2]	0.45*[1]	0.46*[1]	0.41*[1]
Thr	4.70	0.56	0.91	0.53	0.91	0.48	0.88	7.19	0.91	0.79	0.59*[3]	0.84	1.10	1.05
Trp	1.50		0.40*[1]	0.45*[3]	0.67*[2]		0.20*[1]	6.11	0.80	0.84	0.88	0.88	1.08	0.89
Val	4.70	0.60	1.47	0.69	1.13	0.43*[3]	1.17	10.08	1.04	0.98	0.91	0.97	1.30	1.22

Table 5. Protein profile EAA (g amino acid per 100 g protein) of *M. rosenbergii* juvenile muscle (Tidwell *et al*., 1998) and chemical score of protein ingredients. *Limiting amino acid; 1 first limiting; 2 second limiting; 3 third limiting. I Proteínas Marinas y Agropecuarias, S.A. de C.V; II García-Galano *et al.* (2007).

Macrobrachium rosenbergii		Chemical score										
		Squid meal	Shrimp meal	Fish meal[I]	Fish meal[II]	Poultry meal	Feather meal	Sorghum meal	Wheat meal	Soybean meal	*Spirulina* dried	Yeast
Arg	7.89	0.59*[3]	0.86	0.47*[2]	0.82*[1]	0.74	0.83	0.50*[2]	0.62*[2]	0.90	0.91	0.64*[3]
His	3.76	0.38*[1]	0.61*[1]	0.41*[1]	0.88*[2]	0.58	0.45	0.53*[3]	0.72	0.66*[2]	0.50*[1]	0.60*[2]
Ile	4.32	0.61	1.46	0.84	1.04	0.30*[1]	0.95	0.85	0.78	1.03	1.29	1.08
Leu	7.77	0.61	0.88	0.60*[3]	0.93	0.49	0.92	1.66	0.87	0.96	1.11	0.93
Lys	7.82	0.57	1.19	0.66	0.92	0.72	0.27*[2]	0.26*[1]	0.35*[1]	0.78*[3]	0.67*[2]	1.04
Met	2.66	0.68	0.64*[2]	0.65	1.02	0.42*[2]	0.20*[1]	0.65	0.65*[3]	0.46*[1]	0.75*[3]	0.55*[1]
Phe	4.32	0.55*[2]	1.09	0.62	0.95	0.48	1.00	1.21	1.20	1.10	1.12	1.00
Thr	3.98	0.66	1.08	0.63	1.08	0.57	1.04	0.93	0.70	1.00	1.30	1.24
Trp	0.93		0.65*[3]	0.72	1.08		0.32*[3]	1.35	1.42	1.43	1.74	1.44
Val	4.58	0.62	1.51	0.71	1.16	0.44*[3]	1.20	1.00	0.93	1.00	1.33	1.25

Table 6. Protein profile EAA (g amino acid per 100 g protein) of *M. americanum* juvenile muscle (Yamasaki, 2012) and chemical score of protein ingredients. *Limiting amino acid; 1 first limiting; 2 second limiting; 3 third limiting. I Proteínas Marinas y Agropecuarias, S.A. de C.V; II García-Galano *et al.* (2007).

Macrobrachium americanum		Chemical score										
		Squid meal	Shrimp meal	Fish meal[I]	Fish meal[II]	Poultry meal	Feather meal	Sorghum meal	Wheat meal	Soybean meal	*Spirulina* dried	Yeast
Arg	8.40	0.55*[1]	0.81*[2]	0.44*[1]	0.77*[1]	0.69	0.78*[3]	0.47*[2]	0.58*[2]	0.84*[2]	0.86*[3]	0.60*[1]
His	2.03	0.70	1.13	0.75	1.63	1.07	0.84	0.98	1.33	1.21	0.93	1.11
Ile	3.87	0.67	1.63	0.94	1.16	0.33*[1]	1.06	0.95	0.87	1.14	1.44	1.21
Leu	6.45	0.74	1.05	0.73*[2]	1.12	0.59	1.10	2.00	1.05	1.15	1.33	1.12
Lys	6.99	0.63*[2]	1.33	0.74*[3]	1.03	0.80	0.30*[2]	0.30*[1]	0.39*[1]	0.87*[3]	0.75*[1]	1.16
Met	2.36	0.76	0.72*[1]	0.73*[2]	1.14	0.48*[2]	0.22*[1]	0.73*[3]	0.73*[3]	0.52*[1]	0.85*[2]	0.62*[2]
Phe	3.47	0.68*[3]	1.35	0.77	1.18	0.60	1.24	1.51	1.49	1.37	1.39	1.25
Thr	3.17	0.83	1.36	0.78	1.36	0.71	1.31	1.17	0.88	1.25	1.63	1.55
Val	4.07	0.70	1.70	0.80	1.30	0.49*[3]	1.35	1.13	1.05	1.12	1.50	1.40

CS, our results are differ from those reported by Naik *et al*. (2001), who explained that squid meal could completely replace fishmeal without affecting growth for *M. rosenbergii* postlarvae.

The results for *M. americanum* juveniles (Table 6) show the similarity of the amino acid profile of this species with fish meal II, coinciding with EAA requirements for *Litopenaeus schmiti*, reported by Álvarez *et al*. (2004) and Fraga-Castro & Jaime-Ceballos (2011). These authors show that fishmeal in diets cover all EAA except arginine, meanwhile soybean meal satisfies more than half of the EAA, with methionine as first limiting. This suggests that soybean meal is an excellent protein for *M. americanum*, but it needs to be complemented with some meal of animal origin such as fish or shrimp to offset its amino acid deficiencies. It remains, necessary to establish an optimal level of inclusion.

In the case of *M. tenellum* (Table 7), soybean meal contains fewer EAA that do not fit the pattern of the muscle species, corresponding to the reported by García-Ulloa *et al.* (2008) in juvenile *M. tenellum* growth is not affected by replacing fishmeal by soybean meal up to 80%. For this reason, threonine is presented as first limiting and its incorporation is very important for diet design. This coincide with Espinosa-Chaurand *et al.* (2013) for this species, where fish meal presented threonine as the first limiting amino acid, and soybean meal presented methionine as first limiting. But our results differ from that reported for squid meal, which also found threonine as first limiting amino acid, meanwhile Espinosa-Chaurand *et al.* (2013) reported histidine as the first AA limited. Note that, they obtained values higher of CS for squid meal probably because, as discussed by Seligson & Mackey (1984), the calculation of CS and the prediction of the first limiting amino acid for a protein often differ because of the choice of data source and the reference pattern and may contradict data previously validated by bioassay.

Contrarily, fish meal provides high quality protein with a balance of amino acids and fatty acids suitable for the rapid growth of marine organisms (especially carnivores). The use of substitutes has not been as successful in aquatic animals as in land animals, so the availability and quality of fish meal are decisive for the manufacture of aquafeed quality (Cruz-Suárez *et al*., 2000). Although, fish meal is the main source of protein in aquaculture feed, its quality can vary greatly due to manufacture methods and the species of fish used as raw material, among other variables that may affect the content and quality of fish meal (García-Galano *et al*., 2007). Probably, these variables affecting the quality of the same type of ingredient, can be the reason for the differences in CS found between the fish meal I

Table 7. Protein profile EAA (g amino acid per 100 g protein) of *M. tenellum* adults muscle (Espinosa *et al*., 2013) and chemical score of protein ingredients. *Limiting amino acid; 1 first limiting; 2 second limiting; 3 third limiting. I Proteínas Marinas y Agropecuarias, S.A. de C.V; II García-Galano *et al.* (2007).

Macrobrachium		Chemical score									
tenellum	Squid meal	Shrimp meal	Fish meal I	Fish meal II	Poultry meal	Feather meal	Sorghum flour	Wheat flour	Soy meal	*Spirulina* dried	Yeast
Arg 7.11	0.66	0.96	0.52*[2]	0.91	0.82	0.92	0.55*[2]	0.69	1.00	1.01	0.71*[2]
His 2.52	0.57	0.91	0.61	1.31	0.86	0.67	0.79	1.07	0.98	0.75*[3]	0.89
Ile 4.04	0.64	1.56	0.90	1.11	0.32*[1]	1.02	0.91	0.83	1.10	1.38	1.16
Leu 8.08	0.59	0.84*[3]	0.58	0.89*[3]	0.47	0.88	1.59	0.84	0.92	1.07	0.89
Lys 9.60	0.46*[2]	0.97	0.54*[3]	0.75*[2]	0.58	0.22*[2]	0.22*[1]	0.28*[1]	0.63*[2]	0.55*[1]	0.84
Met 2.88	0.63	0.59*[1]	0.60	0.94	0.39*[3]	0.18*[1]	0.60*[3]	0.60*[3]	0.43*[1]	0.69*[2]	0.51*[1]
Phe 4.39	0.54*[3]	1.07	0.61	0.93	0.47	0.98	1.19	1.18	1.09	1.10	0.99
Thr 6.03	0.44*[1]	0.71*[2]	0.41*[1]	0.71*[1]	0.37*[2]	0.69	0.61	0.46*[2]	0.66*[3]	0.86	0.82*[3]
Trp 0.47		1.28	1.43	2.13		0.64*[3]	2.68	2.80	2.82	3.44	2.84
Val 3.94	0.72	1.75	0.83	1.35	0.51	1.40	1.17	1.08	1.16	1.55	1.45

(sardine fishmeal, <60% crude protein, CP) and fish meal II (tuna fishmeal, >60% CP); similarly, the result of low CS for squid meal may have occurred based on its manufacture and source quality even though squid meal is considered an excellent source of protein that competes with fish meal in its applications for feed manufacturing.

Although poultry by-product meals evaluated in the present work showed low values of CS, these meals are considered a good source of amino acids. Additionally, their cholesterol content is important in the formulation of shrimp feed (Cruz-Suárez *et al*., 2007). In this aspect, Yang *et al*. (2004) studied the potential use of renderers' meals (poultry, and meat and bone) as alternative sources of dietary protein, and they found that both could replace up to 50% protein fish meal in diets for *M. nipponense*.

According to our results obtained with the vegetable meals, soybean meal satisfies more than half of the EAA in juvenile prawns of *M. rosenbergii*, *M. americanum* and *M. tenellum*. Additionally, methionine appears as first limiting, coinciding as the limiting amino acid, which has been reported for *Litopenaeus schmitti* by Álvarez *et al*. (2004). According to those authors, soybean meal should only be included in diets in low quantities, due to the deficiency in amino acids, the high presence of anti-nutrients, low digestibility and it induces poor atractability and palatability of feed, which together resulted in lower growth for some species. Previous information does not agree with those reported for juveniles of *M. rosenbergii*, for which it is possible to replace 80% of fishmeal by soybean meal (Hasanuzzaman *et al*., 2009). Despite these good results with soybean meal, there are other waste and by-products from agriculture, livestock and industries that have good nutritional value, low cost and can be easily processed and recycled as aquaculture feed ingredients. One better alternative for feeding prawns is the use of earthworm meal substituting fish and soybean meals as tested by Langer *et al*. (2011), in *M. dayanum*.

On the other hand, among other alternative protein sources are those obtained from unicellular origin such as yeast that presents methionine and arginine as the first limiting amino acids for *M. rosenbergii* juveniles and *M. americanum*, however this meal cover more than half its limiting amino acids. This source is considered an excellent source for shrimp nutrition as it is a good source of protein, essential amino acids, vitamin B complex, folic acid, and a suitable composition of minerals. Additionally, the high content of nucleic acids make them an important source of nucleotide, and β-glucans of the cell wall, and have proven immunostimulatory effects on aquatic species (García-Galano *et al*., 2007). These nutritional properties make it an alternative source of protein to replace fish meal (Bhavan *et al*., 2010), and it has been licensed for use as probiotics in animal feed.

Likewise, *Spirulina* covers more than half of the limiting amino acids for the species studied and presented lysine as the limiting amino acid in all of them. *Spirulina* is considered a rich source of protein, vitamins, minerals, amino acids, fatty acids and antioxidant pigments (Bhavan *et al*., 2010). Thus Jaime-Ceballos (2006), reported that *Spirulina* meal possesses suitable nutritional features for use in aquatic animal feed, and improved attractability. Based on results of this work, it is possible to suggest for *M. amazonicum* postlarvae and *M. rosenbergii* larvae a feed formulation that includes the use of *Artemia* enriched with *Spirulina* and supplemente with some product, which includes whole egg. In the case of *M. rosenbergii* and *M. americanum* juveniles, and *M. tenellum* adults, a feed is suggested that contains soybean meal, fish meal of high quality, shrimp meal, with a supplementary content of *Spirulina* and yeast.

There is a difference in the amino acids most often present as first limiting between sources of animal and plant origin. Based on these differences, it is possible to evaluate complementarities between these sources to achieve an amino acids profile close to that of *Macrobrachium* species. However, CS of a protein only reflects the content ratio between EAA in the evaluated protein and the amino acid content in the reference protein, without taking in account other quality parameters such as digestibility. Therefore, the use of protein digestibility in a corrected amino acid score (PDCAAS), which includes the digestibility of amino acids, would be a more complete approach to the nutritional value of these sources.

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