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Degradation of amino acids by leaching in feeds for shrimp

João Paulo de Sousa Prado[1]; José Marcelino Oliveira Cavalheiro[1]; Thiago Brandão Cavalheiro[1] & Fernanda Vanessa Gomes da Silva[1]

[1]Universidade Federal da Paraíba/UFPB- Campus I-s/n – Cidade Universitária- 58051-110, João Pessoa-PB - Brasil. E-mail: jp_prado@hotmail.com. Autor para correspondência.

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Abstract – The feed for shrimp are one of the most expensive in the aquaculture sector, mainly because this type of feed should have high stability in water. This study aimed to evaluate the stability of amino acids in commercial feeds with different protein contents intended for larval and juvenile shrimp subjected to leaching. The feed samples were exposed to the leaching process during the time period of 04, 08 and 12 hours. The analyses of degradation of amino acids were performed using an elution gradient in HPLC system. In all diets evaluated it was found that lysine and histidine are essential amino acids which suffered less degradation processes. It's important to mention that arginine is considered an important amino acid for growth of shrimp. In both feeds with 35% protein (RA35 and RB35) the losses of arginine were 79 and 89% respectively. The results obtained in this study indicate that the leaching process significantly reduced the content of amino acids in the feeds. The physical structure of the feed doesn't prevent the degradation process of amino acids in the leaching process.

Key Words: *Litopenaeus vannamei, Nutritional quality, Stability.*

Degradação de aminoácidos por lixiviação em rações para camarão

Resumo – As rações para camarão estão entre as mais caras do setor de aquicultura principalmente porque esse tipo de ração deve ter alta estabilidade na água. Este trabalho teve, como objetivo, avaliar a estabilidade de aminoácidos em rações comerciais com diferentes teores proteicos, destinados a camarões na fase larval e juvenil, submetidas à lixiviação. As amostras de ração foram expostas ao processo lixiviatório durante período de tempo de 04, 08 e 12 horas. As análises de degradação de aminoácidos foram realizadas utilizando-se um sistema de HPLC, em modo de gradiente de eluição. Destacadamente, em todas as rações avaliadas, observou-se que a lisina e histidina, foram os aminoácidos essenciais que sofreram menor processo degradativo. É importante ressaltar que a arginina é considerado um aminoácido importante para o crescimento de camarões, e, em ambas as rações com 35% de proteína - RA35 e RB35 -, as perdas deste aminoácido foram de 79% e 89% respectivamente. Os resultados obtidos nesta pesquisa indicam que o processo de lixiviação diminui consideravelmente o conteúdo de aminoácidos das rações. No processo de lixiviação a estrutura física da ração não impede o processo degradação dos aminoácidos.

Palavras-Chave: *Litopenaeus vannamei, Qualidade nutricional, Estabilidade.*

La degradación de los aminoácidos por lixiviación en la alimentación de camarones

Resumen – Las raciones para el cultivo de camarón se encuentran entre los más caros en el sector de la acuicultura, principalmente porque debe tener una alta estabilidad en el agua. Este trabajo tuvo como objetivo evaluar la estabilidad de los aminoácidos en las dietas comerciales con diferentes niveles de proteína, para los camarones en la etapa de larvas y juveniles, sometidos a lixiviación. Las muestras de alimento fueron expuestos al proceso de lixiviatório durante el período de tiempo de 04, 08 y 12 horas. Los análisis de degradación de aminoácidos se realizaron utilizando un sistema de HPLC, el modo de gradiente de elución. En particular, para todas las dietas probadas, se observó que la lisina e histidina son aminoácidos esenciales que se han sometido a menos proceso de degradación. Es importante tener en cuenta que la arginina se considera un aminoácido importante para el crecimiento de camarón, y ambas dietas con 35% de proteína - RA35 y RB35 - la pérdida de este aminoácido eran 79% y 89%, respectivamente. Los resultados obtenidos en este estudio indican que el proceso de lixiviación reduce considerablemente el contenido de alimentos para animales de aminoácidos. En el proceso de lixiviación de la estructura física de la alimentación no se opone a la degradación del proceso de aminoácidos.

Palavras Clave: *Litopenaeus vannamei, Calidad nutricional, La estabilidad.*

Introduction

The feed for shrimp are one of the most expensive in the aquaculture industry, mainly because this type of feed should have high stability in water. The shrimp finds its feed exclusively by smell and taste and not by sight. Unlike fish, shrimp require minutes or hours to locate the feed after it has been distributed in the nurseries. During immersion the pellets lost nutrients and additives that attract shrimp by permanent leaching. After locating the feed the shrimp mince it to be able to ingest it's externally through their small mouths. This results in additional losses by leaching shortly before ingestion (Chamberlain, 2004).

Providing a balanced diet in post-larvae and juveniles phases is a major strategy for the production of healthy shrimp, especially when this has micronutrients that are important components of enzymes that act on the whole body and immune system of shrimp (ABCC, 2005). Leaching might reduce the quality of water in a cultivation system and may also reduce the growth, feed conversion ratio and survival of cultured animals (Obaldo et al. 2002).

The amino acid leaching in water has been evaluated in several studies (Lopez-Alvarado et al. 1994; Baskerville-Bridges & Kling, 2000; Yufera et al. 2002; Onal & Langdon, 2004), these researchers observed that leaching can be extensive and there are major differences between types of food and between different components.

The manufacture of feeds for shrimp presents unique challenges. Shrimp feed must be stable after immersion in water of the nurseries, but must be capable to release compounds attractive to ensure rapid ingestion by shrimp (Hertrampf, 2007). This study aimed to evaluate the stability of amino acids in commercial feeds with different protein contents subject to leaching.

Material and methods

The study was performed at the Laboratory of Fishery Product Development and Flavor Laboratory of the Department of Food Engineering, Campus I, Universidade Federal da Paraíba (UFPB), João Pessoa, Paraíba.

The samples used for this experiment were commercial feeds with protein contents of 35 and 40% with the following characteristics: Extruded feed and subsequently converted into the form of pellets with 1.0 to 1.8 mm diameter with 40% protein (40A feed); extruded feed and subsequently converted into the form of pellets with a diameter of 2.38 mm, with 35% protein (35A feed); extruded feed and subsequently transformed into the form of pellets with 1 to 1.7 mm in diameter, with 40% protein (40B feed) ; extruded feed and subsequently converted into the form of pellets with 2.0 to 2.5 mm in diameter, with 35% (35 B feed).

Subsequently the bigger particles size samples were crushed with use of the cutting mill and the smaller particle size samples were ground by hand using grade and pistil, after grinding the samples were sieved on 200 mesh sieves. Then, the samples were separated for carrying out the chromatographic profiles (control) and leaching process.

To evaluate leaching process of the samples, 60 g of each feed was weighed and placed into plastic containers containing 5000 ml of water of the nurseries over a period of 04, 08 and 12 hours with mild constant stirring. Then, the drain of the water was made with sifter and the pre drying was performed using paper. The feed were left to dry at room temperature. The

separation of the material for analysis of its amino acid composition was made for evaluation of nutrient loss. The amino acid profile was determined in triplicate samples at periods of 4, 8, 12 hours.

The analyses of degradation of amino acids were performed using the methodology used by White et al. (1986) using an elution gradient in HPLC system to the determination of amino acids. The mobile phases employed consisted of mobile phase A: sodium acetate buffer (0.014 M) and mobile phase B: Acetonitrile: Water 60/40. The sample injection (20µL) was performed manually and the detection was 245nm.

The chromatographic separation was performed with an elution gradient at a temperature of 35 º C. Samples equivalent to approximately 400µg of protein were weighed into Pyrex tubes with Teflon screw cap, which was previously washed with 6N HCl solution with deionized water and dried. Adding to the Pyrex tube with a Teflon cap 300µL of a solution of 6N HCl containing 1% phenol, the contents of the tubes were thoroughly rapidly inflated with N2 and sealed with a screw cap. The sealed tubes were placed in an oven at 110 C for 24h ^{o} for hydrolysis. After hydrolysis and cooling of the tubes it was added 20µL of a mixture of methanol: water: triethylamine (2:2:1), the drying and homogenization of the material was performed for 20 minutes. Derivatization of the hydrolyzed was made by mixing methanol: water: triethylamine: PITC (7:1:1:1), the material was homogenized for more 20 minutes and was subsequently dried for 20 minutes. The samples were resuspended in the mobile phase and then injected into HPLC-Varian model 1690 detector with diode drag, Column C18 Waters -x150mm 3.9, and reading at 245nm 5µm.

For the identification of the chromatographic peaks it was used the comparative of the retention times obtained with standards of amino acids (Sigma-AAS-18) under the same chromatographic conditions and the absorption spectra obtained in drag diode detector (DAD). The quantification was performed by external standard.

The results of the analysis in triplicate were statistically analyzed by analysis of variance (ANOVA) and Tukey's test applied between the means at 5% probability using the SPSS version 14.0 (SPSS Inc., 2001) according to Marocco (2007).

Results and discussion

In tables 1, 2, 3 and 4 it can be observed the mean values and percent degradation of amino acids of feed RA35, RB35, RB40 and RA40 at time T0 (control) and after 4, 8 and 12 hours of leaching. According to the analysis results, shown in table 1, there was significant degradation of amino acids in the very first four hours of leaching, with the exception of histidine. It was observed that the highest percentage of degradation at the end of 12 hours of leaching for aspartic acid with 94% of loss. The lowest degradation occurred for histidine at 33% followed by proline with 56% loss.

Table 1 - Mean values and percentage of amino acid degradation of the commercial diet with 35% protein (RA35) subjected to leaching.

Amino acids (mg/100g)	**T0**	**T4**	**T8**	**T12**	**%***
Isoleucine	1,93^{a}±0,11	0,65^{b}±0,03	0,44^{c}±0,04	0,36^{d}±0,03	81
Leucine	3,23^{a}±0,19	1,26^{b}±0,12	0,91^{c}±0,02	0,73^{d}±0,01	77
Arginine	1,96^{a}±0,15	1,26^{b}±0,04	0,53^{c}±0,00	0,42^{d}±0,02	79
Valine	2,16^{a}±0,14	0,72^{b}±0,03	0,45^{c}±0,01	0,34^{d}±0,03	84
Methionine	1,13^{a}±0,02	0,25^{b}±0,01	0,25^{b}±0,01	0,15^{c}±0,01	87
Lysine	4,22^{a}±0,12	2,46^{b}±0,15	2,25^{c}±0,02	1,58^{d}±0,14	63
Phenylalanine	2,23^{a}±0,13	0,78^{b}±0,03	0,34^{c}±0,03	0,32^{c}±0,02	86
Aspartic Acid	6,94^{a}±0,16	1,21^{b}±0,12	0,64^{c}±0,06	0,44^{d}±0,02	94
Glutamic Acid	2,24^{a}±0,15	1,25^{b}±0,12	0,75^{c}±0,02	0,55^{d}±0,02	75
Proline	0,09^{a}±0,00	0,07^{b}±0,00	0,05^{c}±0,00	0,04^{c}±0,00	56
Serine	2,11^{a}±0,10	0,64^{b}±0,02	0,35^{c}±0,01	0,27^{d}±0,03	89
Glycine	3,04^{a}±0,11	0,88^{b}±0,04	0,51^{c}±0,00	0,43^{d}±0,04	86
Threonine	2,25^{a}±0,14	0,56^{b}±0,03	0,44^{b}±0,12	0,32^{b}±0,03	86
Tyrosine	1,35^{a}±0,14	0,44^{b}±0,03	0,41^{b}±0,02	0,24^{c}±0,02	83
Histidine	0,67^{a}±0,05	0,62^{a}±0,04	0,53^{b}±0,05	0,45^{b}±0,04	33
Alanine	0,32^{a}±0,01	0,13^{b}±0,01	0,11^{c}±0,00	0,08^{d}±0,00	75
%Total degradation					77

Different letters in the same row indicate significant differences by Tukey test (α = 95%).
*% Of amino acid leached in 12 hours.

Seven of the nine essential amino acids evaluated had significant losses at the end of 12 hours of leaching with losses above 75%. The lysine lost 63% and histidine was the less degraded with 33% until the end of the process. Lopez-Alvarado & Kanazawa, 1993 have shown that diets containing free crystalline amino acids can lose up to 80% of their amino acids in the first minutes after exposure to the water of the nurseries.

Lysine is an amino acid considered essential for normal growth of the shrimp, which has been proven in several studies (Cowey & Forster, 1971; Fox et al. 1995; Kanazawa & Teshima, 1981; Millamena et al. 1998; Palma et al. 2009; Richard et al. 2010; Shewbart et al. 1972; Teshima et al. 2002). Lysine is usually the most limiting amino acid in the ingredients used to prepare the fish and shrimp feeds, particularly in diets formulated with high levels of vegetable protein (Forster & Ogata, 1998; Harris, 1980; Small & Soares, 2000) or protein ingredients under severe processing conditions (NRC, 2011).

According to Table 2, although the feed (R35B) evaluated has similar characteristics with the feed R35A and the same protein percentage all amino acids had losses of over 60% at the end of leaching and in the first 4 hours of leaching all amino acids had significant degradation, including histidine in feed RA35 that remained stable during the first 4 hours.

Some nutritional deficiencies of essential amino acids have been attributed to lower growth and lower feed efficiency (Wilson, 2002). It's important to mention that arginine is considered an important amino acid for growth of shrimp and both feeds with 35% protein (RB35 and RA35) had losses of 79 and 89% respectively of it.

The amino acid composition of the protein present in the tail of *Litopenaeus vannamei*(Lim, 1993) revealed that the muscle of the tail of the shrimp is particularly rich in arginine and this is the reason why so many diets are limiting in this amino acid. This has been researched many times in experiments on clean water. The amino acid composition of protein present in the tail muscle is a good indicator for the formulation of a feed balanced in terms of amino acid composition corresponding to the amino acid requirement for shrimp. Some authors that evaluate the essential amino acid composition in experimental diets found that arginine is considered the first limiting amino acid, with lysine after it; its demand was examined using free amino acids or intact sources (Fox et al., 1995).

Arginine has been shown to be an essential amino acid for shrimp due to its weak urea activity cycle, which is essential for normal growth of shrimp (Alam et al. 2004; NRC, 2011). Arginine is considered the most limiting amino acid in feeds for Penaeidos shrimps (Millamena et al. 1998). It is also a precursor for the synthesis of creatine and nitric oxide serves as a potent stimulant of insulin and growth hormone, so that it may play an important role in anabolic processes and is involved in the metabolism of nitrogen, creatine and synthesis polyamines and is one of the main substrates for the production of nitric oxide (NRC, 2011; Wan et al., 2006).

According to the analysis results shown in Table 3, lower losses at the end of leaching for 12 hours were observed in the diet (RA40) with 40% protein. Regarding the essential amino acids, in the phenylalanine was evaluated further degradation of the amino acid with 80%, followed by leucine, valine and arginine with losses above 50%.

Table 2 - Mean values and percentage of amino acid degradation in commercial feed B with 35% protein (RB35) subjected to leaching.

Amino acids (mg/100g)	**T0**	**T4**	**T8**	**T12**	**%***
Isoleucine	1,55^{a}±0,07	0,54^{b}±0,09	0,33^{c}±0,06	0,33^{c}±0,07	79
Leucine	3,34^{a}±0,01	0,96^{b}±0,07	0,75^{c}±0,05	0,61^{d}±0,03	82
Arginine	3,13^{a}±0,19	0,76^{b}±0,06	0,51^{c}±0,03	0,35^{d}±0,00	89
Valine	1,95^{a}±0,18	0,65^{b}±0,01	0,38^{c}±0,01	0,34^{d}±0,00	83
Methionine	0,84^{a}±0,01	0,33^{b}±0,03	0,17^{c}±0,02	0,16^{c}±0,02	81
Lysine	6,18^{a}±0,18	2,53^{b}±0,13	1,82^{c}±0,11	1,46^{d}±0,01	76
Phenylalanine	2,05^{a}±0,01	0,51^{b}±0,04	0,33^{c}±0,05	0,22^{d}±0,04	89
Aspartic Acid	3,11^{a}±0,13	1,45^{b}±0,13	0,56^{c}±0,06	0,44^{d}±0,04	86
Glutamic Acid	5,35^{a}±0,10	2,34^{b}±0,00	0,76^{c}±0,00	0,65^{d}±0,00	88
Proline	0,08^{a}±0,00	0,07^{b}±0,00	0,06^{c}±0,00	0,03^{d}±0,00	62
Serine	1,58^{a}±0,15	0,75^{b}±0,09	0,33^{c}±0,04	0,33^{c}±0,05	79
Glycine	2,55^{a}±0,15	0,94^{b}±0,07	0,54^{c}±0,03	0,43^{d}±0,03	83
Threonine	1,51^{a}±0,09	0,76^{b}±0,04	0,44^{c}±0,02	0,33^{d}±0,02	78
Tyrosine	0,94^{a}±0,04	0,43^{b}±0,07	0,26^{c}±0,04	0,28^{c}±0,05	70
Histidine	1,46^{a}±0,12	0,72^{b}±0,00	0,53^{c}±0,00	0,46^{d}±0,00	68
Alanine	0,25^{a}±0,04	0,08^{b}±0,00	0,07^{c}±0,00	0,05^{d}±0,00	80
%Total degradation					80

Different letters in the same row indicate significant differences by Tukey test (α = 95%).
*% Of amino acid leached in 12 hours.

Table 3 - Mean values and percentage of amino acid degradation of the commercial feed with 40% protein (RA40) subjected to leaching.

Amino acids (mg/100g)	**T0**	**T4**	**T8**	**T12**	**%**
Isoleucine	0,94^{a}±0,07	0,95^{a}±0,09	0,72^{b}±0,05	0,52^{c}±0,05	45
Leucine	1,92^{a}±0,09	1,67^{b}±0,06	1,56^{b}±0,06	0,95^{c}±0,05	51
Arginine	2,04^{a}±0,07	1,04^{b}±0,08	0,88^{b}±0,00	0,74^{b}±0,00	64
Valine	1,14^{a}±0,07	1,14^{a}±0,04	0,75^{b}±0,01	0,46^{c}±0,01	60
Methionine	0,44^{a}±0,03	0,45^{a}±0,02	0,32^{b}±0,03	0,25^{c}±0,03	43
Lysine	4,23^{a}±0,09	2,93^{b}±0,09	3,03^{b}±0,02	2,95^{b}±0,09	30
Phenylalanine	1,16^{a}±0,02	0,95^{b}±0,01	0,94^{b}±0,02	0,23^{c}±0,02	80
Aspartic Acid	2,24^{a}±0,09	1,83^{b}±0,02	0,96^{c}±0,08	0,84^{c}±0,08	62
Glutamic Acid	4,12^{a}±0,04	3,27^{b}±0,00	1,23^{c}±0,01	1,02^{d}±0,02	75
Proline	0,09^{a}±0,00	0,08^{b}±0,00	0,04^{c}±0,00	0,03^{d}±0,00	67
Serine	1,16^{a}±0,04	1,05^{a}±0,09	0,92^{a}±0,04	0,43^{b}±0,01	63
Glycine	1,46^{a}±0,07	1,54^{a}±0,09	0,76^{b}±0,05	0,55^{c}±0,05	62
Threonine	1,04^{a}±0,04	0,95^{a}±0,06	0,64^{b}±0,06	0,67$^{b\pm}$0,06	36
Tyrosine	0,65^{a}±0,01	0,53^{b}±0,05	0,57^{b}±0,06	0,43^{c}±0,05	34
Histidine	1,15^{a}±0,09	0,95^{b}±0,07	0,83^{c}±0,01	0,72^{d}±0,07	37
Alanine	0,15^{a}±0,01	0,14^{b}±0,00	0,13^{b}±0,01	0,11^{b}±0,01	27
%Total degradation					52

Different letters in the same row indicate significant differences by Tukey test (α = 95%).
*% Of amino acid leached in 12 hours.

Table 4 shows that there was not observed the same behavior for feed (RB40), with 40% protein in relation with feed RA40 with similar characteristics and the same percentage of protein. The degradation was greater with the amino acid valine, with losses of 93% at the end of leaching. Of the rations evaluated RB40 was the one that preserved histidine the best with the concentration of its composition with losses less than 25%.

Table 4 - Mean values and percentage of amino acid degradation of the commercial feed B with 40% protein (RB40) subjected to leaching.

Amino acids (mg/100g)	**T0**	**T4**	**T8**	**T12**	**%**
Isoleucine	0,57^{a}±0,05	0,55^{a}±0,04	0,44^{b}±0,01	0,14^{c}±0,01	75
Leucine	1,26^{a}±0,01	1,16^{b}±0,08	0,84^{c}±0,02	0,22^{d}±0,02	83
Arginine	0,84^{a}±0,08	0,95^{a}±0,06	0,66^{b}±0,02	0,25^{c}±0,02	70
Valine	0,94^{c}±0,05	0,52^{b}±0,03	0,35^{c}±0,00	0,07^{d}±0,00	93
Methionine	0,44^{a}±0,02	0,25^{b}±0,02	0,22^{b}±0,01	0,13^{c}±0,04	70
Lysine	3,05^{a}±0,08	2,87^{b}±0,00	2,82^{b}±0,05	1,51^{c}±0,08	50
Phenylalanine	0,66^{a}±0,04	0,48^{b}±0,00	0,09^{c}±0,00	0,07^{d}±0,00	89
Aspartic Acid	1,82^{a}±0,05	0,75^{b}±0,03	0,53^{c}±0,05	0,35^{d}±0,04	81
Glutamic Acid	3,43^{a}±0,00	1,02^{b}±0,07	0,75^{c}±0,04	0,46^{d}±0,04	87
Proline	0,08^{a}±0,00	0,05^{b}±0,00	0,05^{b}±0,00	0,03^{c}±0,00	62
Serine	1,06^{a}±0,06	0,65^{b}±0,04	0,45^{c}±0,02	0,24^{d}±0,00	77
Glycine	1,58^{a}±0,05	1,44^{b}±0,05	0,51^{c}±0,01	0,13^{d}±0,01	92
Threonine	0,92^{a}±0,06	0,64^{b}±0,05	0,57^{b}±0,03	0,34^{c}±0,03	63
Tyrosine	0,53^{a}±0,04	0,53^{a}±0,04	0,46^{a}±0,03	0,35^{a}±0,04	19
Histidine	0,84^{a}±0,08	0,83^{a}±0,08	0,91^{a}±0,05	0,56^{b}±0,06	23
Alanine	0,16^{a}±0,01	0,15^{b}±0,00	0,09^{c}±0,00	0,02^{d}±0,00	87
%Total degradation					70

Different letters in the same row indicate significant differences by Tukey test ($\alpha = 95\%$).
*% Of amino acid leached in 12 hours.

There were significant differences in the content of all amino acids analyzed after submergence treatments of the diets with considerable reduction in the amino acid content of the feed .Moreover, the physical structure of the feed cannot be attributed, if crushed or pelletized, to the stability of the amino acid composition because the feeds with 35% protein (pellet) showed more degradation than the samples with 40% protein (crushed). So it was not the physical structure of the ration that provided greater stability of amino acids in order that feeds showed greater degradation crushed. It's important to mention that feed with 35% protein are crushed to then pelleted while the 40% feed are only crushed.

Zerate and Lovell (1997) working with pelleted diets, reported that both protein-bound amino acids, as the synthetic ones, exhibit great losses when in contact with water. So approximately 13% of synthetic lysine (L-Lysine-HCl) is leached in the first 15 seconds in contact with water.

In referring to the manufacturers, the feed A and B have experienced similar degradation in the range of 35% protein, while in the range of 40% the feed A deteriorated about 20% less than the feed B. In all feeds evaluated it was found that lysine and histidine are essential amino acids which suffered less degradation processes.

In this study, degradation points were observed in the amino acids within 12 hours of experiment: 33% (histidine) to 94% (aspartic acid) in diet 35A; 62% (proline) to 89% (phenylalanine and arginine) in the feed 35B; 27% (alanine) to 75% (glutamic acid) in feed 40A and 19% (tyrosine) to 93% (valine) in the feed 40B. The seawater used in the experiment had pH = 7.5. It was then necessary to emphasize the importance of finding the pH of the water of nurseries where shrimp farming is practiced. When comparing degradation periods of four hours for the same amino acids mentioned before, It was found that the degradation points were 7% (histidine) to 83% (aspartic acid) in feed 35A; 12% (proline) to 76% (phenylalanine and arginine) in feed 35B; 7% (alanine) to 21% (glutamic acid) in feed 40A and 0% (tyrosine) to 45% (valine) in the feed 40B. This shows that in the first hour of the experiment the losses processes of amino acids in all feed evaluated are clear without being able to assign which amino acids are more susceptible to degradation.

In studies performed to evaluate the effect of pH in diets used for *Litopenaeus vannamei*, it was demonstrated that these diets show physical and chemical stability at pH values close to neutrality (6.5 - 7.0) in the environment where they operate. However, when the pH is changed of the conditions of neutrality (pH = 8.0) the degradation of essential amino acids is apparent in the experiment period (1h). In this study the authors observed variations of 5% (histidine) to 53% (methionine) (Lim 1993).

Conclusions

The results obtained in this study indicate that the leaching process significantly reduces the content of amino acids in the feed.

The physical structure of the feed does not prevent the degradation process of amino acids in the leaching process.

More research is needed to identify technologies that preserve the amino acids longer during the feeding of the shrimps.

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