



Effect of Amaranth and Quinoa Flours on the Productive Performance of *Litopenaeus Vannamei* Shrimp

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Abstract

Food supplementation with Amaranth and Quinoa flours diet has shown excellent research results in growth when used as a food source in aquatic animals. As a result, the goal of this study was to see how varying percentages of Amaranth and Quinoa flour in diets affected the productivity of the marine shrimp *Litopenaeus vannamei*. In a randomized design consisting of five treatments and three replications, five experimental diets (0%, 15%, 25%, 35%, and 45%) were all shown to be isoproteic (35%) and isoenergetic (3400kcal). Juveniles weighing 1.42g were fed experimental diets and a commercial meal with the same % of protein for 45 days ad libitum three times a day. The analysis of variance revealed a significant difference in the final weight, weight gain, and survival when the percentage of Amaranth and Quinoa flours in the diets was increased. Compared to the control diet, relative values in the 45% ration are higher, although lower, indicating a superior amino acid profile in shrimp and greater productive performance among the rations. The shrimp rated the fatty acid makeup after the trial. Total lipid concentrations differed significantly between the 45 percent supplemented diet and the 35% control diet, with the 45% supplemented diet having a lower concentration. Amaranth in the diets increased polyunsaturated fatty acids, n-6/n-3, and PUFA/SFA ratios, with beneficial results for the desired lipid content under the nutritional aspect for animal and human consumption.

Keywords

Shrimp, diets, Amaranth and Quinoa flours diet, fatty acid composition supplementation.

1. INTRODUCTION

Aquaculture has been considered one of the most efficient ways to reduce the deficit between demand and supply of fish in several world regions. In this context, shrimp farming has emerged as the main segment due to its fast pace and high productivity (FAO, 2018). Despite being recent, South India is in rapid growth, gaining prominence mainly in the

Sothern region with high productions in the states of Andhra Pradesh obtained by the performance of the main cultured penaeid *Litopenaeus vannamei* (Singh, et al., 2013). However, food remains the main obstacle in production costs in cropping systems, representing around 60%, with protein being the most important and expensive nutrient in formulated diets.

Due to this scenario, several researchers have been using a wide variety of ingredients already tested worldwide as a protein source in the feed for aquatic species. However, for shrimp, the proportion has been lower due to the limited application of the results given the diversity of experimental conditions and lack of nutritional characterization of the ingredients, since the protein source requires certain requirements among them, the low polluting potential, desirable nutritional aspects such as high protein content, favorable amino acid profile, high digestibility and acceptable palatability (Widanarni, et al., 2010).

Many vegetable diets have been produced on a large commercial scale, contributing to the development of aquaculture, and their application is associated with nutrition. It can be used directly in the dry or fresh form (as a single component of the diet or as an additive to basic nutrients) in fish consumption. Mollusks and penaeid shrimp, or indirectly as food for live prey in fish feeding, produce energy and obtain nutritious compounds since they have a very active metabolism and low production cost for growing under simple conditions. Its growth can be optimized by temperature, radiation intensity, salinity, agitation, concentration, and nature of nutrients under laboratory conditions (Zelaya, et al, 2007).

Among the vegetable diet already used in aquaculture, Amaranth and Quinoa flour diet have been highlighted by the great nutritional potential of proteins, vitamins, polyunsaturated fatty acids, in addition to a natural mixture of pigments, with available powers that has been used in the feeding of various aquatic animals, mostly fish, bringing significant effects on growth, survival, immune system and color after ingestion (Palombini, et al., 2013).

Traditionally, essential fatty acids can be obtained from animal and plant sources. The choice of the source depends on the desired fatty acid, its availability in the raw material, and the presence and nature of impurities. Among these sources, fish oils are the main ones, but production is insufficient to meet the demand. Amaranth and Quinoa flour diet is a vegetable source with a rich composition in nutrients, mainly in proteins and polyunsaturated fatty acids, which alone or in combinations have been used in the feeding of several aquatic animals an excellent performance in growth—considering the rich chemical composition of these vegetable sources this research aimed to evaluate the influence of different percentages of amaranthus in diets on the fatty acid profile in the muscle tissue of *L. vannamei*.

Therefore, the objective of this study was to evaluate the efficiency of the Amaranth and Quinoa flours diet with different concentrations in diets on the productive performance of shrimp *L. vannamei* and the amino acid profile.

2.0 Material and Methods

2.1 Experimental design and population

The experiment was carried out at the facilities of the Research and Food Processing units, with a duration of 45 days. Polyethylene boxes with a capacity of 30L were used in an open system with constant aeration and artificial lighting with a photoperiod of 12 hours. The design was completely randomized, consisting of five treatments with three replications. *Litopenaeus vannamei* juveniles were acquired from a farm in Ramayapatnam, Andhra Pradesh, with an average weight of 1.42 ± 0.23 g, being acclimatized for two days, gradually decreasing the salinity to 2.5‰, being distributed in their respective treatments in the density of 10 shrimp/treatment.

2.2 Formulation and preparation of diets

Five isoproteic (35% crude protein) and isoenergetic (3,400kcal of ED kg⁻¹) diets were formulated with different percentages of lyophilized Amaranth. Quinoa flour diet: 0%, 15%, 25%, 35% and 45% submitted to the pelleting process, where the dry ingredients were crushed, weighed and mixed in an industrial planetary mixer with the vitamin supplement (premix) and soybean oil, adding water at 60°C until a consistent wet mass is formed. The mixture was introduced into a manual meat grinder to form pellets with a diameter of 2 mm and then dried in an oven with forced air circulation at 80°C for 24 hours and stored in paper bags at room temperature (Table 1).

2.3 Fish Meal

The experimental diets were offered three times a day ad libitum until the shrimp satiety, and for comparative effect, a commercial diet containing 35% crude protein was used. The symphony was performed every two days to remove the remains of feed and feces, and then water samples were taken to measure the physical and chemical parameters of the water.

2.4 Chemical analysis

2.4.1 Determination of total lipids: Aliquots of crushed shrimp muscle tissue (triplicate) and feed samples were used to extract and quantify total lipids, using the method of Folch, et al (1957).

2.4.2 Derivation of the lipid fraction and analysis of the composition of fatty acids: Following Hartman and Lago's approach, the fatty acids in the lipid extract were trans methylated (Lou, et al 2017). Fumed silica capillary columns measuring 60m by

0.25 and 0.25m in diameter were used to identify and measure the concentrations of various fatty acid esters in the sample. The analysis was carried out using the Varian (SCION) 430GC with a flame ionisation detector (FID). The gas utilised was helium with a carrier gas flowing at a rate of 1 mL/min. For 20 minutes, the temperature rose by 2.5°C per minute from 100°C to 240°C, increasing by 2.5°C each minute. Temperatures of 250°C and 260°C were maintained for the injector and detector, respectively. The methyl esters in the samples were compared to Supleco ME19-kit standards to determine the fatty acid content (Fatty Acid Methyl

Esters C6-C22). The methyl ester areas were normalised, and the fatty acid results were presented as a proportion of the total area.

2.4.3 Amino acid composition of farmed shrimp: The amino acid levels of cultured shrimp were evaluated using the White, Hart, and Fry (Prado, et al 2017) methodology in samples that had previously been hydrolysed in double-distilled hydrochloric acid, followed by pre-column extraction of free amino acids with phenylisothiocyanate (PITC). The liquid chromatography method was used to separate the phenylthiocarbamyl-amino acid derivatives (PTC-aa).

Table 1: Formulation and proximal analysis (on a dry basis) of the experimental diets are expressed in percentages

Ingredients	0%	15%	25%	35%	45%
Fish meal ¹	31.56	26.83	23.67	20.52	17.36
Squid flour ²	5	5	5	5	5
Amaranth flour ³	0	18.45	30.75	43.04	55.34
Quinoa flour ⁴	0	---	---	---	---
Fish oil	3.48	2.84	2.42	2	1.58
Liquid lecithin	1	1	1	1	1
Cholesterol	0.5	0.5	0.5	0.5	0.5
Vitamin blend ^{5,7}	4	4	4	4	4
Mineral mix ^{6,7}	1.25	1.25	1.25	1.25	1.25
Carboxymethyl cellulose ⁷	4	4	4	4	4
Antioxidant	0.02	0.02	0.02	0.02	0.02
Antifungal	0.1	0.1	0.1	0.1	0.1
Wheat gluten ⁷	3	3	3	3	3
Chromium oxide ⁷	1	1	1	1	1
Corn starch ⁸	45.09	32.01	23.29	14.57	5.85
Proximal analysis					
Protein	30.89	30.17	29.31	28.93	28.83
Ethereal Extract	6.24	7.16	6.9	7.1	6.83
Humidity	5.82	6.8	6.63	11.09	10.91
Ashes	7.14	7.14	7.04	6.66	6.69
Digestible energy (Kcal)	82	93.2	90.7	64	86

¹Produced by the dry steam method (minimum crude protein 68% c.p.) ICAR-CIBA/Sri Sathvika Aqua Agro Industries Private Limited, Ongole

²Processed in the laboratory by lyophilization (commercial small squid *Loligo sp*)

³Sri Venkateswara Flour Mills, Ongole, Andhra Pradesh

⁴Sattvic Foods Organic Quinoa Flour, Goa

⁵(mg 100g⁻¹ diet): p-aminobenzoic acid, 10; thiamine-HCl, 12; riboflavin, 20; pyridoxine-HCl, 12; choline chloride, 250; nicotinic acid, 75; calcium pantothenate, 50; inositol, 200; Biotin, 0.5; folic acid, 1.5; ascorbic acid, 10; menadione, 4; tocopherol acetate, 40; cyanocobalamin, 0.03; H.H-carotene, 1.15 10⁻³; cholecalciferol, 0.03; vitamin A acetate, 20.

⁶(mg 100g⁻¹ diet): monobasic calcium phosphate, 272; calcium lactate, 640.2; ferric citrate, 60; magnesium sulfate heptahydrate, 274; potassium phosphate, 480; monobasic sodium phosphate, 174; sodium chloride, 86; aluminum chloride, 0.4; potassium iodide, 0.3; copper chloride, 0.2; manganous sulfate monohydrate, 1.6; cobalt chloride hexahydrate, 2.1; zinc sulfate heptahydrate, 7.1; sodium selenite, 2.

⁷Purchased from Urban Platter Vital Wheat Gluten, Mumbai.

⁸Acquired from Urban Platter Baker's Corn Starch, 400g, Mumbai.

2.5 Physical and chemical analysis of water

The physical and chemical analyzes of the water were carried out throughout the cultivation at the

Research Laboratory M/s.Prathyusha Global Trade (P.G.T) Pvt. Ltd ,Ramayapatnam Sea coast, with the reading of dissolved oxygen and temperature being performed weekly by a digital oximeter (Water Quality Analyser - PE 138 - ELICO), pH by a digital potentiometer brand pH Meter LI 120 - ELICO, both bench-top and twice a week salinity by a Esico Digital Salinity Meter, 671 from 0 to 3.5‰. For water quality, daily renewals of 15% of the volume of each aquarium were maintained, and with siphoning, eliminating waste and food remains.

2.6 Growth performance

Biometrics were performed at baseline and every fifteen days to assess growth performance, calculated by: Survival rate (%) = (Final number of shrimp/Initial number of shrimp) x 100; Weight gain (%) = (average final weight – average starting weight); SGR (% day⁻¹) = (ln final weight – ln initial weight) x 100; Feed conversion rate = (feed consumption/weight gain).

Statistical analysis. Shrimp growth variables and water quality parameters were analyzed using an analysis of variance (ANOVA) to determine the effect of protein replacement levels and the influence of treatments at the 5% significance level. In cases where there was a significant difference, the Tukey test was applied to compare the means, at a significance level of 5%, using the Statistica R software version 3.0.

3.0 RESULTS AND DISCUSSION

3.1 Centesimal composition of diets

Table 1 shows the chemical composition of the experimental diets with protein values around 30.89%, a percentage considered ideal for the growth development in the juvenile phase of the shrimp *L. vannamei* (Oujifard, et al., 2012). For moisture, values below 6% commonly found in commercial rations were observed, considered adequate in the formulation of rations for aquatic animals. It avoids excessive drying and decreases the protein quality of the pellets.

The lipid values ranged from 6.7 to 7.1%, following those required by this species in the diets (6 to 7.5%); according to Zafar, et al. (2008), these values are considered ideal. To avoid a reduction in shrimp growth and mortality, in the ash values, the rations were similar to the values measured in commercial rations with a maximum of 13%, a value observed by Davis, et al, (2000) when they analyzed commercial rations intended for shrimp feeding.

Amino acid profile in shrimp, specific and high content of some essential amino acids, was verified in the muscular portion of *L. vannamei* (Table 2), showing a higher incidence of amino acids considered limiting for the formulation of rations (arginine, methionine, and lysine) in shrimp that were fed with rations containing 15% and 45% of Amaranth and Quinoa flours diet. However, when they are compared with the muscle requirement of shrimp and the control diet, the methionine content is deficient in the diet with 15%, considering that the deficiency in one or more amino acids can compromise the nutritional quality of the shrimp, we can see that the ration containing 45% provided the best concentrations of amino acids for the shrimp muscle.

Table 2. Amino acid profile in the muscle of juvenile shrimp fed experimental diets using lyophilized Amaranth and Quinoa flours diet.

Essential Amino acid	*Shrimp	Treatments				
		0%	15%	25%	25%	45%
Arginine	7.5	9.6	8.8	2.3	0.6	8.3
Phenylalanine	3.6	6	4	1.1	2	5.5
histidine	1.9	3	2.1	1.5	1.2	5.6
isoleucine	3.6	5.6	3.9	1.1	1.8	4.4
Leucine	6.5	6.2	5.9	1.8	2.9	6.1
lysine	6.4	12.8	8.6	0.4	1.1	8.5
methionine	2.6	3.5	2.2	0.4	1.2	2.8
tyrosine	5.4	4.2	3.3	0.8	1.6	4.3
threonine	3.4	4	3	0.3	1.7	4
Valine	3.8	5.7	3.9	1.6	2	5.4

* Shrimp muscle requirement (Ju, et al. 2009).

Diets containing amino acids in quantities similar to those found in shrimp muscles, according to Ju et al.

(2009), provide the best growth and survival rates during commercial crops. As a result, feed quality is

not always related to quantity—the total protein in the ration—but to a well-balanced amino acid supplementation.

3.2 Composition of total lipids and fatty acids in diets

The contents of total lipids and fatty acids of the diets containing the different percentages of Amaranth and Quinoa flours diet can be seen in Table 3. A significant difference was observed in the contents of total lipids, with a lower concentration in the diet with 45% supplementation; however, it was no difference. It was observed between the diet containing 35% supplementation and the control diet (0%). These values were within the recommended standards for lipids in formulated diets (5 to 9%) according to Fitzsimmons (2001) for this species. Lee,

et al (2018) obtained 5.16% of lipids in rations formulated for *L. vannamei*; similar results were observed by Morris, et al (2011), who found values from 5.37 to 5.65% in the formulation of rations for this species.

The major fatty acids identified in the diets were oleic acids (18:1n-9) and linoleic (LA, 18:2n-6). High values of n-6 and n-6/n-3 ratios were found, indicating a strong influence in the diet containing 45% of Amaranth and Quinoa flours diet, which has linoleic and linolenic acids in its composition, however, these concentrations can vary according to the type of cultivation. The growth phase is used to obtain the vegetable diet, and soybean oil is also used to prepare rations, an important source of oleic and linoleic acids (Lee, et al 2018).

Table 3: The composition of lipids (%) and fatty acids (g/100g of lipids) of experimental diets with different Amaranth and Quinoa flours diet percentages.

	Diet				
	0%	15%	25%	25%	45%
total lipids	6.9±0.24b	7.1±0.27a	7.3±0.32a	6.8±0.07b	6.7±0.05b
SFA					
11:00	0.05	0.06	0.28	0.58	0.49
14:00	0.83	0.74	0.64	0.62	0.57
16:00	1.3	1.33	1.32	1.32	1.53
17:00	0.12	0.09	0.01	0.12	0.1
18:00	5.47	5.03	4.51	4.36	4.34
19:00	3.18	4.08	4.36	4.19	4.49
20:00	0.06	0.05	0.11	0.16	0.17
21:00	0.12	0.16	0.2	0.26	0.22
23:00	0.11	0.12	0.15	0.15	0.15
TOTAL SFA	11.2	11.6	11.5	11.7	12
MUFA					
14:01	0.39	0.16	0.13	0.32	0.2
16:01	0.03	0.03	0.03	0.03	0.03
17:01	0.06	0	0.02	0.02	0.01
18:1n9	41.1	34.4	32.5	31.4	31.6
18:1n11	2.51	2.08	1.98	2	1.96
20:1n9	0.05	0.18	0.07	0.08	0.04
22:1n9	0.19	0.07	0.04	0.46	0.16
TOTAL MUFA	44.3	36.9	34.7	34.3	34
AGPI					
18:2n6	42.5	50.8	51.6	53.7	54.2
18:3n3	0.13	0.1	0.12	0.17	0.18
20:4n3	0.32	0.31	0.33	0.37	0.33
TOTAL PUFA	42.9	51.2	52	54	54.7
n-6	42.5	50.8	52	54	54.7

n-3	0.45	0.41	0.45	0.54	0.51
PUFA/SFA	3.8	4.4	4.5	4.6	4.5

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 = omega 3; n-6 = omega 6.

3.3 Composition of total lipids and fatty acids in shrimp muscle

In the shrimp muscle, there was an increasing increase in the values of total lipids (Table 3), showing a significant difference, with a lower percentage in the muscle tissue of the shrimp fed with the supplementation of Amaranth and Quinoa flours diet at 35%, with no difference being observed between the supplementation with 45% when compared to the control diet (0%), confirming that the lipid composition of the fish is a direct reflection of the offered diet, and thus may vary according to the species. Lee, et al. (2018) detected in the shrimp *L. vannamei* 0.40 to 1.70% of lipids, while Morris, et al (2011) found in the pink shrimp (native) a variation of 1.0 to 1.4% lipids.

Table 3 shows the fatty acid composition, sums, and ratios between n-6/n-3 and PUFA/SFA in the muscle tissue of *Litopenaeus vannamei* shrimp. Based on the results found, it can be seen that the influence of diet on the fatty acid composition of fish is already a fact. Thus, supplementation with Amaranth and Quinoa flours diet and soybean oil in the rations positively influenced the quality. Of lipids, it shows high concentrations of polyunsaturated fatty acids, omega-6, and lower levels of omega-3 in supplementation with 35% and 45%.

A balance of the proportion of n-6/n-3 in the diet is essential in the metabolism of the human organism,

leading to the prevention of cardiovascular and degenerative diseases and better mental health. Considering that the lipid composition of the fish directly reflects the content of its diet, it was observed that among the n-6/n-3 ratios, the best value was found in the muscle of the shrimp that consumed the ration containing 35% of vegetable diet supplementation (4.1).

For the PUFA/SFA ratio, values higher than 0.45 were found in all shrimp. However, a higher value (0.62) was observed in the shrimp muscle that consumed 35% of the vegetable diet According to the Lee et al., (2018) foods that have an n-6/n-3 ratio above 4.0 and PUFA/SFA greater than 0.45 are recommended for the diet. Lou, et al (2017) found an n-6/n-3 ratio of 4.1 in pink shrimp total lipids.

3.4 Water quality

During the entire experimental period, the physical and chemical parameters of the water did not vary significantly (Table 4) with temperature values between 27.3 and 28.2°C, pH between 7.5 and 7.7, and dissolved oxygen above 4mg/L remaining within the acceptable limit for the cultivation of *Litopenaeus vannamei*. The salinity was maintained at 2.5‰, considered ideal for obtaining better growth; however, this species can adapt to lower salinities, according to Boyd (2002).

Table 4: Means $\pm$ Standard deviation of physical and chemical parameters of the culture water of juveniles fed different diets.

Diets	Temp (°C)	DO (mg.L ⁻¹)	pH
15%	27.70 $\pm$ 0.38 ^a	6.61 $\pm$ 0.22 ^a	7.57 $\pm$ 0.32 ^a
25%	27.58 $\pm$ 0.31 ^a	6.47 $\pm$ 0.16 ^a	7.66 $\pm$ 0.04 ^a
35%	27.86 $\pm$ 0.41 ^a	6.21 $\pm$ 0.25 ^a	7.79 $\pm$ 0.06 ^a
15%	27.84 $\pm$ 0.56 ^a	6.39 $\pm$ 0.12 ^a	7.49 $\pm$ 0.25 ^a
0% (CD)*	28.03 $\pm$ 0.48 ^a	6.42 $\pm$ 0.11 ^a	7.51 $\pm$ 0.19 ^a

*Commercial feed. Equal letters in the same column do not differ statistically ($p > 0.05$).

3.5 Zootechnical performance indicators

Table 5 shows the increasing significant difference in the productive performance of shrimp as the percentage of Amaranth and Quinoa flours diet in the diets increased, evidencing a better result of the diets supplemented with 35% and 45%, however, when compared to the commercial diet, it is observed Better results are obtained in the ration with the 45% supplementation.

Several authors have observed positive results in growth performance with Amaranth and Quinoa flours diet supplementation in diets for some shrimp species. Lee, et al (2018) reported that a 25% increase in this vegetable diet in *Litopenaeus vannamei* shrimp post-larvae diet showed high growth and survival rates. Oujifard, et al. (2012), when using Amaranth and Quinoa flours diet to feed *Penaeus semisulcatus* shrimp, obtained superior results in weight growth. Most researchers have

been using this vegetable diet to feed fish, including tilapia (Apraku, et al., (2017), reporting results with greater efficiency in weight gain and survival rate. For Singh, et al (2013), these positive effects on growth are attributed to the digestibility of nutrients and high nutritional contents present in the Amaranth and Quinoa flour diet.

The average survival of shrimp showed a significant difference; the results were not influenced by the percentage of Amaranth and Quinoa flours diet in the diets, showing high values, which shows the positive effects of this vegetable in favoring better conditions in the culture. Higher feed conversion was

observed in the commercial diet, with no significant difference between the diets supplemented with Amaranth and Quinoa flours diet, indicating satisfactory results according to Ponce, (2006) when considering values from 0.9 to 1.5 as the best feed intake for the species. In study. Oujifard, (2012), when evaluating Amaranth and Quinoa flours diet as an attractant in diets for *L. vannamei*, observed a better feed conversion in animals, noting that this vegetable source can stimulate food consumption in animals even with low content of inclusion in the diet.

Table 5: Zootechnical parameters of *Litopenaeus vannamei* juveniles fed with experimental diets.

Diets	Finished weight (g)	Final length (cm)	weight gain (g)	Food conversion	Survival (%)
15%	2.53±0.31 ^b	7.30±0.30 ^{ab}	1.76±0.35 ^{ab}	0.67 ^{ab}	86.8 ^b
25%	2.71±0.26 ^{ab}	7.36±0.37 ^{ab}	1.93±0.25 ^{ab}	0.63 ^{ab}	95 ^a
35%	2.66±0.28 ^{ab}	7.32±0.32 ^{ab}	1.90±0.29 ^{ab}	0.63 ^{ab}	90 ^a
15%	2.32±0.25 ^b	7.24±0.25 ^b	1.58±0.26 ^b	0.75 ^a	93 ^a
0%	2.80±0.37 ^a	7.88±0.67 ^a	2.05±0.43 ^a	0.58 ^b	85 ^b

Equal letters in the same column do not differ statistically ($p>0.05$). PF- Final weight, CF- Final length, FCR- Feed conversion rate, SGR- Specific growth rate, S- Survival. * Commercial feed.

4. CONCLUSION

Based on the results obtained in the present research, we can conclude that the use of the vegetable diet Amaranth and Quinoa flour diet in the cultivation of marine shrimp *Litopenaeus vannamei* showed positive effects on water quality, as well as a food supplement by providing a greater increase in growth in weight and length, with high shrimp survival in all treatments, however, in the percentage of 45%, an amino acid profile content was observed with more expressive results in the productive performance of shrimp. The addition of the Amaranth and Quinoa flours diet in the rations of the marine shrimp *L. vannamei* provided a better result in the lipid quality and polyunsaturated fatty acids in the shrimp muscle in the 35% supplementation, showing favorable results in the desirable lipid content, under the aspect nutritious, being able to constitute a nutritious source for the elaboration of rations in the feeding of aquatic animals, as well as a source of better lipid proportion for human consumption. It is suggested that Amaranth and Quinoa flours diet be used in future experiments as a food supplement with percentages higher than this research.

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