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Research Article

EVALUATION OF THE IMPACT OF A DIET SUPPLEMENTED WITH DRY BREWER'S YEAST (*Saccharomyces cerevisiae*) AND MEDICINAL PLANTS ON SHRIMP (*Litopenaeus vanammei*) GROWTH

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Abstract

Weight and length gain were estimated in whiteleg shrimp *Litopenaeus vannamei* fed with a bitter dry brewer's yeast (*Saccharomyces cerevisiae*) combined with cereals, such as corn and soya, and medicinal plants, such as guava, basil, and eucalyptus, to achieve optimal development. The present study was carried out at the aquaculture bioassay laboratory, from July to October 2019, with an experimental design that included two treatments, one with VIMIFOS commercial feed and the other with a feed based on dry brewer's yeast and medicinal plants. The feed was administered four times per day at a 7% bodyweight ratio, obtaining similar length gains of around 5.5 cm by the end of the experiment for both treatments, while a mean final weight gain of 9.5 g was obtained with commercial feed, which was higher than the 7.2 g mean final weight gain obtained with brewer's yeast. The organisms fed with brewer's yeast were found to be less light sensitive, indicating that they presented less stress in the presence of light, while those organisms fed with commercial feed tended to jump out of the container. Brewer's yeast-based feed is as efficient as commercial feed, but with the advantage that the production costs are lower.

Keywords: *Litopenaeus vannamei*, brewer's yeast, length, weight, medicinal plants, commercial feed.

INTRODUCTION

Aquaculture is one of the most developed economic activities in the world, as reflected in statistics published by the Food and Agriculture Organization (FAO) of the United Nations, which show a total *Litopenaeus. vannamei* production of over four million tons, with a value of more than 23 million dollars (FAO, 2014). The whiteleg shrimp is native to the Eastern Pacific coast, distributed from Sonora, in northern Mexico, down through Central and South America to its southernmost distribution in Tumbes, Peru, in waters with a temperature normally remaining above 20 °C throughout the year (Wyban, 1991). The white leg shrimp's (*L. vannamei*|) high tolerance to environmental factors means it can tolerate salinity of 0.5-45 psu (practical salinity units), while it grows well at low salinities, from 10 to 15 psu, at spawning densities above 50 org/m^2, conditions in which both the aquatic medium and the hemolymph are is osmotic (McGraw, 2002). Moreover, this species is able both to acclimate itself to a hypotonic environment and maintain its growth and survival rates at levels similar to those it presents in a marine environment. *L. vannamei* is a species characteristic of muddy-bottomed waters between 5 and 72 m in depth, with adults found in completely marine environments, while nursery and grow out occur in estuaries and brackish lagoons. The species is found in waters with annual average temperatures of 20 °C and reaches a maximum length of 230 mm with a 90 mm shell. Adults live and reproduce in the open sea, while post larvae migrate to the coasts for the juvenile stage and then to estuaries, coastal lagoons, and mangroves for the adolescent and pre-adult stages. Males mature at 20 g and females at 28 g, both at an age of six to seven months. Fish meal is used in domestic animal diets, as it is a rich source of crude protein and lipids and can be produced from different fish species, with some fish meal even produced from fish by-products (filleting waste and viscera, etc.). Factors such as bone, ash, and oil content vary nutrient content and digestibility in the final product (Miles, 2006). Additionally, while the meal production process affects protein digestibility (Opstvedt, 2003), the current trend is to reduce the level of fish meal in aqua feed, which has led to the need to find alternative protein sources. Traditionally, fish meal has been the principal protein source, representing 17.6% of global production (Davis, 2004). Little information is available on the feasibility of totally or partially replacing fish meal with other ingredients of both animal and vegetable origin. Depending on the stage of development, there are variations in the protein requirement reported for *L. vannamei*, with Cousin (1991) reporting 30%, Aranyakananda (1993) 15%, and Pedrazzoli (1998) 40%. Forster (2011) studied the effect of soybean meal, replacing fish meal for soybean oil, and finding that shrimp cannot efficiently convert linolenic acid into eicosapentaenoic and docosahexaenoic acid when fish meal or oil is absent from the feed. A product of the corn milling process, cornmeal is low in fiber and ash and contains no anti-nutritional factors (Regost, 1999; Pereira, 2003), while its protein content ranges from 9 to 15%. Yeasts are used as an alternative due to their particle size and adequate water column stability, which enable easy ingestion, while mass yeast production is possible in short time periods at a cost lower than live algal cultures (Coutteau, 1997). Yeasts also present high levels of proteins and B vitamins (Martínez, 1999), although it should be noted that their chemical composition varies depending on the species, the culture medium, and the physico-chemical conditions in which they are developed. The biochemical composition of yeasts is variable, with proteins corresponding to the highest percentages, although this differs on the spectrum of values, with Tacon (1989) andBrown (1996) observing protein content of 15 to 30% and 25 to 37%, respectively. Otero (1999) found a protein content of 40 to 60%, mainly located in the cellular cytoplasm but also in the ribosomes and the cell nucleus, membrane, and wall. With an

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amino acid profile similar to that of soybean meal, yeast can be easily adapted for animal feed and is, moreover, rich in glutamic acid and lysine (up to 8%), although it is relatively low in methionine. Yeast is also naturally rich in B vitamins, such as biotin, thiamine, niacin, and folic acid (Coutteau, 1990). Dry yeast contains 93 to 97% dry material and can contain 40 to 60% nitrogen, 35 to 45% carbohydrates, and 5 to 9% lipids. A significant fraction (up to 12%) of nitrogen is present in the form of nucleic acids, which leads to the production of significant levels of uric acid, which may limit the use of yeasts in high concentrations due to the level of toxicity (Tacon, 2002). The yeast most commonly used as a dietary supplement or substitute in global aquaculture is brewer's yeast (*Saccharomyces cerevisiae)* (Nell, 1996).

In Mexico, the protein quality of brewer's yeast when used as shrimp feed has been studied by evaluating its digestibility *in vitro* using hepatopancreas homogenate taken from *Litopenaeus schmitti*(Forrellat, 1988). Brewer's yeast has been used directly as a feed source due to its high protein value, one example of which being *Saccharomyces cerevisiae*, which has been tested on groups such as rotifers (Nagata, 1992; Walford, 1992; Vallejo, 1995), Artemia sp. (Chien, 1991), Daphnia sp. (Vigano, 1993), shrimp (Roques, 1991; Mura, 1997), and fish (Robertsen, 1990). Lin (2006) studied diets containing medicinal plants by evaluating how digestible the nutrients were for shrimp, finding that medicinal plants reduced protein digestibility and improved lipid digestibility. There is a long history of medicinal plant use in China, with the majority of these plants used as a type of immunological stimulant in aquaculture (RX, 1997; Chansue, 2000). Medicinal plant extracts have been used in aquaculture, especially for controlling bacterial and viral diseases, among which the use of guava leaves (*Psidium guajava*) to treat infectious hematopoietic necrosis in salmon (Silveira, 2000) is notable. Shrimp culture has benefitted from the use of basil (*Ocimum basilicum*), which presents significant antiviral activity against yellow head disease in the shrimp *Penaeus monodon* (Direksaburakom & Rongkumnardwong, 1996; Janwitayanuchit, 2003). Eucalyptus (*Eucalyptus globulus*) is a plant with antimicrobial properties, while basil (*Ocimum basilicum*) provides vitamins A, K, and C and minerals including iron, calcium, manganese, magnesium, and potassium. Basil also contains beta-carotenes, which are antioxidants that protect cells, as well as containing antibacterial properties and flavonoids that protect DNA.

MATERIALS AND METHODS

The present study used postlarval *L.vannamei* specimens obtained from the Playa Juan Ángel shrimp farm in the municipality Úrsulo Galván of the city of Actopan in the state of Veracruz de Ignacio de la Llave, Mexico. The samples were then transferred to the Aquaculture Bioassay Laboratory at the Faculty of Biological and Agricultural and Livestock Sciences, at the Poza Rica-Turpan Region campus of the Universidad Veracruzana (University of Veracruz). Subsequently, 1,051 individuals were acclimated for ten days in two 1000 L (1.68 cm x 1.68 cm x 66 cm) ponds, with recirculated filtered seawater and constant aeration at a temperature of 26 to 30 °C and salinity of 30 to 35 psu. One hundred and seventy-five organisms were then placed in each of the ponds' subdivisions (TA1-TA2-TA3 and TB1-TB2-TB3). After ten days, the organisms in one pond began to be fed with the brewer's yeast-based diet (TA), while those in the second pond began to be fed with commercial feed (TB). For four months, the individuals were fed portions corresponding to 7% of their bodyweight four times per day (7:00, 11:00, 15:00, and 19:00 hr), until they reached a length of 10 to 15 cm. Salinity, oxygen, temperature, and nitrogenated compounds were measured twice a day between 9:00 am and 9:00 pm, while 40% of the water was replaced approximately every two weeks. The SPSS statistics program, version 24, and Statistica, version 10, were used to calculate sexual composition and proportion, average length and weight, and frequency histograms, as well as to determine the breeding seasons and conduct a length-weight regression analysis. The data was then analyzed using the FITSAT II (Fish Stock Assessment Tools, FAO, ICLARM; Pauly and Sparre, 1991) program in order to determine a score, via the Von Bertalanffy individual growth model, for the asymptotic width (L) and growth coefficient (K), using the ELEFAN I extension to then calculate the $L\infty$ and K parameters and their dispersal.

$$Lt + 1 = L\infty\,(1 - K + KLt)$$

where:
$Lt + 1$ = length of time $t + 1$
$L\infty$ = maximum theoretical length attained by the species
K = angular coefficient
Lt = length of time t.

The main ingredients used to prepare the feed were fish meal, wheat, and, in lesser quantities, draff, blood meal, and squid meal. The minor ingredients used were mineral premixes and calcium carbonate, and, in lesser quantities, pigments agglutinants, vitamins, and medicines. Liquid ingredients were added via a spray curtain over the main part of the mixture and not via jets, as direct jets produce caking (lumps) in the mixture, which, in turn, can trap minor ingredients and prevent homogeneous distribution in the mixture. Liquids such as oils and lecithin, as well as soluble ingredients, must be introduced via their own system separate from the water line. A completely random experimental design was developed to evaluate the inclusion of brewer's yeast and medicinal plants (Experiment A) in diets for juvenile whiteleg shrimp (*L. vannamei).* The commercial feed used was VIMIFOS (Natural Force), in a 20-kilo presentation which bore no nutritional information, for which reason a bromatological analysis was conducted.

RESULTS

The maximum, minimum, and average values were taken for the physical and chemical variables of the water during the experiments, while the average temperature from July to October was 28.3^{o} C for both treatments. Salinity was recorded at 31.99 psu for the yeast treatment and 32.09 psu for the commercial treatment. The minimum oxygen values in July were 1.3 for the yeast treatment group, which maintained an average of 3 to 4 mg/l, as did the commercial treatment group (although this varied in July, August, and October), which presented minimum oxygen values of below 2.7 mg/l. The VIMIFOS commercial feed registered the highest protein content, 44.40%, and the lowest fiber content, 3.99%, while the brewer's yeast treatment registered 44.34% protein and 5.38% fiber (Table 1). By the end of the study period, the minimum and maximum weights for the brewer's yeast treatment were 2 g and 13 g, respectively (Table 2).

Table 1. Proximate composition and gross energy of the feed used in brewer's yeast and commercial feed treatments.

Ingredients %	VIMIFOS commercial feed (Natural Force)	Dry brewer's yeast
Dry material	91.35	94.85
Moisture	8.65	5.15
Protein	44.40	44.34
Ether extract	15.15	11.90
Total ash	8.84	6.38
Crude fiber	3.99	5.38
Nitrogen-free elements	27.61	32.00
Gross energy	525.25	518.1

Table 2. Average weights (g) for *L. vannamei* obtained via the brewer's yeast

Month	N	Mean	Standard error of the mean	Mínimum	Máximum
July	30	0.6667	0.03156	0.50	0.90
August	120	1.5000	0.06251	0.50	4.00
September	180	4.2556	0.16033	1.00	12.00
October	120	7.2667	0.18962	2.00	13.00
Total	450	4.0844	0.13661	0.50	13.00

Mean monthly weights were also obtained via the commercial feed treatment, giving a general mean of 5.98 g. At the beginning of July, the minimum and maximum weights registered were 0.50 and 2 g, respectively, while, for October, the last month of the experiment, the minimum and maximum weights registered were 5 g and 15 g, respectively (Table 3).

Table 3. Average weights (g) for *L. vannamei* obtained via the commercial treatment

Month	N	Mean	Standard error of the mean	Mínimum	Máximum
July	30	0.8667	0.09338	0.50	2.00
August	120	2.5833	0.13153	0.80	7.00
September	180	6.7111	0.14495	2.00	14.00
October	120	9.5583	0.18402	5.00	15.00
Total	450	5.9800	0.16068	0.50	15.00

The general mean length obtained via the brewer's yeast treatment was 7.88 cm, based on a mean length of 4.73 cm at the beginning of the treatment in July and a mean length of 10.07 cm at the end of the treatment in October. The initial minimum and maximum lengths were 2.9 cm and 6.5 cm, respectively, while the final minimum and maximum lengths were 6 cm and 12.7 cm, respectively (Table 4).

Table 4. Average length (cm) for *L. vannamei*, obtained via the brewer's yeast treatment

Month	N	Mean	Standard error of the mean	Mínimum	Máximum
July	30	4.737	0.1623	0.50	2.00
August	120	5.881	0.0946	0.80	7.00
September	180	8.292	0.1022	2.00	14.00
October	120	10.071	0.0970	5.00	15.00
Total	450	7.886	0.0996	0.50	15.00

The initial minimum and maximum lengths obtained via the commercial treatment were 3.3 cm and 7.8 cm, respectively, while the final minimum and maximum lengths were 6.4 cm and 13.1 cm, respectively. The general mean length obtained was 9.06 cm, based on a mean length of 5.44 cm at the beginning of the treatment in July and a mean length of 11.02 cm at the end of the treatment in October (Table 5). The linear regression indicated a positive relationship between the length and weight of the organisms for both treatments, presenting similar values of 0.913 for the commercial treatment and 0.926 for the brewer's yeast treatment.

Table 5. Average length (cm) for *L. vannamei* obtained via the commercial treatment

Month	N	Mean	Standard error of the mean	Mínimum	Máximum
July	30	5.443	0.1827	3.3	7.8
Augyst	120	6.963	0.1445	3.5	10.1
September	180	9.761	0.0673	6.0	12.0
October	450	11.024	0.983	6.4	13.1
Total	450	9.064	0.1015	3.3	13.1

The highest concentration for the brewer's yeast treatment was observed at lengths of 5 cm and weights of approximately 2.5 g, while the highest concentration for the commercial treatment was observed at weights of approximately 2 g. The weight correlation for the two treatments, with an interval of standard error of 2, is shown is Figure 5. The initial total weight for the treatments was approximately 1 gram for 22 July. The final total weight observed for both treatments varies significantly, with the data for the commercial treatment revealing an average weight gain of over 10 g, while this was 7.5 g for the brewer's yeast treatment **(**Figure 1).

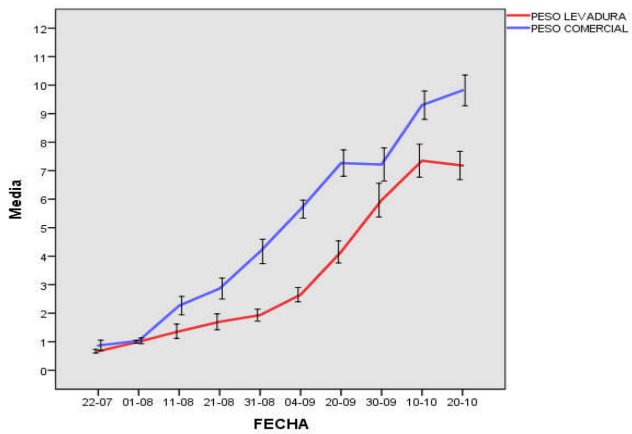

Figure 1. Comparative data for weight gain in relation to the two treatments applied

The relationship for length between the two treatments, with an interval of standard error, is shown in Figure 6. The initial total length observed for the brewer's yeast treatment was approximately 5 cm for 22 July to 01 August, while this was approximately 6 cm for the commercial treatment. The lengths observed for both treatments varies significantly, with the data obtained for the commercial treatment group revealing an average length gain of over 11 cm, while this was 10 cm for the brewer's yeast treatment group (Figure 2).

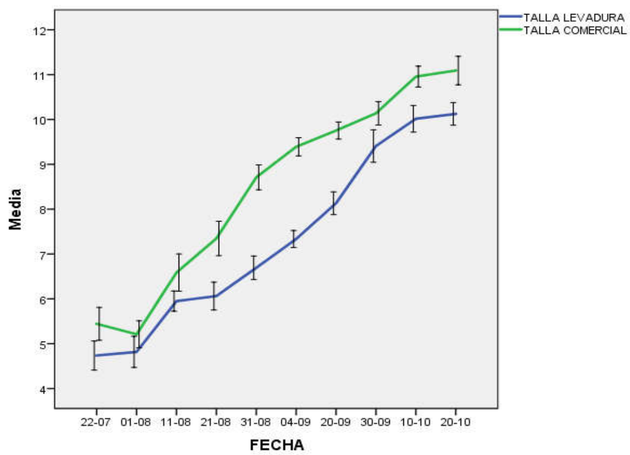

Figure 2. Comparative data for length gain in relation to the two treatments applied

DISCUSSION

The general conditions for the evaluation of the treatments applied in the present study are within the standards established by Wyban (1991), who states that, normally, shrimp grow at temperatures above 20 °C throughout the year and present a high tolerance to environmental factors, such as the 0.5-45 psu salinity noted by McGraw (2002). The values observed in the present study were obtained at a temperature of 28.3 °C for both treatments throughout the culture cycle, with a salinity of 31.99 psu for the brewer's yeast treatment and 32.09 psu for the commercial treatment. Despite the constantly changing climate, featuring rains, strong winds, and heat, the temperature remained within a good range. Soya was found to comprise 52.45% of the protein in the treatment based on dry brewer's yeast. With Storebakken (2000) indicating that soya products are generally high in protein, ranging from 45 to 60%, this dietary ingredient contributes an optimum amount of protein for the growth and survival of *L. vannamei* shrimp. This is a low-cost feed compared to fish meal or products containing protein of animal origin. Dry brewer's yeast was found to contain 44.34% protein, a finding that concurs with the results obtained by Otero (1999), namely that yeast provides 40 to 60% of the ideal protein intake for *L. vannamei* shrimp. The results obtained for the yeast treatment group concur with those obtained for the commercial treatment group in terms of the percentage, 44%, of protein provided by both treatments, with the significant difference being that the yeast treatment presents 10% savings in terms of cost. Greco *et al*. (2000) note that these organisms are able to grow to 20 times their length and 500 times their biomass as they develop from nauplius to adult state over the course of 15 molts. This was observed between the organism's eighth to twelfth day, with lengths ranging from 8-20 mm, thus concurring with results of the present study, which obtained similar lengths for both treatments. In the four months in which the present study was conducted, average weight gains of 0.5, 1.25, 1.75, 2.0, 2.5, 4.0, 6.0, 7.26, and 7.0 g were recorded. While no statistical differences were observed, the means reflected values above those expected, while an average weight gain of 4.8 g was observed by the end of the experiment.

Conclusion

The results obtained in the present study show that, in order to grow efficiently in semi-intensive culture systems, *L. vannamei* requires the application of nutritional regimes with a high final level of protein or feed with a balanced nutritional content and a 40 % protein level throughout the grow-out cycle. For this reason, the use of yeast (*S.cerevisiae)* and soybean meal in shrimp diet is recommended. These ingredients contain a high protein content (44.3% for yeast and 52.4% for soybean meal), which would reduce, either partially or totally, the amount of fish meal in feed, thus favoring the conservation of feed and the reduction of both feed consumption and production costs. The application of moderated feed schedules can reduce both costs, by 20 %, and environmental deterioration. Both treatments applied in the present study revealed similar weight and length gains, confirming the benefits of partially or totally replacing meals of animal origin, thus generating lower production and storage costs. Although the physico-chemical parameters were similar, the organisms fed commercial feed presented a higher level of light sensitivity and more propensity to jumping than those administered feed containing vegetable protein.

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