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Research Article

A STUDY OF THE EFFECT OF THE BIO-PROTEIN RESULTING FROM THE TREATMENT OF BIO-MARINE RESIDUES ON GROWTH OF THE SHRIMP *Palaemon longirostris*

Izdihar Ammar[1]., Moufid Yassin[2]., Badr Al Ali[3] and Rabee Raya[4]

[1,3,4] Food Biotechnology Department Pharmacy Faculty, Tishreen University, Lattakia, Syria
[2]Department of Marine biology at HIMR, Tishreen University, Lattakia, Syria

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ABSTRACT

Some microorganisms isolated from the marine environment have been used to treat the remains of marine organisms. These organisms were characterized by their ability to secrete an enzymatic spectrum which separate the compounds in the marine organisms residues. Also they were characterized by building their protein-rich biomass through their metabolism.

Results showed the highest percentage of the total protein in fermented biomass by Micrococcus variants reached to 42.11%. It can be used as a substitute for traditional vegetable and animal protein in animal feed through the application of three fodder mixtures where the produced protein replaces soybean protein and fishmeal protein. Also its effect on the growth of the shrimp Palaemon longirostris was studied.

Most of the studied treatments showed a converging of the results. The best of which was the second mixture where the resulting biomass substituted soybean with fishmeal as protein sources for the results of (CLI %, PER, GGE%). The mean value of the coefficientof increased the length of the carapace was 20.76%, and the protein efficiency ratio was 0.99. The total growth efficiency was 37.45%.

INTRODUCTION

Disposal of marine organisms remains (peels of shrimp, crabs, algae, and fish residues) is an environmental problem, where burning it is not a viable solution, and its natural disintegration is slow. Protein, chitin and calcium carbonate form 90% of dry weight of marine residues (Pal *et al*., 2014). Its use as an immediate food faces several problems, the most important of which is its containment of low protein content and lack of utilization of the nutrients present in it. Therefore, these residues need chemical and biological processes that increase the utilization of their nutrients (Septinova *et al.*, 2012).

Several technologies have been developed to reuse these residues with its important chemical compounds, including single-cell protein production, which is an environment friendly product and can be used or offering in fodder mixtures for shrimps and other farmed animals.

This technology is based on the growth of a microorganism on residues, which helps in recycling it. These organisms are characterized by its high protein content in addition to hydrocarbons, vitamins and amino acids, especially methionine and lysine which are limited in animal and vegetable food (Gour *et al*., 2015).

High cost of the used fodder is one of the main problems of shrimp farming. Where the nutrition of shrimp form about 60% of the production cost in these farms (Pontes *et al.*, 2015). Fishmeal is the best food source for its high protein content of 68%. It is rich in amino acids, fatty acids, vitamins and minerals, but the difficulty of its availability and the high prices make it a difficult food.

One of the reasons for reducing the financial burden of shrimp farms is finding cheap protein sources compared to fishmeal, and have high nutritional values such as vegetable protein and single-cell protein. So it was possible to abandon the fish protein in the shrimp food if a rich source and nutrient of protein is available, which provides food requirements for the animal (Ahmadi *et al.,* 2010).

Shrimp prefers the bottom food in the sea floor, not the floating one. Size of the fodder grains should be between 20-30% of the volume of shrimp mouth, where the very small size can lead to

Corresponding author:* **Izdihar Ammar
Food Biotechnology Department Pharmacy Faculty, Tishreen University, Lattakia, Syria

negative results because a large part of the individual's energy will be lost in the search for the largest number of grains. Whereas the large ones can caused apnea. So it was necessary toregard the size of fodder grains.

The disadvantages of over-fodder are fodder dispersal and increase pollution, which leads to lower levels of dissolved oxygen and therefore increased need to it, in addition to increasing the spread of bacteria and other diseases (Craig & Helfrich, 2009).

Protein is the most important factor in the nutrition of crustaceans for its important role in the growth of crustaceans, survival and high cost on the other hand. Protein nutritional requirements for shrimp vary from one species to another and range from (20-42%) of the provided food (Amaya *et al*., 2007).

The energy increasing ratio in food leads to a decrease in growth treatments due to low protein and intake food and the direction of the processes of metabolism aimed at demolition rather than construction. On the other hand, the high level of fat (high level of energy) reduces the intensity of the stability of the fodder in water and reduces the consumed food while increases the coefficient of protein consumption (ANPU) with increasing the proportion of protein in food (Maghsoudloo *et al.,* 2012).

In this study, marine life residues, accumulated on the coast and from the residues prepared as a food meal, were collected, including crustaceans (crabs and shrimps) to treat them by microorganisms isolated from the same residues. These organisms are characterized by its ability to build their protein-rich biomass by metabolizing the organic compounds in these residues. Several fodder mixtures were applied, where the resulting biomass formed the protein source.Its effect on the growth of one of the shrimp species spread locally was studied, the species is *Palaemon longirostris*, which lives in shallow water near the shoreline along the Syrian coast. This species has an economic importance, is found permanently in the markets of some European and Asian countries and also its small individuals are used as fish bait (Sezgin *et al.,* 2007).

The research was conducted to enrich the bio-marine residues with protein by treating them with microorganisms to use it as a fodder component and application of several fodder mixes and replacing the produced protein apposition soy protein and fish meal. then monitoring its effect on the growth of the species *Palaemon longirostris*.

The importance of this study is due to the high efficiency in converting marine organisms residues from non value matter to a specific protein replaces traditional vegetable and animal protein (soybean and fish meal) in fodder of shrimp.

MATERIALS AND METHODS

Preparation of Marine Organisms Residues for Biochemical Treating

Remains of marine organisms were collected from the coast of Lattakia Governorate (next to the Sports City), which included the remains of crab and shrimp husks. Then washed with water, dried in the drying oven at 60 ° C for 24 hours, then grinded by an electric grinding machine to obtain small sizes granules, and sifted on sieves with different diameters to achieve homogeneity in the size of the crushed residues, and separated into granules with different diameters between 250 µm to 2 millimeters). The hydrolysis methods were used, either with acidic hydrolysis using of HCl (1.25N) or alkaline hydrolysis using of NaOH (1N) (Sibi *et al*., 2013).

Protein Enrichmenton a Solid Nutrient Medium from the Marine Organisms Remains

A colony of *Micrococcus varians* (a marine microorganism which separated marine life residues isolated from marine water) was incubated on the nutrient agar medium for 24-hours at 30 ° C. The colony was harvested using a physiological serum (sodium chloride 0.9% or 9 g / l). 1 ml of the suspension was taken and cells number was calculated usingcell count slide (NEUBAUER). The concentration (10^8 cell/ ml) was used. It was added to the media consisting of the sterile residues which is the remains of the shrimp according to the following percentage: (1:2 w/v) (50% sterile sea water: sterile residues). It was sealed with cotton and cellophane and incubated at 30 ° C for two weeks.After the incubation end, it dried at 60 ° C for 24 hours and stored at 4 ° C for protein analysis using the Keldal method and the pyrite method (AOAC, 2005).

Applianceof Several Fodder mixes and Monitoring their Effect on the Growth of Palaemon Longirostris

Control the Experiment Conditions on the Used Organism

Young members of *Palaemon longirostris* were collected from the natural habitat (Lattakia beach),removed to the prefabricated aquariums in the laboratory, glass basins (35 * 30 * 88 cm). Each of it contains 40 liters of marine water and equipped with oxygen pumps and filters. The initial weight and length of the carapace were measured for each shrimp, and individuals were placed at an average of 10 individuals in the pelvis. The experiment lasted 40 days (during the summer of 2018). In this period, two meals were given, morning and evening (The weight of each meal is 10-15% of the individual's weight).The water was completely changed each week and the conditions were adjusted as shown in Table (1).

Table 1 Conditions of water quality and the followed procedures during the experiment

Number of individuals per basin	10 **individuals**
The amount of sea water in the basin	40litters
Number of fodder times	Twice a day
The amount of fodder each time	10- 15 % of body weight in each time
Experiment long	40 days
Degree of temperature	24- 27 °C
Salinity	37.7
pH	7.5-8
Rate of dissolved oxygen in water	7-6mlg/ L
NO_2	0.009mlg/ L
NO_3	0.16mlg/ L
NH_3	0.115 mlg/ L

Preparation of the Fodder Mixture

Three fodder mixtures were prepared, each containing approximately 38.9% protein. It was well mixed with 100 ml distilled water and dried at 60 ° C. It was grinded to granules with different diameters to fit the size of the shrimp mouth using different diameter sieves (600 Micrometers- 1 mlm) (500- 600 micrometers). Table (2) shows the components of these mixtures.

Table 2 Composition and percentages of applied fodder mixture

Composition and percentages of the first fodder mixture (the total protein rate 38.92 %)		**Composition and percentages of the second fodder mixture (the total protein rate 38.98 %)**		**Composition and percentages of the control fodder mixture (the total protein rate 38.95 %)**	
The produced biomass	45 g (protein rate of it is 18.94 %)	The produced biomass	57 g (protein rate of it is 24 %)	fishmeal	22 g (protein rate of it is 14.98 %)
soybean	45 g (protein rate of it is 19.98 %)	fishmeal	22 g (protein rate of it is 14.98 %)	soybean	54 g (protein rate of it is 23.97 %)
Wheat bran	3g	Wheat bran	3g	Wheat bran	3g
maize	3g	maize	14g	maize	17g
Vegetable oil	3g	Vegetable oil	3g	Vegetable oil	3g
Vitamins and minerals	1g	Vitamins and minerals	1g	Vitamins and minerals	1g

The Studied Growth Treatments

1. WG % (Weight Gain): = (mean final wet weight- mean initial wet weight/ mean initial wet weight) × 100.
2. SGR % (specific growth rate) = (ln average final weight – ln average initial weight) / number of days) × 100.
3. FCR (food conversion ratio) = total dry feed intake (g) / wet weight gain (g)
4. PER (protein efficiency ratio) = wet weight gain (g) / dry protein intake (g)
5. GGE % (Gross growth efficiency) = (The final wet weight - Initial wet weight/ Total weight of consumed fodder) × 100.
6. CLI % (carapace length increase) = (mean final orbital carapace length mean - initial orbital carapace length / mean initial orbital carapace lengt)) × 100. (Maghsoudloo *et al.,* 2012).

RESULTS AND DISCUSSION

The most important characteristic of solid agriculture is the high productivity, simplicity of its requirements and ease of application, which is characterized by the ability of microorganisms to produce an enzymatic spectrum, its metabolism of the composition of the waste of compounds, and the growth on it in abundance, and its ability to increase protein in the mediumcontaining of the residues that grow on it as a substrate or carbon and nitrogen source (Amar, 2001).

Results of the current study showed that the use of agriculture on solid nutrient medium gives a higher biomass and increases the total protein content in the marine life residues before treatment from 18.5% to 42.11% after its fermentation. The increase in protein ratios is due to the growth and widespread of bacteria in the fermented substrate, which also causes an increase in total protein (the protein of the bacteria and the protein of the residues that have not yet broken down).

Effect of fodder mix on the growth of species Palaemon longirostris

Good nutrition considersan essential for high and health productivity.Food given for shrimp should contain proteins, carbohydrates, fats, vitamins and minerals to ensure healthy growth. Table (3) shows the values of the studiedgrowth coefficient(CLI%, WG%, SGR %, FCR, PER, GGE%) when using the first fodder mix. The mean weight increasingwas 1.94 g as the initial weight to 2.53 gas a final weight within 40 days of the experiment

Table 3 Values of the studied standardsof the first fodder mixture

Individuals	**CLI %**	**WG%**	**SGR%**	**PER**	**FCR**	**GGE %**
1	11.57	19.41	2.82	1.21	2.23	44.66
2	25.39	14.36	1.30	0.55	4.86	20.55
3	29.83	28.53	2.52	1.08	2.50	39.92
4	19.60	63.38	2.25	0.96	2.81	35.57
5	6.93	30.30	1.00	0.43	6.32	15.81
6	20.79	45.55	1.02	0.87	3.04	32.8
7	28.57	50	1.02	0.87	3.04	32.8
8	18.18	55.55	1	0.85	3.12	32.00
9	15.11	39.72	0.72	0.61	4.31	23.2
10	19.73	69.64	0.97	0.82	3.20	31.2
mean	19.57	41.64	1.46	0.82	3.54	30.85

Value ofCLI % (carapace length increase) ranged between (6.93 - 29.83%) and the average value of 19.57%. The values of WG % ranged from 14.36 to 69.64%, the average value was 41.64%. SGR % was between 0.72 - 2.82 with an average value of 1.46. FCR attained between (2.23- 4.86) with an average value of 3.54. PERvalues reached to (0.43 - 1.21) with an average value of 0.82.Where GGE % valueswere (15.81 - 44.66%) with an average value of 30.85%.Table (4) shows results of the effect of the second fodder mixture on the growth of studied shrimp, in which the soybean protein was substituted with the produced biomass. The mean weight increase was from 4.80 g as initial weight to 6.41 g as a final weight.

Table 4 Values of the studied standards when using the second fodder mixture

individuals	**CLI %**	**WG%**	**SGR %**	**PER**	**FCR**	**GGE %**
1	23.00	42.78	4.32	1.15	2.54	43.25
2	22.16	31.18	3.62	0.96	3.03	36.25
3	19.21	29.56	3.6	0.96	3.05	36
4	22.66	29.65	3.9	1.04	2.82	39
5	15.11	25.15	4.17	1.11	2.63	41.75
6	17.20	35.22	4.50	1.20	2.44	45
7	19.23	19.50	3.72	0.99	2.95	37.25
8	24.41	28.57	3.00	0.8	3.66	30
9	22.65	27.05	3.62	0.96	3.03	36.25
10	22.01	53.60	2.97	0.79	3.63	29.75
mean	20.76	32.22	3.74	0.99	2.97	37.45

CLI % recorded values between 15.11 - 24.41% with an average value of 20.76%. WG % was between (19.50 - 53.60%) with an average value of 32.22%. The average values of SGR % ranged from 2.97 to 4.50 with an average value of 3.74. FCR attained (2.44- 3.66) with an average value of 2.97. PER recordedvalues between 0.79- 1.20 with an average value of 2.97. GGE % was between 29.75 and 43.25 % with an average value of 37.45% (table 4).Results of the control fodder mixture containing the fishmeal and soybean (Table 5) show the following: mean weight increase was from 4.99 g as an initial weight to 6.47 g as a final weight.

Table 5 The values of the studied standards when using the control fodder mixture

individuals	CLI %	WG%	SGR%	PER	FCR	GGE%
1	15.63	40.99	5.17	1.25	2.12	47
2	20.39	70.61	3.42	0.82	3.21	31.13
3	19.37	37.67	3.92	0.96	2.76	36.13
4	20.79	26.40	3.87	0.93	2.83	35.22
5	16.28	21.25	3.12	0.75	3.52	28.40
6	20.83	32.28	4.10	0.88	2.68	37.27
7	20.39	31.99	4.37	1.06	2.51	39.77
8	16.50	30.87	3.62	0.87	3.03	32.95
9	14.79	33.08	4.42	1.07	2.48	40.22
10	21.07	27.06	3.27	0.79	3.35	29.77
Mean	18.60	35.22	3.92	0.93	2.84	35.78

CLI % values ranged between (14.79 - 21.07%) with an average value of 18.60%.WG% was between (26.40 - 70.61%) with an average value of 35.22%. SGR% values were between 3.12 - 5.17 with an average value of 3.92. FCR reached to (2.12- 3.52) with an average value of 2.84. PERwas between 0.75 - 1.25 with an average value of 0.93. GGE %recorded between (28.40 - 40.22%) with an average value of 35.78%.

Figure (1) shows a comparison between the results of the effect of the three used mixtures on the growth of studied shrimp.There is a convergence in the values of (CLI%) for all mixtures with a slight increase in this parameter when using the second mixture. The second mixture also showed an increase in grossgrowth efficiency (GGE%) and protein efficiency ratio (PER). But for the percentage of (WG)%, the average increase of the first fodder mixture was the best while the control mixture was the best in SGR % and FCR. The mean weight increase was 1.94 g as the initial weight to 2.53 g as the final weight of the first mixture. While the mean weight increase of the second one attained4.80 g as the initial weight to 6.41 g as the final weight. Itwas the best. When the control mixture was used, the mean weight increase was 4.99 g as the initial weight to 6.47 g as the final weight during 40 days of the experiment.

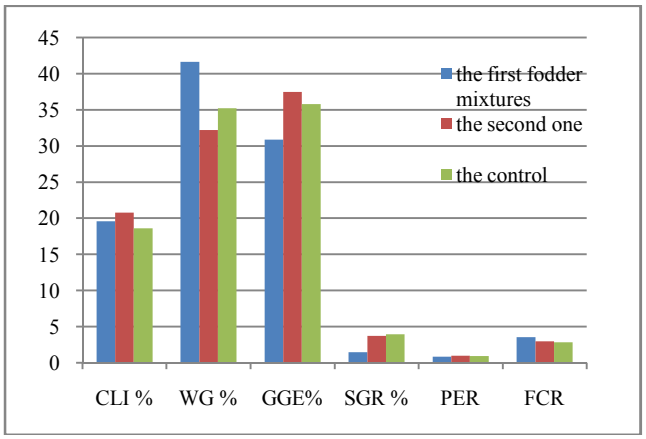

Figure 1 Effect of different fodder mixtures on the average values of the studied growth standards of the species Palaemon longirostris

It is evident from the above that the process of replacing the traditional protein with the produced biomass did not negatively affect the growth of shrimps. Most of the studied treatments showed a close approximation in the results. The best was when using the second mixture where the biomass replaced the soybean with the fishmeal as protein sources (PER, CLI %, GCE %).

Since the current study is the first on the species *Palaemon longirostris*, the results were compared with the results of similar studies on other species of shrimps such as the species *Litopenaeus vannamei* (Maghsoudloo *et al.,* 2012). The protein source was fish meal and soybeans. The values of CLI %were higher and ranged between (47.58-70.07%) and SGR values ranged between (2.60-3.37). While FCR values were (2.43-4.47). PER values were (0.68-1.15) and they were close to the values of our study.

In another study, the effect of fishmeal alone as a protein source with the effect of a marine protein mixture (fish and some marine molluscs) was compared to the growth of another species: *Farfantepenaeus paulensis*. The food consumption and growth rate were better in the mix (FCR = 1.98 and PER = 1.08).While the fish meal alone attained (FCR = 2.69 and PER = 0.76) (Cavalli *et al.*, 2004).

Results of the application of marine yeast as a protein source with a protein ratio ranging from (40.2-55.4%) on the growth of the species *Fenneropenaeus indicus* showed that the best results of the treatments were achieved using protein ratio 44.3%. The ratio attained (FCR = 0.61- 1.45), (SGR = 8.95), (PER = 3.37) (GGE = 165.23) (Sarlin,2005).

In a similar study on the species *Litopenaeus vannamei*, there was an increase in weight from 0.21 g as aninitial weight to 2.10 g as a final weight during 28 days of the experiment where fishmeal and shrimp were the protein source (Mente, 2003). There was also an increase in weight from 0.37 g to 4.23 g within 42 days of the same species using fishmeal as a protein source (Davis and Arnold, 2000). It also increased its weight from 0.36 g to 2.02 g in 30 days using fishmeal and soybeans (Rosas *et al.,* 2001). The single-cell protein was also tested for its effect on the growth of the species *Litopenaeus vannamei.* The food conversion coefficient was between (1.70 - 1.95) (Tlusty *et al.,*2017).

The replacement of fishmeal with single-cell protein in shrimp food was succeedand without notable effect on weight gain, survival rate, and growth standards (Leblanc and Silverman, 2018). The highest value of food conversion coefficients to the species *Penaeus indicus* was (2.12) with protein content of 40% in the used food (Ashokkumar *et al.,* 2011).

Conclusion

1. The highest percentage of total protein in the biomass fermented with bacteria *Micrococcus variants* attained 42.11%
2. The mean weight increase was 1.94 g as an initial weight to 2.53 g as a final weight of the first mixture, while it was 4.80 g as an initial weight to 6.41 g as a final weight of the second one, and it was the best.While for the control mixture, the increasewas 4.99 g as aninitial weight to 6.47 g as a final weight within 40 days of the experiment.
3. Most of the studied treatments showed a close approximation of the results. The second was the best in which the producing biomass replaced soybean with fishmeal as protein sources for the results of (CLI %, PER %, GGE%).The mean value of CLI was 20.76%.The mean value of PER was0.99 and GGE was 37.45%.

The residues of marine organisms accumulated on the beach or from the food industry that can contaminate the environment are valuable raw materials that can be used as medium to reach a protein-rich biomass with a wide spectrum of amino acids in percentages that can be a real substitute for traditional protein, especially plant.

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