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Effects of Avizyme® multi-enzyme addition to diets containing different amounts of canola mealon body composition of western white shrimp (*Litopenaeus vannamei*(*Boone,1931*))

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ABSTRACT

This survey was conducted to quantify the effect of addition of Avizyme® multi enzyme to the body composition of western white shrimp (*Litopenaeus vannamei)*carrying the experiment based on initial average weight of 2.5±0.5 (gr). The experiment was conducted based on absolute casual program including 7 and 3 repetition for each attendants including a control attendants (free- canola, free Avizyme® -multi enzyme and 30% of fish meal) and six attendants at the level of 15,30,45 percent plant protein along with 0.5% Avizyme® multi enzyme and free-Avizyme® multi enzyme In the begging of the period, 525 shrimps were casually introduced in 21 plastic tanks of 50 liter each (25 shrimp in each tank). Shrimps were fed three times a day at 8, 14, 20 o'clock for about 8 weeks in a water with average temperature of 31.60 ± 0.60$C^0$and water salinity of 41 ± 0.8 (ppm).In the begging of culturing process, 40 Shrimps were randomly selected and also at the end, from each repeat 10 shrimps were captured and after de-skinning and freezing they transferred to the laboratory for analyses of body. The results at the end 60 days of period showed that from humidity characteristic point of view and the shrimp body protein, no meaningful statistical differences were observed between the Control and testing attendants (P>0.05). But, From the protein characteristic aspect, fiber and body ash there were meaningful differences based on this, the lipid characteristic in testing attendants 1,2,3 with enzyme and similar attendants without enzyme and the control attendant, there were meaningful differences (P<0.05). The body ash characteristic between attendants 2 with 5 and 3with 6 in relation to control attendant was meaningful statistic differences.

Introduction

Considering the rapid population increase, accessing to food and improving the quality seems essential. Oilseeds after grains are the second major food source in the world[1].Canola is a name stemmed from a variety of modified Canola seed which possess less than 30 micro mole per gram of dried substance and lower oric acid (less than 2%) less than it is found in Canola [2]. Canola is rich in sulfur and amino acids necessary for fish diet food [3]. In the recent years the cultivating Canola and its usage in the aquaculture farmingare increasing and in Iran and the parts of the world[4]. Canola allocates about 4% of protein world trade [5].Canola seed contain 40% oil and the produced from oil extracting process contain 35% to 40% protein [6]. Canola grows mostly in Canada ,U.S.A, Asia, Europe and Australia. Canola, at the present, after Soya is the most important vegetable oil food source and this plant in the last 20 years, has put behind the sunflower, peanut and the cotton seed world productions [6]. The results of research studies have shown that farming expansion of Canola in Iran is possible and by cultivating olive inn parallel can lead to ease down the dependency of vegetable oil. The production of this plant has reached from 50.65 Tons in 1993 to 117.323 thousand ton in 2010. Among the nation's provinces, two provinces, Mazandaran and Golestan with equal share allocate 50% of the total production of Canola in the country. Ardebil and Hamedan Provinces with 13723 and 4784 tons productions respectively are in the third and 4thposition of Canola production in the country[5]. The price of Canola per unit protein is usually less than soya[7].Canola is a good source of necessary minerals compare with other plant oil seeds. Canola is particularly good source of Selenium and Phosphor[8,9]. Shrimp's feed is one of the major factors of increasing cost of shrimp production. Therefore, choosing the right type of shrimp, fast growing rate, resistivity against the unfavorable environmental condition, the conversion nutrition factor, and possibility of using inexpensive feed source is of major signifigance. In this regard, the western white shrimp (*Litopenaeus vannamei)* as a valuable nutritional food source fed from plant protein has been respected [10,11,12,13] The most expensive part of the food is the protein part of it in which the higher level of animal protein has a determinant effect on the economy of production turnover [14].The high level share of plant protein in the *LP.Vannamei* diet compare to other cultured shrimp and in general the low level protein level needed, is the strong point in expanding and breeding of this type [15]. For this reason, *LP.Vannamei* due to its low cost and high compatibility level replaced the other type

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of cultured shrimp in the world [16].What is under consideration now, is the compactness, resistivity against the unfavorable environmental conditions particularly feeding the *LP.Vannamei* diet with animal protein instead of vegetable protein [17].The above statistic shows that the world production of *LP.Vannamei* has constanly increased from 8000 tons in 1980 to 194000 tons in 1998. After a in significant decrease in its production in 1999 and considerable decrease in 2000, due to Whitespot disease in Latin America, a rapid increase in production took place in Asia reaching to 1386000 tons in 2004 and 2,300,000 tons in 2010 [5].Feeding enzymes to shrimps and fishes is one of the major nutritional advances in the aquaculture sector since last few years. Exogenous enzymes are now extensively used throughout the world as additives in animal diets. Also, supplementation with enzymes can help to eliminate the effects of anti nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved performance of fish/shrimps [18,19]. The Researchers have used nutritional enzyme to assist the protein digest and the phosphor and carbohydrate in the canola. [20,21,22].This research shows that the consumption of enzyme will assist a proper digest of canola nutrition particularly the digestibility of dissolvent starch-free Polysaccharide (NSP). Enzymes are assisting tools in making the non-nutritional ingredients inactive and increase the value contents of the vegetable protein in the food. The above researchers also pointed out that adding enzyme to diets could increase the proper food consumption, decrease the cost of food and decrease the excretion of nutritional ingredients to environment [23].

Material and methods

This research was conducted at the country shrimp research port center in Bushehr in the summer of 2011 (july and August). One thousand *LP.Vannamei* needed were taken from the Delvar private aquaculture pool site. First, the shrimps were transferred to 3 tanks of 300 liters each and after one week of adaptation, 525 of shrimp's offspring with an initial average weight of 2.5(gr) were distributed along with 7 attendants having three repetitions for each attendant to 21 plastic tanks of 50 liter. The water of tanks was drained with regard to the turbidity of water up to 50 percent each day before the feeding time. Geodesy of shrimps was done every 15 days after draining 90% of the water out first. Aeration of each tank was done using a stone-air and the period of light exposure was done with 12 hrs of darkness and 12 hrs of natural light. The chemical physics of water such as temperature (3.60 ± 0.6 ^{C}), oxygen (5 ± 0.1ppt), salinity (41 ± 0 .8 ppm) and 7.6 ± 3 pH were all measured during the test period. All the tanks were siphoned off for the excretion and leftover and cleaned completely while every data was recorded.

After setting up the culturing process system, in order to evaluate the effect of enzyme addition to the Canola at all different levels on the growth characteristics, 7 type of diets (3 pilot attendants at three levels of 15,30,45 percent of Canola along with 0.5% enzyme and three pilot attendants at three levels of 15, 30,45 percent free enzyme canola) and one Control attendant (free canola and enzyme with 30% of fish meal) with equal digestible protein and energy were fed to the shrimps. Before the start of test, shrimps were in adaptation plight with a commercial diet for one week and then for 8 weeks they were fed with experimental diet (table 1). Feeding was done manually and based on the body percentage with three daily meals at 8, 14, 20 o'clock. Every morning before feeding, all the food leftovers and excretion were siphoned and unused pallets were siphoned in the 300 micron sieve and their dried weight was measured as an unused feed.

According to the analyses, the amount of ingredients used was such that15, 30, 45 percent of plant protein to be obtained (table 2).After determining the necessary ingredients, making diet feeds by using laboratory equipment at the country's shrimp research center port site at Bushehr took placed. First the prime ingredients were weighed by a digital scale with a precision of 0.01(gr). Before attempting to mix the ingredients, they were milled and passed through a sieve of 300 micron. Then the initial ingredients were weighed and put into a mixer of 2 Kg volume. The ingredients were first mixed up dry for 15 minutes and then 60˚ C water was added with35%of its dry weight, fish oil and soya lecithin were further added. The mixing continued for another 15 minutes. After that the feed was passed through a grounding machine with a head of 2 mm, and then the noodle feeds were spread out with hand on an aluminum foil and put beside of a drying machine. After this stage, the noodle feed were broken into smaller pieces and put into plastic containers in different diet portion and kept in the refrigerator for as long as the research period. The feed packages used for short time consumption were left in the same working area in the plastic containers.

Shrimp can be taken up after two month of breeding. First, weighing each tank of shrimps was done and then at the end of each repetition period the shrimps were selected and were sent to the laboratory for the analyses of their body.

For the analyses purpose, 40 shrimps were selected randomly in the beginning and at the end of the breeding period all the shrimps in the tanks were selected and after de-skinning and grounding, the approximate disintegration based on (AOAC,1995) standard was done[24]. All the aforesaid tests were performed in the nutrition laboratory of the Hooverrash fish and shrimp food production factory. To carry this part, Kjehldal (Kjehldal, 1883) method was used in accordance with (AOAC, 1990) utilizing micro Kjehldal(1883) device[24,25,26]. After three times of distillation of digestive processing and Titration on samples, by using nitrogen percentage formula the samples from each repetition determined and multiplied by 6.25 in order to calculate the crude protein.

To carry this part, an innovative method introduced by (Soxhelet, 1848) in accordance with (AOAC 1990) was performed using Soxhele automatic device made in Sweden. After extrusion of lipids from the sample by solvent and subtracting the secondary weight from the initial weight, a sample of lipid volume was obtained and through ratio calculation the percentage was determined as such[26,27]:

Percentage of Crude Lipid =(Lipid Extrusion/ sample weight)*100

The sample's ash was measured utilizing (AOAC, 1990) method and the Electric furnace. To achieve that, the samples were put in the German Herius furnace of 550 C temperature for 4 hours. The weight of grey color residue was indicative of the volume of sample's ashes and through ratio making, the percentage of this compound was determined as such[26]: percentage of ash weight =(ash weight/sample weight)*100.

To do this measurement, (AOAC, 1990) method was used. Accordingly, samples of Attendants from three repetitions were put in the oven of 105 C for 24 hours and its humidity was calculated as such[26]: Percentage of humidity= (sample initial weight- sample secondary weight)/sample initial weight*100

Variance dual analyses (ANOVA) were used to do these analyses. A comparison of aggregate of attendants with the aid of Tukey Test at the level of 5%($P<0.05$) was carried. The analysis of data was done using SPSS17 and drawing the graphs was done by using Excel under Windows.

Table 1. Research pilot attendants*

Attendants	Control	Attendants1	Attendants2	Attendants3	Attendants4	Attendants5	Attendants6
Type	0%Canola meal&0% Multi enzyme+30% fish meal	15% canola meal with0.5% multi enzyme	30% canola meal with0.5% multi enzyme	45% canola meal with 0.5% multi enzyme	15% canola meal without multi enzyme	30% canola meal without multi enzyme	45% canola meal without multi enzyme

*All the initial materials except Canola and Avizyme®® multi enzyme Were obtained from Hovarash factory in Bushehr province and the canola obtained from Gilan province Vahdat feed factory and the Avizyme® multi enzyme from Ariadaleman factory in Tehran. Avizyme® is a multi purpose multi enzyme which is produced by Biochem Germany which includes 8000 unit/gram Protease enzyme, 600 unit /gram xaylanaz and 800unit /gram Amylase.

Table 2(A): Percentage of using Ingredients &composition of diets

	Control	Attendants 1	Attendants 2	Attendants 3
Item	1- canola meal 0% 2- fish meal 30% 3- shrimp head meal 17% 4- wheat flour 36% 5- Gluten 9% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2%	1- canola meal 15% 2- fish meal 25% 3- shrimp head meal 15% 4- wheat flour 28.5% 5- Gluten 8% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2% 10- Avizyme® multi enzyme 0.5%	1- canola meal 30% 2- fish meal 15% 3- shrimp head meal 12% 4- wheat flour 23.5% 5- Gluten 11% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2% 10- Avizyme® multi enzyme 0.5%	1- canola meal 45% 2- fish meal 5% 3- shrimp head meal 18% 4- wheat flour 19.5% 5- Gluten 14% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2% 10- Avizyme® multi enzyme 0.5%
Total	100%	100%	100%	100%

Table 2(B): Percentage of using Ingredients &composition of diets

	Attendants 4	Attendants 5	Attendants 6
Item	1- canola meal 15% 2- fish meal 25% 3- shrimp head meal 15% 4- wheat flour 29% 5- Gluten 8% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2%	1- canola meal 30% 2- fish meal 15% 3- shrimp head meal 12% 4- wheat flour 24% 5- Gluten 11% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2%	1- canola meal 45% 2- fish meal 5% 3- shrimp head meal 18% 4- wheat flour 20% 5- Gluten 14% 6- soya lecithin 2% 7- fish oil 2% 8- Mineral supplement 2% 9- VitaminSupplement 2%
Total	100%	100%	100%

Table 3.The results of the analysis of different levels of canola meal diets

Item	Control	Attendants 1	Attendants 2	Attendants 3
1	Protein 36.64%	Protein 36.49%	Protein 36.27%	Protein 35.68%
2	Lipid 7.64%	Lipid 7.26%	Lipid 7.98%	Lipid 6.68%
3	Fiber 2.66%	Fiber 3.30%	Fiber 4.47%	Fiber 5.58%
4	Ash 9.12%	Ash 8.33%	Ash 7.66%	Ash 6.70%
5	Moisture 9.10%	Moisture 8.97%	Moisture 8.25%	Moisture 9.10%
6	Nitrogen Free Extract (NFE) 34.84%	Nitrogen Free Extract (NFE) 35.65%	Nitrogen Free Extract (NFE) 35.37%	Nitrogen Free Extract (NFE) 36.26%
Item		Attendants 4	Attendants 5	Attendants 6
1		Protein 36.55%	Protein 36.33%	Protein 35.74%
2		Lipid 7.26%	Lipid 7.98%	Lipid 7.68%
3		Fiber 3.31%	Fiber 4.47%	Fiber 5.59%
4		Ash 8.34%	Ash 7.67%	Ash 6.71%
5		Moisture 9.04%	Moisture 9.35%	Moisture 9.80%
6		Nitrogen Free Extract (NFE) 35.50%	Nitrogen Free Extract (NFE) 36.20%	Nitrogen Free Extract (NFE) 34.48%

Table 4(A).Effect of different levels of Canola meal on the body composition of young western white shrimp (*Litopenaeus vannamei*) (average ± SD) during the 60 days of culturing Process

Body Composition (%)					
Diet testing	Protein	Lipid	Fiber	Ash	Humidity
Beginning of period	21.5 ±0.40^{d}	0.1 ±0.12^{d}	0.6 ± 0.05^{d}	2 ±0.19^{d}	75.1 ± 0.66^{d}
Attendant (1) 15% Canola With enzyme	24.6±0.6^{a}	0.13±0.05^{c}	0.37±0.11^{b}	2.5±0.01^{a}	72.9±1.1^{a}
Attendant (2) 30% Cano With enzyme	23.8±0.9^{a}	0.13±0.05^{c}	0.37±0.06^{b}	2.67±0.29^{a}	73.3±1.2^{a}
Attendant (3) 45% Canola With enzyme	23.9±0.4^{a}	0.53±0.15^{ab}	0.37±0.06^{b}	1.8±0.26^{c}	73.5±1.04^{a}
Table 4(B).Effect of different levels of Canola meal on the body composition of young western white shrimp (*Litopenaeus vannamei*) (average ± SD) during the 60 days of culturing Process.					
Attendant (4) 15% Canola without enzyme	23.5±0.6^{a}	0.3±0.17^{bc}	0.1±0.001^{c}	2.65±0.3^{a}	72.5±0.5^{a}
Attendant (5) 30% Canola Without enzyme	23±0.1^{a}	0.4±0.1^{abc}	0.4±0.001^{ab}	2±0.01^{bc}	72.9±0.06^{a}
Attendant (6) 45% Canola without enzyme	23.5±0.4^{a}	0.23±0.06^{c}	0.1±0.001^{c}	2.33±0.29^{ab}	72.4±0.3^{a}
Control attendant	24.7±3.1^{a}	0.63±0.3^{a}	0.53±0.15^{a}	1.77±0.23^{c}	73.2±0.46^{a}

The numbers in one column designated with alphabet having different statistical meaning (P<0.05).

Result

Generally speaking, the obtained results included food diet disintegration (Table3), shrimp body analyses (Table 4 and diagram 1) and water parameters. The amount of digestive energy of the food rations (the food pallet to be used for the test) was 3500 Kcal equal for each kilogram and the ratio of Calcium to Phosphor was chosen as 1.5 to 1.

During the test period, the water parameters were measured and on the average during the entire period the water temperature was 31.60 ±.60^{C}, the oxygen dissolved in water was 5± 0.1 ppt, and water pH at 7.6± 3, water salinity at 41± 0.8 ppm were also measured. The humidity of body varied from minimum of 72.4 % for the attendant6 to the max73.5 for the attendant 3, the body crude protein from min 23% on attendant 3 to max of 24.7 on Control attendant, the body lipid from min0.13% on attendant 1and 2 to max0.63 on Control attendant. The body ash was also measured from min 1.77 % on Control attendant to max 2.67 on attendant 2 and crude fiber from min 0.1% on attendant 4 and 6 to max .53 % on Control attendant (table 4).

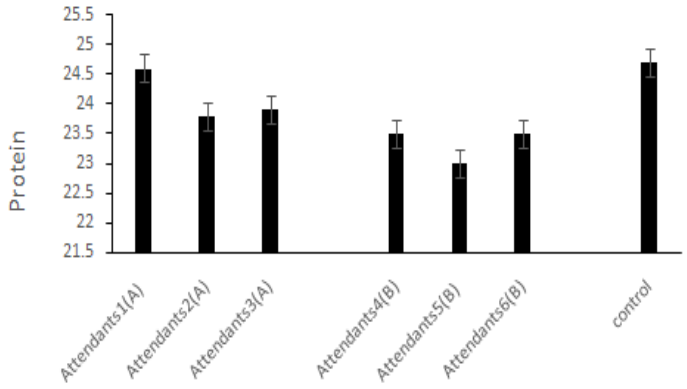

Fig 1: Approximate Percentage of shrimp's body protein (A:with enzyme and B:without multi enzyme)

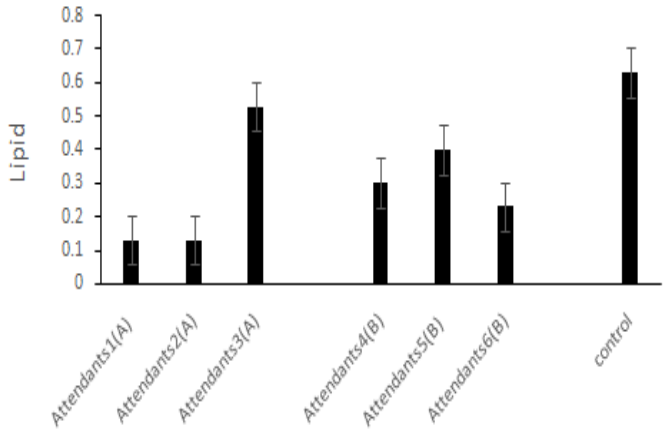

Fig 2: Approximate percentage of shrimp's body lipid (A:with enzyme and B:without multi enzyme)

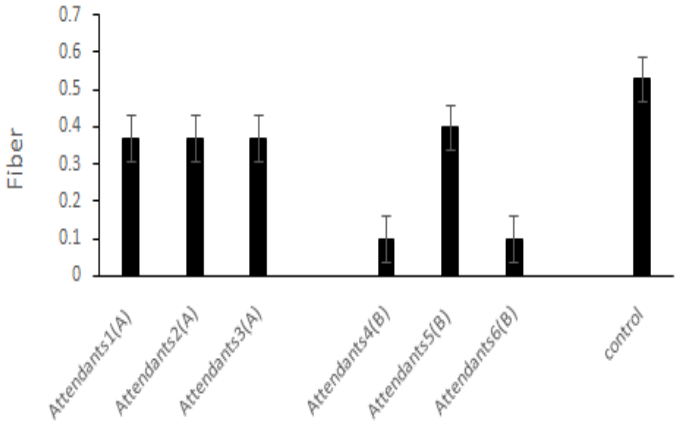

Fig 3: Approximate percentage of shrimp's body fiber (A:with enzyme and B:without multi enzyme)

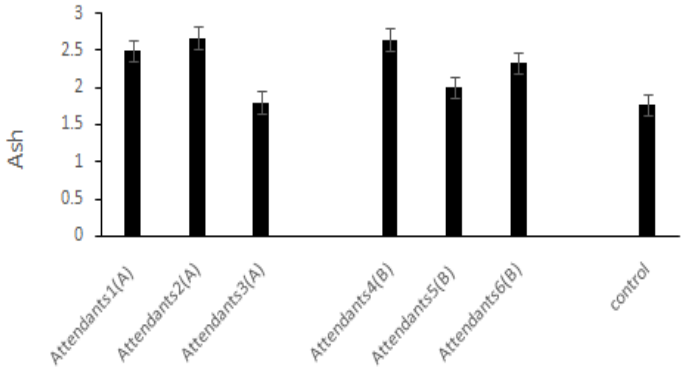

Fig 4: Approximate Percentage of shrimp's body ash (A:with enzyme and B:without multi enzyme)

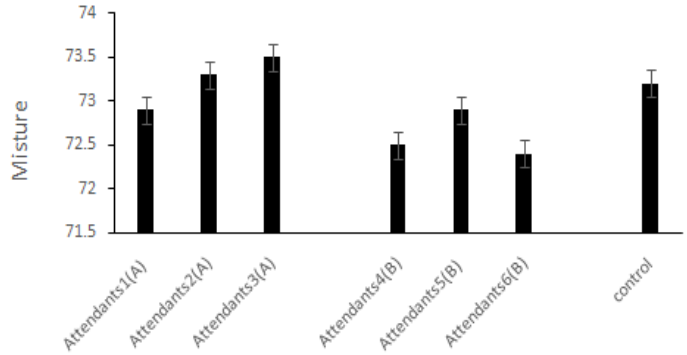

Fig 5: Approximate Percentage of shrimp's body Moisture (A:with enzyme and B:without multi enzyme)

Discussion

In the present research, the rate of protein in attendants' shrimp's body containing Avizyme® multi enzyme and the rate in ones without Avizyme® multi enzyme did not demonstrate any meaningful statistical differences with one another and with the Control attendant. But, with an increase of Canola meal on the feed diet the amount of protein in the body decreased. On the other hand, the presence of enzyme supplements in the attendants with some degree of Canola meal causes an increase in the final protein level in the body composition to the attendants without multi enzyme, but no meaningful statistical differences observed between different attendants and the control ($P>0.05$). In regard to the lipid in the body meaningful differences observed between the attendants with enzyme supplements and the Control attendants and the ones without enzyme supplements either ($P<0.05$). In this regard the increase in Canola meal in the feed diet caused a slight increase of lipid in the body composition. The presence of Avizyme® enzyme supplements in the diets containing the Canola meal caused a decrease of this factor in the tissue compare with the attendants of without supplements enzyme.

Results of several studies indicate that high levels of plant protein sources in the diet can be especially crust aceans such as shrimp*LP. Vannamei*omnivorous species without having a negative impact on growth performance should be used [28,29]. The study showed that30% and 45%canola mealdietwith0/5% Avizyme® multi enzyme 's performance, it has the growth and survival of shrimp body length.In the studies that [30].Factors affecting nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed a plant protein–based diet supplemented with microbial phytase showed the addition of phytase to the diet of rainbow trout caused a significant difference in the amount of ash and minerals (P, Ca, Mg, Fe and Zn), The results is consistent of the present study[31]. The use offish meal with vegetable protein composition of canolameal and soybean meal in the diet (*Marsupenaeus japonicas)*on growth performance and body composition were assessed and reported that 20 to40% of fish meal with a mixture offlour,soybean meal and canola meal without adversely affecting growth, improved performance and enhanced of shrimp body composition, The results correspond this study. (Bautista-Teruel et al,2003) using food as a source of vegetable protein instead of chickpea flour, defatted soy flour in diets for tiger shrimp (*Penaeus monodon*) examined, Reported that the addition of chickpea flour plant protein diets for black tiger shrimp has significant effects on body composition (fat, fiber, ashes), The results of this study are consistent with current research[32].(Felix & Selvaraj, 2004) reported that anti-nutritional factors, have enzymes that can deactivate utilities and increase the nutritional value of feeds by plant protein sources[32]. These researchers have pointed out that the addition of enzymes in foods can increase the food, cost of food and reduce the excretion of nutrients to the environment. (Ricque-Marie et al, 1998) studied Effect of phytase on phosphorus digestibility and protein in western white shrimp shrimp (*Litopenaeus vannamei*), And have reported that the addition of enzyme Add shrimp to phosphorus availability is increased protein digestibility[33]. (Fox et al,2006) The use of phytase in the diet used to feed fish, including fish and shrimp have recommended.Been reported and suggested that these enzymes can be used to access phosphorus diet containing high amounts of plant protein sources increase[34].In (Lim et al, 1997) research on the nutritional value of rich and non rich fiber Canola on the diet of (*Litopenaeus vannamei)* for 56 days reported that using a rich fiber Canola to the level of 30% and low fiber Canola up to15% did not show any undesirable effect on growth characteristic, yet, using higher level of Canola results in slow growth rate and feed consumption by shrimps[35]. In addition, the humidity between the shrimps fed by high level of Canola and different attendants meaningful differences were observed, although not a meaningful differences were observed in terms of lipid, ash and protein among the attendants but by raising the level of protein did cut down the humidity and the highest humidity measured on diets with high level of Canola. These results do not comply with her results of this present research. (Webster et al, 1997) showed in a survey that when soya meal was used instead of Fish meal in the diet of (*Ictalurus furatus*)no meaningful difference body composition including protein, lipid and body humidity produced by different attendants which their diets were replaced with soya meal instead of fish meal was observed[36]. Similar observations (Dsouza et al ,2006) concerning the effect of the substitution soy meal in portions of 20%,40% instead of fish meal in the diet of *(Onchrohynchus mykiss)* was taken which confirms the results of this present research in terms of the body composition[37]. In the present study, the protein content of shrimp body treatments, without multi-enzyme &Avizyme® multi enzyme together with the control treatments were not significantly different, But with the increase in dietary canola meal protein stabilization in the body decreases. On the other hand, there are different levels of enzyme supplementation in the treatment of canola meal protein causes the body to become more without multi enzyme shrimp was compared treatment. The amount of lipid and enzyme supplements contain a statistically significant difference between treatment and control without enzyme supplementation was observed. In this case, the elevated levels of canola meal diets with an insignificant increase in body fat, The presence of enzyme in diets containing canola meal reduced free enzyme treatments were compared in the context of these factors.

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