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Influence of herbal growth promoter AquaPro in *Penaeus monodon* culture system

M. NavinChandran[1], A.M. Suganya[2], P. Berciyal Golda[2], G. Immanuel[2], A. Palavesam[1] *

[1]Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli-627012.

[2]MNP laboratory, Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam-629502, Tamilnadu, India.

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Abstract

Laboratory feeding trial was performed for a duration of 90 days in *Penaeus monodon* post larvae (PL-20) to assess the growth promoting efficacy of AquaPro, a powdered herbal supplement of Indian herbs and research Pvt Ltd). AquaPro was administered along with the basal diet to shrimp at different concentrations (250, 500, 750 and 1000mg/100g) and were marked as F1, F2, F3 and F4 diets, respectively. A control diet (C) lacking AquaPro was also prepared simultaneously and used. The prepared diets were fed to the shrimp twice daily at *ad libitum* for 90 days. The results indicated that the maximum weight gain of 4.60 ± 0.013 g was recorded in F4 diet fed *P. monodon*; whereas, a low weight gain of 2.395 ± 0.014 g was noticed in control diet fed shrimp. The better feed conversion efficiency (FCE) of 73.60 ± 0.415% and specific growth rate (SGR) of 4.501 ± 0.17% were also noticed in F4 diet fed *P. monodon* when compared with control diet fed shrimp. The tested biochemical components in the muscle tissue of *P. monodon* at the end of the experimentation showed marked variation among control and experimental diet fed groups. In conclusion, it is suggested that *P. monodon* fed on F4 diet (1.0g AquaPro/100g basal diet) not only accelerated growth responses but also influenced the tissue biochemical constituents.

Keywords: Aqua Pro, SGR, FCE and FCR.

Introduction

Herbal drugs and herbs are having the capability of acting as growth promoter in addition to offering protective function against infection. Nowdays, the herbal drugs are also gaining popularity in aquacultural practices; since they are cost effective, ecofriendly with nill side effects. Moreover, they can be also used as a safe alternative to antibiotics that have potential negative impacts (Govind Pandey *et al.*, 2012). Even though, hormones have a positive role in growth of prawns their application in aquacultural practices cannot be encouraged due to their residual effects in the cultured organisms (Sambhu and Jayaprakas, 2001). People at global level; have realized the ill effects of antibiotics and are now interested in using natural products (Fanci, 1993). More than 80% of the world's population depends on traditional medicine for their health care (Diallo *et al*., 1999). Further, it was reported that herbal extracts have active principles and also possess antioxidant and anti microbial activities (Kotiya Anil *et al*., 2011; Prasad and Variyar, 1993). Herbal extracts can be administered orally but its effectiveness depends upon the use of correct dosage and hence dose optimization is essential (Yin *et al*., 2006). Because of its antioxidant and antimicrobial activities herbal extracts are widely used as the supplementary feed additive in aquaculture system to enhance disease resistance as well as to promote growth. As growth promoters, even at a very low concentration herbal extracts enhanced feed utilization efficiency (Viola and Aricli, 1987). The growth promoting properties of herbal extract mixture has also been reported by several authors (Citarasu *et al*., 2003; Immanuel *et al*., 2004; Michael Babu *et al*., 2008; Venketramalingam *et al*., 2007). Hence, the present investigation has been performed to test the growth promoting capability of AquaPro in *P. monodon* indoor culture system.

Materials and Methods

Collection of *P. monodon* post larvae

P. monodon post larvae (PL-20) were obtained from the commercial hatchery and they were transported carefully to the laboratory in oxygenated polythene bag with least disturbance. Then the Post larvae were acclimatized in one tonne capacity FRP tank provided with well aerated seawater at 25ppt salinity. The PL were fed on starter feed regularly (CP Nova) for a duration of 10days. The well acclimatized larvae were then used for experimentation.

AquaPro

AquaPro premix is a herbal feed supplement that was formulated and developed by Indian herbs research and supply co ltd, Saharanpur, Uttar Pradesh. It contained natural herbs without any chemical additives and so it was tested in shrimp culture system.

Experimental design

Healthy *P. monodon* post larvae selected from the stock tank were segregated into five groups, each group consisting of 20 individuals and they were reared in 100l capacity glass tanks at 25ppt salinity. The entire experiment was performed in triplicates for a duration of 90 days. One group served as the control whereas, the rest of the four groups served as the experimental (F1-F4). During experimentation, shrimp larvae were fed regularly on respective control and experimental diets at the rate of 2 times per day at *ad libitum*. The control diet contained basal diet and lack AquaPro; whereas, the experimental diets contained AquaPro at 250mg, 500mg, 750mg and 1000mg, concentrations along with basal diet.

Water quality analysis

During the culture period, water quality parameters were maintained at a optimum level by providing 50% of water exchange daily. Temperature was recorded by digital thermometer whereas; salinity and pH of water sample was recorded by salinity refractometer (Atago) and digital pH meter respectively. The dissolved oxygen content and ammonia were also estimated regularly by following the standard method of APHA (1995).

Feed formulation and preparation

Feed ingredients mentioned in Table 1 were used for the preparation of respective control and experimental diets followed by the method described by Navin chandran *et al*., (2014).

Growth responses

Growth responses were measured during the end (90th day) of experimental duration and the overall growth performances were calculated using the formula given below.

$$\text{Growth (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Food Conversion Ratio (FCR)} = \frac{\text{Total amount of feed given (g dry weight)}}{\text{Total production of shrimp (g dry weight)}}$$

$$\text{Specific Growth Rate (SGR \%)} = \frac{\text{In final weight (g)} - \text{In initial weight (g)}}{\text{Experimental period (days)}} \times 100$$

Where ln= natural log.

$$\text{Food Coversion Efficiency (FCE \%)} = \frac{\text{Wet weight of the fish produced (g)}}{\text{Dry weight of the feed given (g)}} \times 100$$

Biochemical analysis

Biochemical analysis such as protein, carbohydrate and lipid were performed in the diet and muscle samples of *P. monodon* following the methodology (Lowry *et al*.,1951; Roe, 1995; Folch *et al*., 1957).

Table -1: Quantity of feed ingredients (g/100g dry weight) used for the preparation of control (C) and experimental diets (F1- F4)

Feed ingredients	Type of diet / Amount of feed ingredients				
	Control	F1	F2	F3	F4
Fish meal (g)	47	47	47	47	47
Soya meal (g)	12	12	12	12	12
Ground nut oil cake (g)	25	25	25	25	25
Rice bran (g)	6	6	6	6	6
Tapioca powder (g)	3	3	3	3	3
Seaweed (g)	2	2	2	2	2
Vitamin and mineral (g)	1	1	1	1	1
Cod liver oil (ml)	2	2	2	2	2
Stickon (ml)	2	2	2	2	2
AquaPro (mg)	-	250	500	750	1000

Results

Biochemical analysis of diet

In the formulated diets, more or less a uniform biochemical constituent was maintained. The protein content varied from 36.40 ± 0.34 to 36.62 ± 0.56% dry weight. Likewise, the carbohydrate and lipids contents were respectively ranged between 12.66 ± 0.12 to 12.80 ± 0.16 % dry weight and 7.20 ± 0.05 to 7.40 ± 0.06 % dry weight (Table 2).

Table -2: Biochemical composition (% dry weight) in diet of *P. monodon* fed on control (C) and AquaPro added experimental diets (F1- F4)

Diets	Biochemical constituents (% dry weight)		
	Protein	Carbohydrate	Lipid
Control	36.40 ± 0.34	12.72 ± 0.14	7.40 ± 0.06
F1	36.62 ± 0.42	12.68 ± 0.08	7.36 ± 0.04
F2	36.45 ± 0.60	12.66 ± 0.12	7.32 ± 0.03
F3	36.62 ± 0.56	12.80 ± 0.09	7.26 ± 0.03
F4	36.60 ± 0.66	12.80 ± 0.16	7.20 ± 0.05

Mean ± SD is the average of three individual estimates

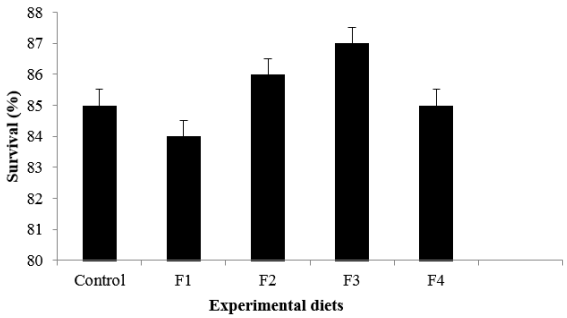

Fig.1: Survival (%) of *P. monodon* fed on control (c) and AquaPro added diets (F1- F4) (Each value is the Mean ± S.D of triplicate analysis)

Water quality parameters

In culture tanks, the temperature ranged between 32.00 ± 0.12°C to 32.00 ± 0.16°C in control (C) and experimental (F1-F4) tanks during the experimentation; whereas, the pH showed very low fluctuations among treatments i.e. it ranged from 8.14 ± 0.17 to 8.27 ± 0.21 in the control and experimental tanks, respectively. The salinity of the culture system was maintained at constant level of 20 ± 0.00ppt throughout the experimentation. The dissolved oxygen content varied between 5.14 ± 0.31mg/l and 5.95 ± 0.36mg/l in culture tanks during the experimentation. The ammonia content was in the range of 0.14 ± 0.012 to 0.29 ± 0.011mg/l (Table -3).

Table -3: Overall water quality parameters recorded in control (c) and experimental tanks (F1- F4) during 90 days of experimentation

Water quality parameters	Control	F1	F2	F3	F4
Temperature **(º C)**	32.00 ± 0.12	32.00 ± 0.14	32.00 ± 0.13	32.00 ± 0.14	32.00 ± 0.16
pH	8.14 ± 0.17	8.21 ± 0.16	8.16 ± 0.19	8.27 ± 0.21	8.17 ± 0.18
Salinity	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
Dissolved oxygen (mg/l)	5.24 ± 0.31	5.44 ± 0.21	5.62 ± 0.32	5.95 ± 0.36	5.14 ± 0.42
NH_3 (mg/l)	0.21 ± 0.014	0.14 ± 0.012	0.27 ± 0.012	0.24 ± 0.013	0.29 ± 0.011

Mean ± SD is the average of three individual estimates

Table- 4. Overall growth responses of *P. monodon* fed on control (c) and AquaPro added experimental diets (F1- F4)

Growth parameters	**Control**	**F1**	**F2**	**F3**	**F4**
Average initial weight (g wet weight)	0.085 ± 0.003	0.080 ± 0.002	0.079 ± 0.003	0.090 ± 0.004	0.080 ± 0.002
Average final weight (g wet weight)	2.480 ± 0.012	2.532 ± 0.013	2.670 ± 0.017	2.90 ± 0.014	4.680 ± 0.013
Growth (g wet weight)	2.395 ± 0.014	2.452 ± 0.012	2.591 ± 0.013	2.810 ± 0.014	4.60 ± 0.013
Food consumed (g dry wt)	4.48 ± 0.12	4.13 ± 0.14	4.80 ± 0.13	4.90 ± 0.15	6.25 ± 0.16
SGR (%)	3.748 ± 0.14	3.838 ± 0.18	3.911 ± 0.14	3.859 ± 0.13	4.501 ± 0.17
FCR	1.870 ± 0.021	1.684 ± 0.014	1.853 ± 0.010	1.744 ± 0.019	1.359 ± 0.024
Growth (%)	2.661 ± 0.031	2.724 ± 0.042	2.879 ± 0.036	3.122 ± 0.041	5.111 ± 0.051
FCE (%)	53.46 ± 0.324	59.37 ± 0.326	53.97 ± 0.414	57.35 ± 0.512	73.60 ± 0.415

Table -5: Biochemical composition (% dry weight) of muscle tissue of *P. monodon* fed on control (C) and AquaPro added experimental diets (F1- F4) during the 90th day analysis.

Experimentation	**Biochemical constituents (**% dry weight)		
	Protein	Carbohydrate	Lipid
Control shrimp	41.53 ± 1.02	20.33 ± 0.82	6.24 ± 0.18
Experimental shrimp (F1)	44.14 ± 1.04	22.14 ± 0.75	6.80 ± 0.24
Experimental shrimp (F2)	46.18 ± 1.07	23.12 ± 0.63	7.21 ± 0.36
Experimental shrimp(F3)	48.02 ± 1.04	23.46 ± 0.72	8.24 ± 0.32
Experimental shrimp(F4)	48.53 ± 1.03	25.51 ± 0.81	8.94 ± 0.36

Mean ± SD is the average of three individual estimates

Overall growth performance of *P. monodon*

P. monodon fed on diets added with various concentrations of Aquapro showed variations between experimental diets concerning growth responses. For instance, the growth ranged between 2.452 ± 0.012g to 2.810 ± 0.014 g/ wet weight in F1 to F3 diets and a maximum weight gain of 4.60 ± 0.013 g/ wet weight was noticed in F4 diet fed *P. monodon.* However, a lower weight gain of 2.395 ± 0.014/ wet weight was attained by control diet fed shrimp. Feed conversion efficiency gradually increased with the increasing concentration of AquaPro, but it was very high 73.60 ± 0.415% in F4 (1000mg) diet fed group. A better FCR of 1.359 ± 0.024 was recorded in F4 diet fed shrimps when compared to control and other AquaPro treated groups. The specific growth rate showed minimum variation between control and F1-F3 treated groups and it was between 3.748 ± 0.14 to 3.911 ± 0.14%; Whereas, it was high 4.5.1 ± 0.17% in F4 diet fed shrimp (Table 4).

Biochemical analysis in muscle tissue of *P. monodon*

Biochemical analysis recorded in the muscle tissue of *P. monodon* showed variations among control and AquaPro added diets as shown in Table 5. The protein content in the muscle tissue of control diet fed *P. monodon* was 41.53 ± 1.02% dry wt; whereas, in AquaPro treated groups, (F1-F4) it ranged from 44.14 ± 1.04 to 48.53 ± 1.03% dry weight with a maximum value recorded in F4 diet fed shrimp. Similar trend was also recorded for carbohydrate and lipid contents. For instance, control diet fed shrimp displayed low carbohydrate and lipid contents of 20.33 ± 0.82% dry weight and 6.24 ± 0.18% dry weight respectively. But in AquaPro (F1-F4) added diets fed shrimp the carbohydrate content ranged between 22.14 ± 0.75% to 25.51 ± 0.81% and the lipid content ranged from 6.80 ± 0.24% to 8.94 ± 0.36% respectively with a maximum value registered by F4 diet fed shrimps.

Survival analysis

Survival of *P. monodon* fed on control and AquaPro treated shrimps (F1-F4) showed minimum variation and it ranged from 84.00 ± 1.72% to 87.00 ± 1.84% as depicted in Fig 1.

Discussion

The results of the present study inferred that AquaPro at a concentration of 1000mg/100g obviously increased the growth performance in *P. monodon*. A linear increase in growth of *P. monodon* was recorded with the raise in concentration of AquaPro in the diet. Hiam El – Desouky *et al.* (2012) reported that *Macrobrachium rosenbergii* fed on different doses of *zingiber officinalis* and *cyanodon dactylon* showed a significant increase in specific growth rate (SGR) and food conversion ratio (FCR) when compared to control diet fed groups. Further, Wu *et al.* (1998) reported that chinese herbal medicine supplemented along with grass carp diet promoted a better weight gain, feed conversion efficiency and specific growth rate. Also in consistence with the above studies, *P. monodon* fed on AquaPro at a concentration of 1% showed a significant increase in SGR and FCR i.e. 4.501 ± 0.17% and 1.359 ± 0.024 respectively.

Keshwanth and Jayaram (1998) reported that prawn post larvae fed on nurtipro aqua significantly increased growth and final average weight. Kotiya Anil *et al.* (2011) also reported that plant extracts have tremendous role in improving the aquatic animal's growth. Earlier studies have also demonstrated the growth promoting capability of herbal products in fishes (Shadakshari, 1993; Unnikrishnan, 1995 and Jayaprakash and Euphrasia, 1997). Parallel to the above findings in the present study AquaPro fed on F4 diet displayed a maximum weight gain of 4.60 ± 0.013g wet weight. In correlation with this result, Seung – cheol *et al*. (2007) reported that addition of different herbal extracts led to elevated growth performance in red Seabream *Pagrus major*. Citarasu *et al*. (1998) concluded that *P. indicus* post larvae fed on herbal products stressol I and II resulted in an increased survival and growth but in our present investigation *P. monodon* post larvae fed on control and AquaPro treated groups did not show much variation with respect to survival but the growth performance alone showed variation.

Feed conversion efficiency (FCE) is an important factor indicating food utilization (Jayaprakash and Euphrasia, 1997). In the present investigation FCE was higher 73.60 ± 0.415% in F4 diet fed shrimp and a comparatively lower FCE of 53.46 ± 0.324% was registered in control diet fed *P. mono*don. Herbs may be incorporated along with diets as a mixture or as an individual supplement (Czeh *et al*., 2009). Herbs have a promising role in aquaculture and many reports are available regarding their growth promoting potential in fishes (Direkbusarakom, 2004; Sasmal *et al*., 2005). The positive role of AquaPro in improving growth performance in *P. monodon* can be correlated to NutiPro-aqua which is a soya based herbal growth promoter that enhanced the digestive enzyme activity and finally led to the improved growth performance in *Macrobrachium rosenbergii* (Keshawanth and Jayaram, 2000). Similar reports were shown by Shadakshari (1993) and Jayaprakash and Euphrasia (1997) on livol (IHF-1000) that consisted of a consortium of different plant ingredients, which were significantly increased the digestion and led to a better production in cultivable fishes.

Dada and Olughemi (2013) reported that *Clarias gariepinus* fingerlings treated on commercial feed additives AquaPro and Aquabooster led to a better growth performance. *Labeo rohita*

fed on diet containing Immuplus, a polyherbal formulation enhanced the growth, total protein and globulin (Kumari *et al*., 2007). Likewise, in the present study *P. monodon* fed on AquaPro a herbal supplement showed enhancement in tested biochemical components. Accordingly, the protein content in the muscle tissue was higher in F4 diet fed *P. monodon* 48.53 ± 1.03% dry weight during the 90th; whereas, in control diet fed *P. monodon* it was low i.e. 41.53 ± 1.02% dry weight. However, in the other AquaPro treated groups (F1-F3) the muscle protein content was higher and it ranged from 44.14 ± 1.04 to 48.02 ± 1.04% dry weight when compared to control diet. The carbohydrate and lipid contents also showed a similar variation. Accordingly, in control diet fed *P. monodon* it was 20.33 ± 0.82% dry weight and 6.24 ± 0.24% dry weight respectively; whereas, in (F1-F4) treated groups it ranged from 22.14 ± 0.75 to 25.51 ± 0.81% dry weight and 6.80 ± 0.24 to 8.94 ± 0.36% dry weight respectively with a maximum value attained by F4 diet fed shrimp. Further investigation is warranted in optimizing the dose of AquaPro that will promote both the growth performance and immune enhancement in shrimps.

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