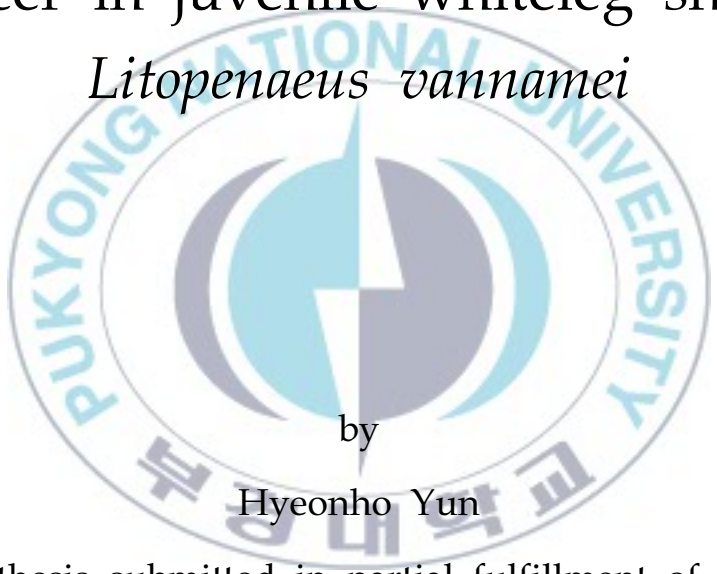
Thesis for Degree of Master of fisheries science

Reevaluation of the dietary protein requirement and the effects of dietary PROTIDE® as a fishmeal replacer in juvenile whiteleg shrimp, *Litopenaeus vannamei*



by

Hyeonho Yun

A thesis submitted in partial fulfillment of the requirement for the Degree of Master of Fisheries Science in the Department of Fisheries Biology, Graduate School Pukyong National University

February 2012

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치하기 흰다리 새우 *Litopaeneus vannamei*에 있어서 사료내 단백질 요구량 재평가와 PROTIDE®의 어분 대체 효과에 관한 연구

Advisors : Prof. Sungchul C. Bai and

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Reevaluation of the dietary protein requirement and the effects of dietary PROTIDE® as a fishmeal replacer in juvenile whiteleg shrimp, *Litopenaeus vannamei*

A Dissertation

by

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Contents

요 약 문

본 논문은 국내 주요 양식 어종인 흰다리 새우에 있어서 반 순환 여과 시스템을 사용하여 최대 성장을 위한 사료내 단백질 요구량의 재규명과 PROTIDE®의 어분 대체원으로써의 효과를 평가하고자 수행되었다. 6주간의 실험 후 증체율과 Broken line analysis의 결과 사료내 최적의 단백질 요구량은 43.3%보다는 크고 44%보다는 작을 것으로 판단되었으며, 9주간의 실험 후 증체율, 일간성장률, 사료전환효율, 단백질전환효율과 생존율의결과 치하기 흰다리 새우용 배합사료내 PROTIDE는 어분을 10~20%까지 대체 할 수 있을 것으로 판단되었다.

실험 1: 치하기 흰다리 새우에 있어서 사료내 단백질 요구량 재평가

총 6주간의 본 실험은 반 순환여과 시스템에 있어서 치하기 흰다리 새우의 최대 성장을 위한 사료내 단백질 요구량을 재평가하기 위하여 실시하였다.

1주간의 예비 사육 후, 평균무게 1.0 ± 0.1g (mean ± SD)인 치하기 흰다리 새우를 사용하여 가용 에너지가 17.0kJ로 동일한 4개의 사료(조단백함량 33%, 38%, 44%, 54%)를 제작하였다. 사료는 건중량 기준으로 어체중의 약4%를 공급 하였으며 각각의 구간마다 3반복으로 한 탱크당 30미씩 사육하였다.

6주간의 사육 후, CP44구와 CP54구는 CP33구보다 유의하게 높은 증체율과 일간성장률을 보였지만, CP44와 CP54구들 간에는 유의한 차이가 없었다. 단백질 전환효율에 있어서 CP33, CP38구와 CP44구는

CP54구에 비해 유의하게 높은 값을 보였지만, 서로간에 유의한 차이가 없었다. 사료전환효율에 있어서 CP44구는 CP33구에 비해 유의하게 낮은 값을 보였지만, CP33, CP44구와 CP54구들 사이에는 유의한 차이가 없었다. 전어체와 가식부 조단백질 분석, 가식부 아미노산 분석 결과 CP38%구, CP44%구와 CP54%구는 CP33%구보다 높은 단백질, Glu, Ile와 Leu값을 보였지만, CP38구, CP44%구와 CP54%구들 사이에는 유의한 차이가 없었다. Broaken line analysis의 결과 최적 성장을 위한 사료내 단백질 함량은 43.3%인 것으로 나타났다.

따라서, 흰다리 새우 치하의 최적 성장을 위한 사료내 단백질 함량은 43.3%보다는 높고 44%보다는 낮을것으로 사료된다.

실험 2 : 치어기 흰다리 새우에 있어서 사료내 PROTIDE®의 어분 대체 효과

총 9주간의 실험은 반 순환여과 시스템에서의 치하기 흰다리 새우 사료내 어분 대체원으로써 PROTIDE®(PRO)의 어분대체효과를 평가하기 위함이다. 조단백이 45%, 가용 에너지가 16.4kJ로 동일한 5개의 사료를 제작 하였다. 1주간의 예비 사육 후, 평균무게 0.15 ± 0.02g (mean ± SD)인 치하기 흰다리 새우를 사용하여 한 수조당 50마리씩 4반복배치 하였으며 사료는 어체중의 7%를 건조중량 기준으로 공급하였다. 실험 사료중 20%의 어분은 PROTIDE로 각각 0%, 10%, 20%, 30%, 40% (PRO_0, PRO_2, PRO_4, PRO_6, PRO_8)씩 대체 되었다.

9주간의 사육 후, PRO_0구와 PRO_2구는 PRO_6구와 PRO_8구에 비해 유의하게 높은 증체율과 일간성장률을 보였지만, PRO_0, PRO_2와 PRO_4구들 간에는 유의한 차이가 없었다. PRO_0과 PRO_2구는 PRO_6구

와 $PROP_8$구에 비해 유의하게 높은 단백질전환효율을 보였다. PRO_6구와 PRO_8구는 PRO_0구와 PRO_2구에 비해 유의하게 높은 사료전환효율과 생존율을 보였지만, 생존율에 있어서 PRO_0구, PRO_2구와 PRO_4구들, 그리고 PRO_4구, PRO_6구와 PRO_8구들 간에는 유의한 차이가 없었다.

따라서, 증체율, 일간성장율, 사료전환효율, 단백질전환효율과 생존율의결과 치하기 흰다리 새우용 배합사료내 PROTIDE는 어분을 10~20%까지 대체 할 수 있을 것으로 판단된다.

Reevaluation of the dietary protein requirement and the effects of dietary PROTIDE® as a fishmeal replacer in juvenile whiteleg shrimp, *Litopenaeus vannamei*

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Abstract

These present studies were conducted to reevaluate the dietary protein levels for the maximum growth and to evaluate the optimum dietary levels of PROTIDE® (PRO) as a fish meal replacer for juvenile whiteleg shrimp, *Litopenaeus vannamei*, reared in the semi-recirculation system. After six weeks of first feeding trial observations demonstrated, the optimum dietary protein levels could be greater than 43.3%, but less than 44% based on the weight gain

and broaken line analysis, and after nine weeks of second feeding trial, PROTIDE® could be used to replace the fish meal higher than 10%, but less than 20% based on the weight gain, feed conversion ratio, protein efficiency ratio and survival rate.

Experiment 1. Reevaluation of the dietary protein requirement in juvenile whiteleg shrimp, *Litopenaeus vannamei*

A six weeks of feeding trial was conducted to reevaluate the dietary protein levels for the maximum growth of juvenile white leg shrimp, *Litopenaeus vannamei*, reared in the semi-recirculation system at FFNRC. After 1 week of the conditioning period, one of four isocaloric (17.0 kJ energy g^{-1} diet) diets containing 33, 38, 44, and 54% crude protein (CP) was fed to shrimp at approximately 4% of wet body weight on a dry matter basis to triplicate groups of 30 shrimp averaging 1.0± 0.1g (mean ± SD). After 6 weeks of the feeding trial, weight gain and specific growth rate of shrimp fed 44% and 54% CP diet were significantly higher than those of shrimp fed 33% diet ($P<0.05$), but there were no significant difference among the shrimp fed 44% and 54% diets ($P>0.05$). Protein efficiency ratio of shrimp fed 33%, 38% and 44% CP diets were significantly higher than those of shrimp fed 54% diet ($P<0.05$), but no significant difference among the shrimp fed 33%, 38% and 44% CP diets ($P>0.05$). Feed conversion ratio of shrimp

fed 44% CP diet was significantly lower than those of shrimp fed 33% CP diet ($P<0.05$), But no significant difference among those of shrimp fed 38%, 44% and 54% diets ($P>0.05$). Glu, Ile and Leu of edible portion amino acid compositions and whole body protein contents of shrimp fed 38%, 44% and 54% diets were significantly higher than those of shrimp fed 33% CP diet ($P<0.05$), however, there were no significant difference among those of shrimp fed 38%, 44% and 54% CP diets ($P>0.05$). Broken line analysis of weight gain indicated that 43.3% protein is the optimum dietary level to promote maximum growth in juvenile shrimp. Therefore, our findings suggested that the optimum dietary protein level could be greater than 43.3%, but less than 44% for maximum growth in juvenile whiteleg shrimp.

Experiment 2. The effects of dietary PROTIDE® as a fishmeal replacer in juvenile whiteleg shrimp, *Litopenaeus vannamei*

A nine weeks of feeding trial was conducted to evaluate the optimum dietary levels of PROTIDE®(PRO) as a fishmeal replacer in juvenile whiteleg shrimp, *Litopenaeus vannamei* diet. Five experimental diets were formulated to be isonitrogenous (45% crude protein, CP) and isocaloric (16.4 kJ energy g^{-1} diet). After 1 week of

feeding trial, quadruplicate groups of 50shrimp averaging 0.15±0.02g (mean ± SD) were fed one of 5 experimental diets at approximately 7% of wet body weight on dry matter basis. Experimental diets containing 20% of fishmeal were formulated with PROTIDE replacing 0%, 10%, 20%, 30% and 40% of fishmeal protein (PRO_0, PRO_2, PRO_4, PRO_6, PRO_8, respectively). After 9 weeks of feeding trial, weight gain and specific growth rate of shrimp fed PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed PRO_6 and PRO_8 diets ($P<0.05$), but there were no significant difference among those of shrimp fed PRO_0, PRO_2 and PRO_4 diets, and among those of shrimp fed PRO_4, PRO_6 and PRO_8 diets ($P>0.05$). Protein efficiency ratio of shrimp fed PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed PRO_6 and PRO_8 diets ($P<0.05$). Feed conversion ratio (FCR) and survival rate of shrimp fed PRO_6 and PRO_8 were significantly higher than those of shrimp fed PRO_0 and PRO_2 diets ($P<0.05$), but no significant difference among survival rate of shrimp fed PRO_0, PRO_2 and PRO_4, and among survival rate of shrimp fed PRO_4, PRO_6 and PRO_8 diets ($P>0.05$). Therefore, these results clearly indicated that PROTIDE® could be used to replace the fish meal higher than 10%, but less than 20% based on the weight gain, feed conversion ratio, protein efficiency ratio and survival rate in juvenile whiteleg shrimp.

Ⅰ. Introduction

A massive expansion of farming areas along with the intensification has lead to the dramatic increase in the annual domestic production of whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931) from a nil value 661MT in 2006 to 2,705MT in 2010 (KOSTAT 2011). The global production of whiteleg shrimp has increased from 146,362MT in 2000 to 2,329,534MT in 2009 (FAO, 2010), and receiving increasing attention worldwide as a potential species of intensive farming in ponds (Hopkins et al., 1993; Burford et al., 2003), raceways (McAbee et al., 2003; Browdy & Moss 2005) and in floating cages (Zarain-Herzberg et al., 2010). Farmers prefer raising the pacific white shrimp, because the species is easier, quicker, and more profitable to grow than other commonly farm-raised shrimp (Hedlund 2007). The excellent disease resistant quality compared to other shrimp species has encouraged several shrimp farmers along the coast line to shift to whiteleg shrimp farming.

Paralleling the growth of the shrimp industry has been an expansion in feed production, production intensity and increase in the levels of feeding. While some commercial shrimp diets are widely used and very popular among shrimp farmers, it is doubtful whether any of these can be considered as a true complete feed. Growth rates of tank-reared shrimp that depend on prepared diets

for 100% of their nutritional needs do not match the growth rates that are commonly observed in natural pond environments.

The dietary protein requirement of penaeid shrimp is an important nutritional factor and considered as is a major limiting nutrient for growth. Protein is one of the primary cost components of prepared feeds, which account for over 50% of the production cost in modern shrimp farming. Additionally, protein content of the feed and dietary availability can affect water quality via nitrogen excretion. Protein that is utilized for energy and not deposited for growth contributes to the release of nitrogen metabolites into the culture medium (Cho et al., 1994). Several studies estimated the optimum protein requirement of *L. vannamei* and the reported level varied widely between 20% and 55% (Colvin & Brand 1977; Smith et al., 1984; Aranyakananda & Lawrence 1993; Kureshy & Davis 2002). Existing a wide variation in reported protein requirement for whiteleg shrimp leads to contradiction and confusion for shrimp farmers. Protein requirements of the shrimp vary highly with respect to changes in the biotic and abiotic factors present in the system (Guillaume 1997). Dietary characteristics such as protein quality, processing method, nutrient digestibility and nutrient ratios may also affect the requirements of protein (Guillaume 1997). Apart from all these factors, pollutions which leads to continuously changing environment is one of the vital factors affecting the nutritional requirement of animals including shrimps. Therefore it is needed to reevaluate and find out a correct level of protein,

required to promote the optimum growth and survival of juvenile whiteleg shrimp.

On the other side, expansion of world shrimp production in combination with other economic factors has led to a reduction of shrimp prices in domestic as well as international sea food market. Market experts predict these trends to continue. Therefore it became imperative to reduce the production cost. The ongoing trends to replace fishmeal with animal by-products meals and plant based meals gaining momentum with a view to develop "environment-friendly" feeds containing the least amount of protein necessary for optimal growth. To develop better feed management practice and improve the economics of feeds in shrimp farming has received attention worldwide. One possible solution could be the formulation of diet with high quality but lower cost ingredients, preferably from locally available resources. The formulation of nutritionally rich but inexpensive diet can be achieved by utilizing and experimenting upon a variety of locally available low priced materials (Usha Goswami and Goswami, 1982).

Fishmeal (FM) has traditionally been used as the major protein source for marine feeds because it is an excellent source of essential nutrients such as protein and indispensable amino acids, essential fatty acids, cholesterol, vitamins, minerals, attractants and unidentified growth factors (Swick et al., 1995; Samocha et al., 2004). But it is one of the most expensive ingredients in formulated

shrimp diets. Expansion of world shrimp production in combination with other economic factors has led to a reduction of shrimp prices in domestic as well as international sea food market. Market experts predict these trends to continue. Therefore it became imperative to reduce the production cost. To develop better feed management practice and improve the economics of feeds in shrimp farming has received attention worldwide. Lee and Bai (1997) noted that the world supply of fish meal increased only about 27% during the past 20 years and fish meal output by the major fish meal producing countries actually declined. Because of limiting supply of fish meal around the world, the cost of producing fish would be expected to increase. The success of the aquaculture industry will depend in part on reduction in fishmeal (FM) use in fish feeds. The ongoing trends to replace fish meal with animal by - products meals and plant based meals gaining momentum with a view to develop "environment-friendly" feeds containing the least amount of protein necessary for optimal growth. Several studies have been conducted to replace or reduce its inclusion level in fish and shrimp diets by various inexpensive alternative plant protein sources (soybean meal, corn gluten meal, soy protein concentration etc) and animal protein sources (meat and bone meal, blood meal, feather meal, poultry by-product meal, lysine by product etc). Partial replacement of fishmeal in fish feed (Bai et al., 1997; Carter and Hauler, 2000; Lee and Bai, 1997 ; Lee et al., 2002) and considerable success in partially or totally replacing FM with plant protein sources in various shrimp species (Lim and Dominy, 1990; Piedad-Pascual et

al., 1990; Penaflorida, 1995; Eusebio and Coloso, 1998; Sudaryono et al., 1999; Alvarez et al., 2007) has been reported. Complete replacement of fishmeal with other protein sources reported to produce low growth rates, especially in crustaceans and carnivorous fish, may be due to the poor digestibility and variable quality of alternative protein source.

As an alternative strategy to antibiotics, many reports have been published regarding application of probiotics in feeding and health management in aquaculture. However, because of high cost, potential impact on the environment, regulatory issues, food safety and challenges regarding incorporation into modern extruded feeds, large-scale application of probiotics in the water has been limited. Nucleotides have essential physiological and biochemical functions including encoding and deciphering genetic information, mediating energy metabolism and cell signaling as well as serving as components of coenzymes, allosteric effectors and cellular agonists (Carver and Walker, 1995; Cosgrove, 1998). The roles of nucleotides administered by feeding have been debated for many years. Nucleotides have been considered as non-essential nutrient, because neither overriding biochemical malfunctions nor classical signs of deficiency are developed in human or animal models. However the conception has been challenged by successive research publications suggesting that dietary nucleotide deficiency may impair liver, heart, intestine and immune functions. Nucleotide supplementation has

been one important aspect of research on clinical nutrition and functional food development for humans. Therefore, it has been considered that study on nucleotide supplementation in shrimp diets is an important issue to verify its potential.

Although initial efforts in the evaluation of dietary supplementation of nucleotides for fishes could be traced to the early 1970s, research at that time had mainly focused on the possible chemo-attractive effects of these compounds (Mackie, 1973; Kiyohara et al., 1975; Mackie and Adron 1978). Worldwide attention on nucleotide for fishes was aroused by the reports of Burrels et. al., 2001a, who found that dietary supplementation of nucleotides enhanced resistence of salmonids against viral or bacterial disease. To date, research pertaining to nucleotide in fish nutrition has shown consistent and encouraging results for fish health management, although exact mechanism of nucleotides remain hypothetical and systematic research on fish as far from complete (Li and Gaclin III, 2006; Li et al., 2004a, b). Research on nucleotide nutrition in shrimp is needed to provide insights concerning interactions between nutrition and physiological responses as well as practical solutions to reduce basic risks from infectious diseases with a view to reduce the fishmeal level and reduce the feed cost.

PROTIDE® is commercially popular as fermented IMP (inosine monophosphate) by product, has been demonstared as a good replacer of fishmeal to inhance the growth rate and stimulate the

immune responses in olive flounder, *Paralichthys olivaceous* and red sea bream, *Pagrus major*. Various experiments conducted to replace fishmeal with PROTIDE, emphasizing to investigate its potential in shrimp nutrition also. PROTIDE is residual product after extracting the energy through sugarcane oxidation. It is cheaper than fishmeal and nutritionally rich with 63.6% of protein concentrate, 8.8% of lipid, 10.0% of ash ,2.8% of fiber, considerable amino acid profiles and specially 2% of nucleotide.

Therefore, in first experiment four isocaloric diets containing 33, 38, 44, and 54% crude protein (CP) were formulated and 6 weeks of feeding trial was conducted to reevaluate the dietary protein requirements of juvenile whiteleg shrimp, *Litopenaeus vannamei.* The present study also aimed at the development of nutritionally balanced and environment-friendly but inexpensive formulated feed to promote the optimum growth and survival of juvenile whiteleg shrimp.

In second experiemnt, five experimental diets were formulated and 9 weeks of feeding trial was conducted to study the effects of dietary PROTIDE® as a fishmeal replacer in juvenile whiteleg shrimp, *Litopenaeus vannamei.*

Ⅱ. Reevaluation of the dietary protein requirement in juvenile whiteleg shrimp, *Litopenaeus vannamei*

abstract

A six weeks of feeding trial was conducted to reevaluate the dietary protein level for the maximum growth of juvenile whiteleg shrimp, *Litopenaeus vannamei*, reared in the semi-recirculation system at FFNRC. After 1 week of the conditioning period, one of four isocaloric (17.0kJ energy g^{-1} diet) diets containing 33, 38, 44, and 54% crude protein (CP) was fed to shrimp at approximately 4% of wet body weight on a dry matter basis to triplicate groups of 30 shrimp averaging 1.0± 0.1g (mean ± SD). After 6 weeks of the feeding trial, weight gain and specific growth rate of shrimp fed 44% and 54% CP diet were significantly higher than those of shrimp fed 33% diet ($P<0.05$), but there were no significant difference among the shrimp fed 38%, 44% and 54% diets ($P>0.05$). Protein efficiency ratio of shrimp fed 33%, 38% and 44% CP diets were significantly higher than those of shrimp fed 54% diet ($P<0.05$), but no significant difference among the shrimp fed 33%, 38% and 44% CP diets ($P>0.05$). Feed conversion ratio of shrimp fed 44% CP diet was significantly lower than those of shrimp fed 33% CP diet ($P<0.05$), But no significant difference among those of shrimp fed 33%, 44% and 54% diets ($P>0.05$). Glu, Ile and Leu of

edible portion amino acid compositions and whole body protein contents of shrimp fed 38%, 44% and 54% diets were significantly higher than those of shrimp fed 33% CP diet ($P<0.05$), however there were no significant difference among those of shrimp fed 38%, 44% and 54% CP diets ($P>0.05$). Broken line analysis of weight gain indicated that 43.3% protein is the optimum dietary level to promote maximum growth in juvenile shrimp. Therefore, our findings suggested that the optimum dietary protein level could be greater than 43.3%, but less than 44% for maximum growth in juvenile whiteleg shrimp.

Materials and Methods

Experimental Design and Diets

The experiment was conducted at the Institute of Fisheries Science, Pukyong National University, Busan, Korea. The compositions of experimental diets are shown in Table 1. Four experimental diets containing fishmeal, dehulled soybean meal, squid liver powder and wheat gluten meal as a protein sources were prepared to contain protein levels at 33%, 38%, 44% and 54% at the expense of wheat flour, soybean oil and fish oil. The diets were formulated to be isocaloric, containing 17kJ g^{-1} energy based on calculation (Garling & Wilson1976; NRC 1997). Procedures for diet preparation and storage were followed as previously described by Bai & Kim (1997). After thoroughly mixing the dry ingredients, soybean oil and fish oil together with 30% well water were added and further mixed to make a mash. Laboratory pelletizing machine was used to obtain uniform pallets of 2mm-diameter sizes. The formulated diets were stored at -20°C (Wet pellets) until use.

Animal husbandry and Experimental design

Juvenile whiteleg shrimp were procured from Tae-an hatchery of the National Fisheries Research & Development Institute. Before the starting the experiment, all shrimps were reared in a 1000L tank and fed *adlibitum,* a commercial shrimp diet for 1 week. After 1 week of conditioning period, 30 Shrimp measuring initial averaging 1.0± 0.1g (mean ± SD) were randomly distributed into each of 12

plastic aquaria (30-L) and covered with plastic. Each aquaria was randomly assigned to one of the three replicates of the four dietary treatments. Semi-recirculation system was used in the experiment and water flow was maintained at 3L/ minutes. The diets were fed to triplicate groups of fish to satiation, approximately at 4% of total body weight per day for 6 weeks. Shrimp were fed 4 times a day at 9:00, 12:00, 15:00 and 18:00, seven days a week. Total shrimp weight in each aquarium was determined every 2 weeks and the feeding rate was adjusted accordingly. Water temperature was maintained at 25°C by using a heater. Dissolved oxygen was maintained above 6.0 mgL^{-1}, salinity 32±1 psu and the pH 7.5± 0.3, in during the experiment.

Siphoning was done every morning in each rearing tank; dead shrimp were removed, weighed, and recorded daily.

Sample collection and analysis

At the end of the feeding trial, all shrimp were weighed and counted to calculate percentage weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate. Proximate composition analysis of the experimental diets, whole body and edible were performed by the standard methods of AOAC (1995). Samples of the diets and shrimp were dried at 105°C to a constant weight to determine their moisture content. Ash content was determined by incineration at 550°C. Protein was determined using the kjeldahl method (N x 6.25) after acid digestion, and crude lipid was ascertained by soxhlet

extraction using the soxhlet system 1046 (Tacator AB, Sweden) after freeze-drying the samples for 20h. Amino acid analysis of edible protion was were performed by ninhydrin method (Sykam Amino Acid Analyzer S433, Sykam; Eresing, Germany).

Statistical Analysis

All data were analyzed by one-way ANOVA (Statistix 3.1; Analytical Software, St. Paul, MN, USA) to test the effects of the dietary treatments. When a significant treatment effect was observed, an LSD test was used to compare means. Treatment effects were considered at $P<0.05$ level of significance. Broken line analysis (Robinson Norton & Baker, 1979) was used to test the dietary protein requirement (SAS procedure NLIN, 1996).

Results and Discussion

Growth performance

Growth trial was conducted without interruption, water quality, or any disease problems. The experimental diets were well accepted by the shrimp; no rejection of the feed was observed. After 6 weeks of the feeding trial, WG of shrimp fed the CP33 and CP38 diets were significantly lower than those of shrimp fed CP44 diet, but there were no significant difference among the shrimp fed, CP44 and CP54 diets (Table 2). The clear trend linear increase in weight gain with the increase in protein concentration up to 44% and showed a slight decrease with a further increase to 54%. Improved growth and feed efficiency with increasing dietary protein levels have been described in many other species (Tabacheck 1986; Bai et al., 1999). Several studies reported varying the protein requirements (15 to 48%) for *L. vannamei* under different conditions (Colvin & Brand 1977; Smith et al., 1984; Aranyakananda & Lawrence 1993; Kureshy & Davis 2002; Liu et al., 2005; Hu et al., 2008). Observed growth pattern in present experiment is similar and comparable with previous studies of whiteleg shrimp. S. Xia et al., 2010 obtained the highest daily weight gain and specific growth rate 114.7±0.06 and 1.24±0.06 respectively from 60 days of experiment with sub adult shrimp. Authors did not find any significant difference in terms of SGR among the shrimp fed various protein levels and reported the dietary protein requirement could be between 39% and 47% for sub adult whiteleg shrimps. Kureshy and Davis (2002) showed the

protein requirement for maximum growth with *L. vannamei* to be higher than 32%, and observed that a 48% DP level yielded better feed efficiency. Liu et al., (2005) found better growth performance and feed conversion ratio among whiteleg shrimp juveniles fed a diet with a 40% protein level. However, some researchers have reported a lower protein nutrition requirement. For example, Colvin & Brand (1977) found better feed conversion for penaeid shrimp juveniles fed a 25% CP diet compared with shrimp fed diets with 30%, 35% or 40% DP levels. Smith et al. (1984) indicated that maximum growth is obtained when shrimp are fed a diet exceeding 36% protein. Aranyakananda and Lawrence (1993) concluded that maximum growth can be obtained with a DP level of 15% among shrimp fed *ad libitum*. Hu. et al. (2008) reported that a diet containing 34% protein is optimum for better growth. The differences can be attributed to the variations in body weight, culture system, stocking density, environmental factors, etc. (Brito et al., 2001)

Shrimp size, stocking density rearing conditions WG (114.77) and SGR (1.78) obtained at 44% dietary protein encouraging. While the broken-line model (Fig. 1) indicating, the optimum dietary protein level for supporting maximum weight gain could be 43.3% for juvenile whiteleg shrimp. In general broken-line falls below the actual requirement, while the difference is negligible and 44% can be considered as the required protein level to promote the optimum growth in juvenile whiteleg shrimp.

There were no clear trends in survival rate of shrimps fed the various experimental diets. Obtained percentage survival (86.7) at 44% of crude protein level is almost similar with the survival reported in several previous studies. While Initial weight (1.0 ± 0.1g) of shrimp used in the present experiment was comparatively lower than shrimp size used in various other experiments. Shrimp size (weight) affects growth response relative to the protein content of the diet. Smith et al. (1984) studied the response of three sizes of *L. vannamei* (4.0, 9.8, and 20.8 g) fed diets containing 22%, 29%, and 36% crude protein for a period of 30 days. Dietary protein content only affected weight gain for 4.0g shrimp, with a significant increase in weight gain corresponding to the increase in dietary protein content. Authors reported that a dietary protein level in excess of 36% would be required to yield maximum weight gain of 4.0g shrimp. Experimenting with initial size of 6g whiteleg shrimp, Survival (92.93±0.17) reported by S. Xia et al. 2010 at 43% dietary protein level, seems to be slightly higher than the survival (86.7) obtained at 44% level in present experiment. Duration of study, initial size and variation in other experimental conditions could be some of the important factors associated with the comparatively less survival obtained in the present experiment.

In general, the FCR showed a similar linear increasing trend to WG. Shrimp fed the CP44 diet had the numerically lowest FCR (1.69) among all the dietary treatments, which was significantly

different from the shrimp, fed CP33 diet. However, there were no significant difference in terms of FCR, among the shrimp fed CP38, 44 and 54 diets. Food conversion ratio at 44% of dietary protein levels better reported in several other experiments (S. Xia et al., 2010; Kureshy and Davis, 2002) at optimum dietary protein level crude protein level and better than the FCR reported in several other experiments for *Litopenaeus vannamei*.

No clear significant trend (Table 2) was observed in terms of PER among the shrimp fed 4 different protein diets. Significantly lowest PER was obtained with the shrimp fed CP54, while there were no significant differences among the shrimp fed CP33, CP38 and CP44. The significant decline of PER with the increase in dietary protein after 44%, clearly indicating that surplus protein was being metabolized rather than deposited as growth. Similar results were reported by Huang et al. (2003); Liu et al. (2005); Hu et al. 2008 and S. Xia et al. 2010. Usually, dietary protein at a low level could be efficiently utilized for protein synthesis by shrimp. The inefficient conversion of protein to growth reflects the continued catabolism of protein for energy in the absence of any protein sparing effect at high protein levels. Several dietary factors may effect the PER. First, part of the dietary factors may be metabolized for energy when dietary non-protein nutrients (carbohydrate and lipids) are not improved or utilized properly. For instance, the poor ability of shrimp to utilize high dietary lipid levels lowered PER. Second, different protein sources with different protein quality may result in

different PER values (Hajra at al., 1988). In the present study, protein sources were same in different diets to maintain similar amino acid profiles.

Proximate analysis

The proximate composition of whole body and edible portion are shown in Table 3. No clear significant trend was observed in lipid, moisture and ash content but edible protein content followed the increasing trend with dietary protein up to 44% CP afterwards slightly decreased with the further increase in dietary protein to 54%. The amino acid requirements for shrimp have not been well defined because shrimp do not efficiently utilize crystalline amino acids from the purified diets used to study amino acid requirements. As a general rule, however, the amino acid requirements of a species closely mirror the amino acid composition of their muscle tissue (Lim and Persyn, 1989). The amino acid composition of shrimp feeds is largely based on the amino acid composition of shrimp muscle (Akiyama et al., 1991). In fact, Kaushik (1998) did not find any significant difference in the total whole body amino acids of two different sizes of European sea bass, gilthead sea bream and turbot. In the present study, the whole body total amino acid composition (% dry sample) of the juvenile shrimps, was not significantly altered by dietary treatments, except for some amino acids at higher level among the shrimp fed CP44. The same observation reported in fishes also such as rainbow trout (Mohanty and Kaushik, 1991) and Japanese flounder (Alam et

al., 2002). The small differences of certain amino acid levels in the whole body among the dietary treatments found in the present study may be due to the differences in the tissue levels of free amino acids as also observed in rainbow trout (Kaushik and Luquet, 1980). Dall and Smith (1987) also showed that concentration of whole muscle total amino acids in tiger prawn *P. esculentus* changed only slightly when starved, but concentrations of various free amino acids changed significantly. In the present study, an improved essential amino acid profile was observed among the shrimp fed CP44. But no significant trend was observed in amino acid profile with respect to various level of dietary protein. The highest amount of protein retained in the edible shrimp muscle (Table 4) among the shrimp fed CP44, could be related to a greater rate of protein synthesis in the shrimp that received dietary protein at 44% level, which demonstrates an improved amino acid profile

In conclusion, our results are in consistent with other previous studies conducted to evaluate the optimum protein requirement of whiteleg shrimp, *Litopenaeus vannamei*. 44% of dietary protein appears to provide the most appropriate dietary amino acid profiles for the maximum growth of juvenile White leg shrimp. Broken line analysis indicating 43.3% dietary protein is good enough to promote optimum growth. In general broken line falls below the actual requirement, while it is negligibly different from the actual requirement. Dietary protein at 44% level seems to be most appropriate to promote optimum growth of juvenile whiteleg

shrimp. The remarkable conclusion, ingredients and their composition used to formulate the feed containing 44% of crude protein, can be used to formulate nutritionally rich but inexpensive feed to make culture of whiteleg shrimps a better economic enterprise. Additionally, after further refinement in ingredients composition with the supplementation of probiotic/prebiotic can be employed to formulate the functional feeds for shrimp farming.

Therefore, our findings suggested that the optimum dietary protein level could be greater than 43.3%, but less than 44% for maximum growth in juvenile whiteleg shrimp.

Tables and Figures

Table 1. Composition and proximate analysis of the experimental diet (% of DM basis)[*1]

Ingredient (%)	Diets			
	CP33	CP38	CP44	CP54
Fish meal [*2]	9.5	12.7	15.9	22.3
Dehulled soybean meal [*2]	18.8	25.4	31.9	44.8
Squid liver powder [*2]	5.0	5.0	5.0	5.0
Wheat gluten meal [*2]	4.0	5.0	6.0	8.0
Wheat flour [*2]	50.3	40.0	29.8	9.4
Corn starch [*2]	5.0	5.0	5.0	5.0
Soybean oil [*2]	1.5	1.5	1.5	1.5
Fish oil [*3]	2.9	2.5	2.1	1.4
Vit. premix [*4]	2.0	2.0	2.0	2.0
Min. premix [*5]	0.5	0.5	0.5	0.5
CaP-monobasic [*6]	0.5	0.4	0.3	0.1
Proximate analysis (% of dry matter basis)				
Moisture	13.38	11.46	11.9	12.92
Crude protein	33.3	37.8	43.9	53.9
Crude lipid	6.1	6.5	6.6	6.3
Crude ash	5.0	5.9	6.8	8.3

[*1]Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

[*2]Suhyup feed C. Uiryeong, Korea

[*3]Jeil feed Co. Hamman, Korea

[*4]Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150; Choline bitate, 3000l Inositol, 150; Menadion, 6; Niacin, 150; Pyridoxine · HCl, 15; Rivoflavin, 30; Thiamine mononitrate, 15; dl-α-Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; Cobalamin, 0.06

[*5]Contains (as mg/kg in diets) : NaCl, 437.4; $MgSO_4 \cdot 7H_2O$, 1379.8; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; $MnSO_4$, 0.016; $FeSO_4$, 0.0378;

$CuSO_4$, 0.00033; Calciumiodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025

[*6] Sigma-Aldrich Korea Yongin, Korea

Table 2. Percent weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth ratio (SGR) and survival rate in whiteleg shrimp fed 4 different protein levels for 6 weeks (% of DM basis)[*1]

	Diets[*2]				Pooled SEM[*4]
	CP33	CP38	CP44	CP54	
WG[*3]	84.0^{c}	88.3^{bc}	114.8^{a}	104.8^{ab}	4.62
FCR[*3]	2.38^{a}	2.10^{ab}	1.69^{b}	1.99^{ab}	0.11
PER[*3]	1.12^{a}	1.17^{a}	1.15^{a}	0.87^{b}	0.05
SGR[*3]	1.42^{c}	1.47^{bc}	1.78^{a}	1.66^{ab}	0.05
Survival[*3]	85.0^{a}	93.3^{a}	86.7^{a}	93.3^{a}	2.41

[*1] Values are means from triplicate groups of shrimp where the means in each row with a different superscripts are significantly different ($P<0.05$).

[*2] Refer to the table 1.

[*3] WG = (final weight - initial weight) x 100/initial weight

[*3] FCR = (dry feed intake/wet weight gain) x 100

[*3] PER = weight gain/dietary protein intake

[*3] SGR = (ln final weight - ln initial weight)/days

[*3] Survival rate = (total shrimp - dead shrimp)×100/total shrimp

[*4] Pooled SEM = Pooled standard error of mean (SD/$\sqrt{n}$)

Table 3. Whole body and Edible portion proximate composition of juvenile whiteleg shrimp fed 4 different protein levels for 6 weeks (% of DM basis)[*1]

		Diets[*2]				Poolde SEM[*3]
		CP33	CP38	CP44	CP54	
Moisture	Whole	78.31^{ab}	77.00^{b}	79.11^{ab}	79.71^{a}	0.44
	Edible	77.07^{a}	76.60^{a}	76.02^{a}	76.78^{a}	0.25
Crude lipid	Whole	3.04^{a}	3.31^{a}	2.22^{b}	2.54^{ab}	0.18
	Edible	2.01^{b}	2.90^{a}	2.20^{ab}	2.17^{ab}	0.15
Crude protein	Whole	76.27^{c}	81.43^{a}	79.74^{ab}	78.08^{bc}	0.77
	Edible	91.76^{b}	92.17^{ab}	94.18^{a}	92.75^{ab}	0.41
Ash	Whole	13.5^{ab}	12.66^{b}	13.17^{ab}	13.85^{a}	0.19
	Edible	6.21^{a}	5.90^{a}	5.99^{a}	6.27^{a}	0.09

[*1]Values are means from triplicate groups of shrimp where the means in each row with a different superscripts are significantly different ($P<0.05$).

[*2] Refer to the table 1.

[*3] Pooled SEM = Pooled standard error of mean ($SD/\sqrt{n}$)

Table 4. Edible portion amino acid composition of juvenile white leg shrimp fed 4 different protein levels for 6 weeks(% of DM basis)[*1]

	Diets[*2]				Pooled SEM[*3]
	CP33	CP38	CP44	CP54	
Asp.	12.45[a]	13.89[a]	14.19[a]	13.03[a]	0.35
Thr.	3.53[a]	3.14[a]	3.40[a]	3.45[a]	0.10
Ser.	2.68[a]	3.09[a]	3.15[a]	3.02[a]	0.09
Glu.	10.41[b]	11.82[a]	12.2[a]	11.82[a]	0.29
Pro.	3.93[a]	4.50[a]	4.38[a]	4.51[a]	0.12
Gly.	9.26[a]	9.30[a]	8.86[a]	8.40[a]	0.24
Ala	4.85[a]	4.89[a]	4.76[a]	4.88[a]	0.06
Val.	3.37[a]	3.47[a]	3.64[a]	3.42[a]	0.05
Ile.	3.14[b]	3.35[ab]	3.33[ab]	3.48[a]	0.05
Leu	5.53[b]	5.77[a]	5.91[a]	5.86[a]	0.06
Tyr.	2.36[a]	2.25[a]	2.34[a]	2.35[a]	0.03
Phe.	2.93[a]	2.97[a]	3.27[a]	3.10[a]	0.06
His.	2.02[a]	2.11[a]	2.04[a]	2.1[a]	0.02
Lys.	6.17[a]	5.76[a]	6.27[a]	6.63[a]	0.19
Arg.	6.44[a]	5.83[a]	6.67[a]	6.83[a]	0.22
Cys.	0.77[a]	0.76[a]	0.78[a]	0.80[a]	0.01
Met.	0.50[d]	0.62[c]	0.67[b]	0.74[a]	0.03

[*1]Values are means from triplicate groups of shrimp where the means in each row with a different superscripts are significantly different ($P<0.05$).

[*2] Refer to the table 1.

[*3] Pooled SEM = Pooled standard error of mean ($SD/\sqrt{n}$)

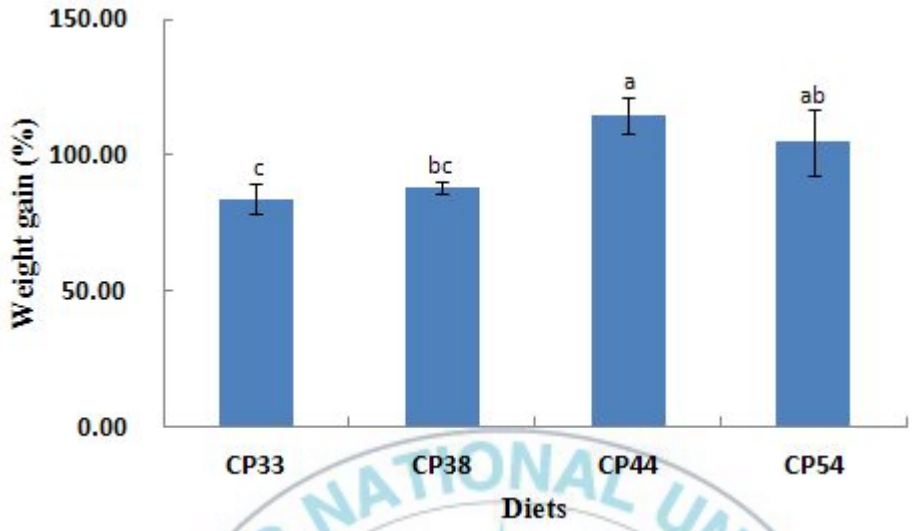

Fig. 1. Weight gain in juvenile whiteleg shrimp fed 4 different levels of dietary protein during 6 weeks.

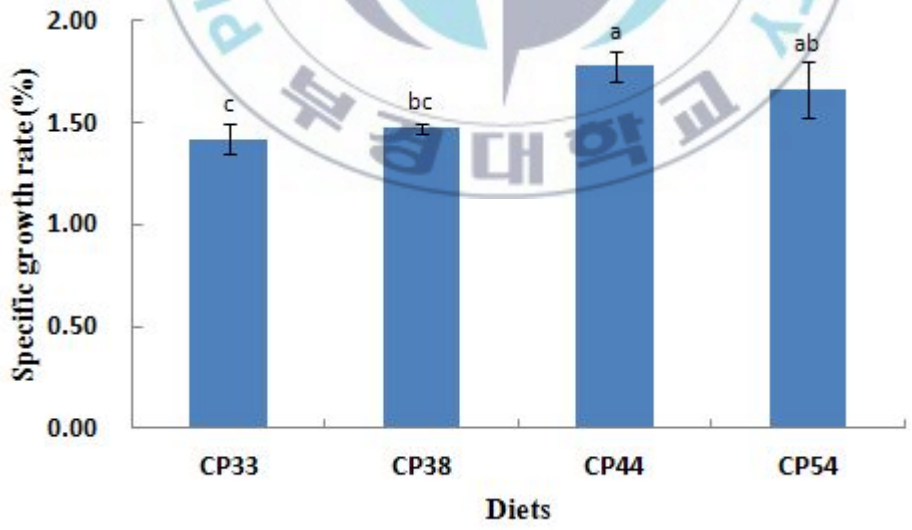

Fig. 2. Specific growth rate in juvenile whiteleg shrimp fed 4 different levels of dietary protein during 6 weeks.

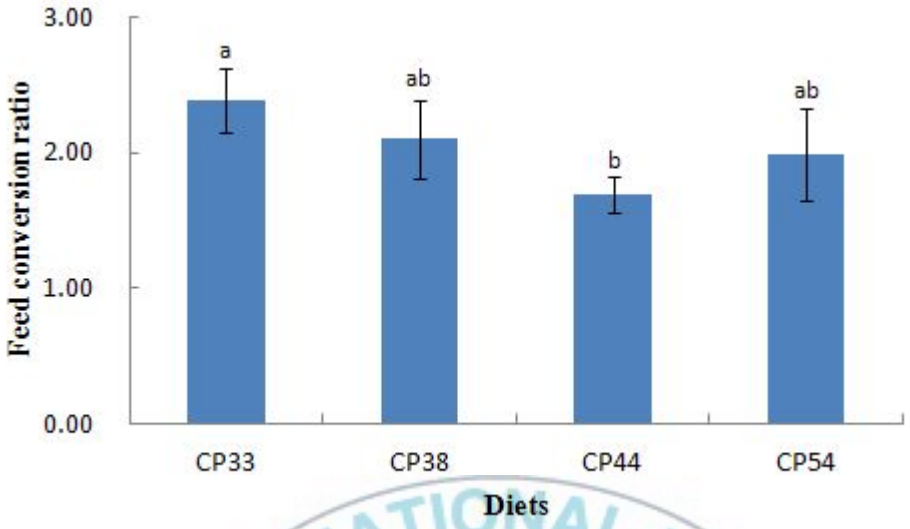

Fig. 3. Feed conversion ratio in juvenile whiteleg shrimp fed 4 different levels of dietary protein during 6 weeks.

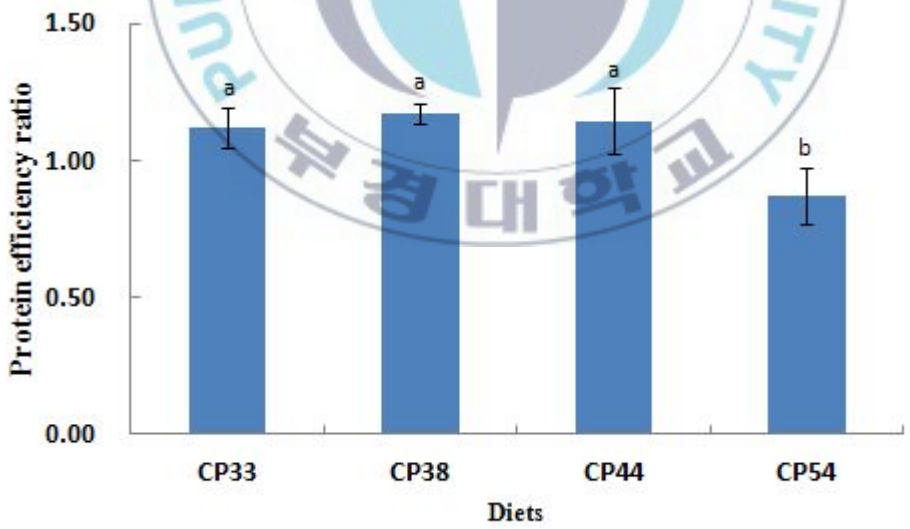

Fig. 4. Protein efficiency ratio in juvenile whiteleg shrimp fed 4 different levels of dietary protein during 6 weeks.

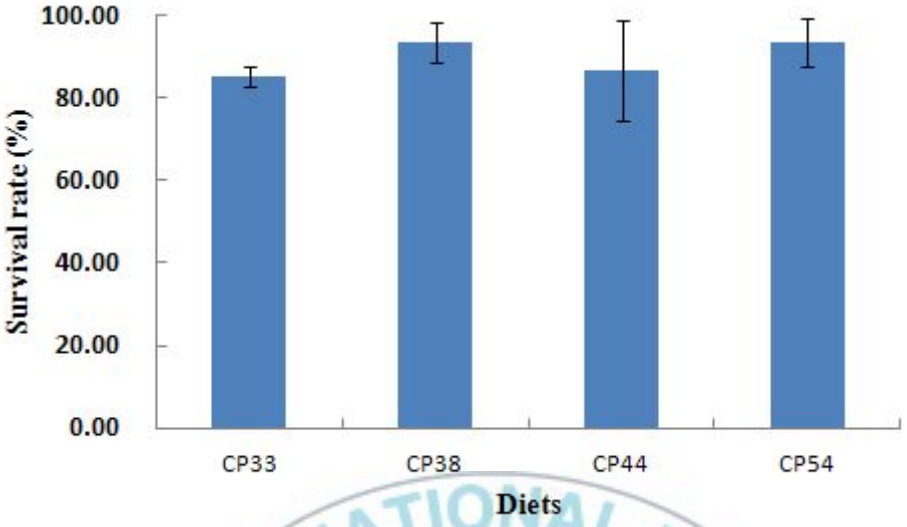

Fig. 5. Survival rate in juvenile whiteleg shrimp fed 4 different levels of dietary protein during 6 weeks.

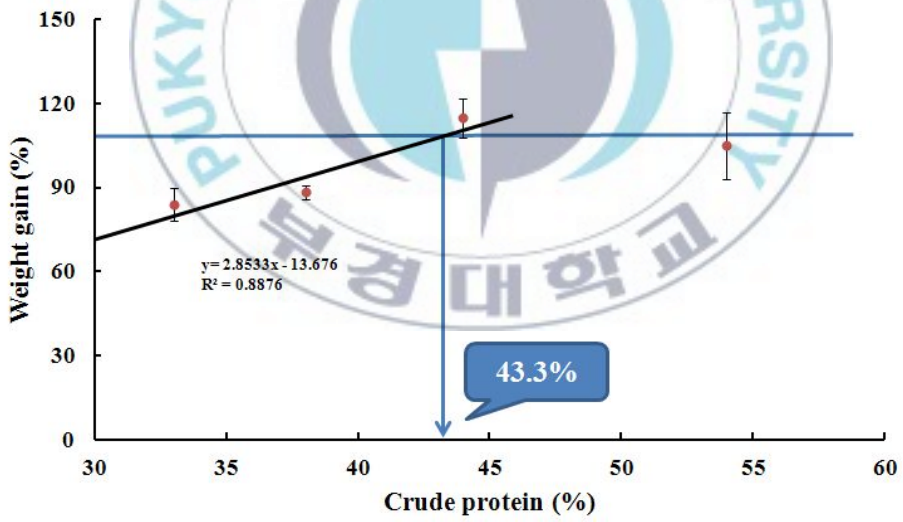

Fig. 6. Broken-line model of percent weight gain in juvenile whiteleg shrimp fed 4 different levels of dietary protein during 6 weeks.

Ⅲ. The effects of dietary PROTIDE® as a fishmeal replacer in juvenile white leg shrimp, *Litopenaeus vannamei*

Abstract

A nine weeks of feeding trial was conducted to evaluate the optimum dietary levels of PROTIDE®(PRO) as a fishmeal replacer in juvenile white leg shrimp, *Litopenaeus vannamei* diet. Five experimental diets were formulated to be isonitrogenous (45% crudeprotein, CP) and isocaloric (16.4 kJ energy g^{-1} diet). After 1 week of feeding trial, quadruplicate groups of 50 shrimp averaging 0.15± 0.02g (mean ± SD) were fed one of 5 experimental diets at approximately 7% of wet body weight on dry matter basis. Experimental diets containing 20% of fish meal were formulated with PRO replacing 0%, 10%, 20%, 30% and 40% of fish meal protein (PRO_0, PRO_2, PRO_4, PRO_6, PRO_8, respectively). After 9 weeks of feeding trial, weight gain and specific growth rate of shrimp fed PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed PRO_6 and PRO_8 diets ($P<0.05$), but there were no significant difference among those of shrimp fed PRO_0, PRO_2

and PRO_4 diets, and among those of shrimp fed PRO_4, PRO_6 and PRO_8 diets ($P>0.05$). Protein efficiency ratio of shrimp fed PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed PRO_6 and PRO_8 diets ($P<0.05$). Feed conversion ratio (FCR) and survival rate of shrimp fed PRO_6 and PRO_8 were significantly higher than those of shrimp fed PRO_0 and PRO_2 diets ($P<0.05$), but no significant difference among survival rate of shrimp fed PRO_0, PRO_2 and PRO_4, and among survival rate of shrimp fed PRO_4, PRO_6 and PRO_8 diets ($P>0.05$). Therefore, these results clearly indicated that PROTIDE® could be used to replace the fish meal higher than 10%, but less than 20% based on the weight gain, feed conversion ratio, protein efficiency ratio and survival rate in juvenile whiteleg shrimp.

Materials and Methods

Experimental Design and Diets

The experiment was conducted at the Institute of Fisheries Science, Pukyong National University, Busan, Korea. The compositions of experimental diets are shown in Table 1. Five experimental diets containing fishmeal, PROTIDE®(PRO), dehulled soybean meal, squid liver powder and wheat gluten meal as a protein sources were prepared to contain protein level at 45% at the expense of wheat flour, soybean oil and fish oil. Five experimental diets were formulated to be isonitrogenous (45% crude protein, CP) and isocaloric (16.4 kJ energy g^{-1} diet) based on calculation (Garling & Wilson 1976; NRC 1997). Experimental diets containing 20% of fishmeal were formulated with PROTIDE replacing 0%, 10%, 20%, 30% and 40% of fishmeal protein (PRO_0, PRO_2, PRO_4, PRO_6, PRO_8, respectively). Procedures for diet preparation and storage were followed as previously described by Bai & Kim (1997). After thoroughly mixing the dry ingredients, soybean oil and fish oil together with 30% well water were added and further mixed to make a mash. Laboratory pelletizing machine was used to obtain uniform pallets of 2mm-diameter sizes. The formulated diets were stored at -20°C (Wet pellets) until use.

Animal husbandry and Experimental design

Juvenile whiteleg shrimp were procured from Tae-an hatchery of the National Fisheries Research & Development Institute. Before the

starting the experiment, all shrimps were reared in a 1000L tank and fed *ad libitum,* a commercial shrimp diet for 1 week. After 1 week of conditioning period, 50 Shrimp measuring initial averaging 0.15± 0.02g (mean ± SD) were randomly distributed into each of 20 plastic aquaria (30-L) and covered with plastic. Each aquaria was then randomly assigned to one of the four replicates of the five dietary treatments. Semi-recirculation system was used in the experiment and water flow was maintained at 3L/ minutes. The diets were fed to quadruplicate groups of fish to satiation, approximately at 7% of total body weight per day for 9 weeks. Shrimp were fed 4 times a day at 9:00, 12:00, 15:00 and 18:00, seven days a week. Total shrimp weight in each aquarium was determined every 3 weeks and the feeding rate was adjusted accordingly. Water temperature was maintained at 27°C by using a heater. Dissolved oxygen was maintained above 6.0 mgL^{-1}, salinity 32±1 psu and the pH 7.5± 0.3, in during the experiment.

Siphoning was done every morning in each rearing tank; dead shrimp were removed, weighed, and recorded daily.

Sample collection and analysis

At the end of the feeding trial, all shrimp were weighed and counted to calculate percentage weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate. Proximate composition analysis of the experimental diets, whole body and edible were performed by the standard methods of AOAC (1995). Samples of the diets and shrimp

were dried at 105°C to a constant weight to determine their moisture content. Ash content was determined by incineration at 550°C. Protein was determined using the kjeldahl method (N x 6.25) after acid digestion, and crude lipid was ascertained by soxhlet extraction using the Soxhlet system 1046 (Tacator AB, Sweden) after freeze-drying the samples for 20h. Amino acid analysis of edible protion was performed by ninhydrin method (Sykam Amino Acid Analyzer S433, Sykam; Eresing, Germany).

StatisticalAnalysis

All data were analyzed by one-way ANOVA (Statistix 3.1; Analytical Software, St. Paul, MN, USA) to test the effects of the dietary treatments. When a significant treatment effect was observed, an LSD test was used to compare means. Treatment effects were considered at $P<0.05$ level of significance. Broken line analysis (Robinson Norton & Baker, 1979) was used to test the dietary protein requirement (SAS procedure NLIN, 1996).

Results and Discussion

Growth performance

Growth trial was conducted without interruption, water quality, or any disease problems. The experimental diets were well accepted by the shrimp; no rejection of the feed was observed. After 9 weeks of the feeding trial, weight gain and specific growth rate of shrimp fed PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed PRO_6 and PRO_8 diets (Table2), but there were no significant difference among those of shrimp fed PRO_0, PRO_2 and PRO_4 diets, and among those of shrimp fed PRO_4, PRO_6 and PRO_8 diets. Protein efficiency ratio of shrimp fed PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed PRO_4, PRO_6 and PRO_8 diets. Feed conversion ratio (FCR) of shrimp fed PRO_0 and PRO_2 were significantly lower than the shrimp fed PRO_4, PRO_6 and PRO_8 diet. Survival rate of shrimp fed PRO_6 and PRO_8 diets were significantly higher than those of shrimp fed PRO_0 and PRO_2 but there were no significant difference among shrimp fed PRO_0, PRO_2 and PRO_4 diets, and similarly among shrimp fed PRO_4, PRO_6 and PRO_8 diet.

Usually, fish can consume feeds more rapidly but shrimp are slow feeder and nutrients loss due to leaching is common obstacle in shrimp farming. In during the experiment, PROTIDE was observed to be soluble in the water easily which is demonstrated by decreased FCR, WG, SGR and PER with subsequent increase in

dietary PROTIDE. On the other hand, survival rate increased with the increase in dietary PROTIDE.

The future of the aquaculture industry is believed to depend in part on reduction in fish meal (FM) use in fish feeds. The ongoing trends to replace fish meal with animal by‐products meals and plant based meals gaining momentum with a view to develop "environment-friendly" feeds containing the least amount of protein necessary for optimal growth. Several studies have been conducted to replace or reduce its inclusion level in fish and shrimp diets by various inexpensive alternative plant protein sources (soybean meal, corn gluten meal, soy protein concentration etc) and animal protein sources (meat and bone meal, blood meal, feather meal, poultry by-product meal, lysine by product etc). Partial replacement of fish meal in fish feed (Bai et al., 1997, 1998; Carter and Hauler, 2000; Lee and Bai, 1997 ; Lee et al., 2002) and considerable success in partially or totally replacing FM with plant protein sources in various shrimp species (Lim and Dominy, 1990; Piedad-Pascual et al., 1990; Penaflorida, 1995; Eusebio and Coloso, 1998; Sudaryono et al., 1990; Alvarez et al., 2007) has been reported. Complete replacement of fish meal with other protein sources reported to produce low growth rates, especially in crustaceans and carnivorous fish, may be due to the poor digestibility and variable quality of alternative protein sources.

In recent years, the concept of 'functional foods' has developed in

human and animal nutrition. Functional foods, which first emerged in Japan, have been defined as 'foods or dietary components that may provide a health benefit beyond basic nutrition' (IFIC, 2004). The presence of biologically active components of functional foods can have an impact on health benefits or other desirable physiological effects in addition to their nutritional function. The term 'functional foods' covers a broad range of products including, for example, DHA- and selenium-enriched eggs, selenium-enriched pork, stanol- and sterol-enriched margarine, etc. Also included under this category are dietary 'probiotics' and 'prebiotics'.

As an alternative strategy to antibiotics, many reports have been published regarding application of probiotics in feeding and health management in aquaculture. A probiotic is defined, in the strict sense, as "a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract" (Roberfroid, 2000). It should be noted that the term has also been widely and incorrectly applied in aquaculture, and especially shrimp culture, to include the use of live microbes to beneficially alter the microbial balance in the culture system itself. However, because of high cost, potential impact on the environment, regulatory issues, food safety and challenges regarding incorporation into modern extruded feeds, large-scale application of probiotics in the water has been limited.

Nucleotides have essential physiological and biochemical functions including encoding and deciphering genetic information, mediating

energy metabolism and cell signaling as well as serving as components of coenzymes, allosteric effectors and cellular agonists (Carver and Walker, 1995; Cosgrove, 1998). The roles of nucleotides administered by feeding have been debated for many years. Nucleotides have been considered as non-essential nutrient, because neither overriding biochemical malfunctions nor classical signs of deficiency are developed in human or animal models. However the conception has been challenged by successive research publications suggesting that dietary nucleotide deficiency may impair liver, heart, intestine and immune functions. Nucleotide supplementation has been one important aspect of research on clinical nutrition and functional food development for humans. Therefore, it has been considered that study on nucleotide supplementation in shrimp diets is an important issue to verify its potential.

Obtained weight gain from the second experiment is higher than the observed weight gain pattern in the first experiment, which demonstrates the beneficial effect of Protide on whiteleg shrimp nutrition. Our results are in favor of several previous experiments reported the beneficial effects of nucleotide in different species of fin fishes.

PROTIDE® containing 2% of nucleotide and gaining momentum in the ongoing trend of fish meal replacement with the objective to develop functional feed. However a nucleotide study of shrimp is incomplete and basic. Although initial efforts in the evaluation of dietary supplementation of nucleotides for fishes could be traced to

the early 1970s, research at that time had mainly focused on the possible chemo-attractive effects of these compounds (Mackie, 1973; Kiyohara et al., 1975; Mackie and Adron, 1978). Worldwide attention on nucleotide for fishes was aroused by the reports of Burrels et. al., 2001a, who found that dietary supplementation of nucleotides enhanced resistence of Salmonids against viral or bacterial disease. To date, research pertaining to nucleotide in fish nutrition has shown consistent and encouraging results for fish health management, although exact mechanism of nucleotides remain hypothetical and systematic research on fish as far from complete (Li and Gaclin III, 2006; Li et al., 2004a, b). Research on nucleotide nutrition in shrimp is needed to provide insights concerning interactions between nutrition and physiological responses as well as practical solutions to reduce basic risks from infectious diseases with a view to reduce the fish meal level and reduce the feed cost.

Protide supplement at 2.5% level has been reported to replace 14% of fish meal in diets for Juvenile Olive flounder *Paralichthys olivaceous* without growth performance or feed efficiency depression. In another experiment with Red sea bream *Pagrus major* Protide supplement at 3-5% reported to replace fish meal (7-12%) without any depression on growth performance. Results obtained from the present experiment are consistent with other reports for protide, in terms of weight gain data indicated the potential of protide to replace the fish meal higher than 10%, but less than 20% in juvenile whiteleg shrimp *Litopenaus vannamei.* The clear trend of increase in survival rate with subsequent increase in Protide

supplement suggesting the efficacy of protide to replace the fish meal up to 20%.

Proximate analysis

The proximate composition of whole body and edible portion are shown in Table 3. There were no clear trend in the crude lipid and ash contents of all the shrimp diets. In the edible portion crude protein contents, shrimp fed PRO_4, PRO_6 and PRO_8 diets were significantly higher than those of shrimp fed PRO_0 and PRO_2. In the whole body crude protein contents, shrimp fed PRO_4, PRO_6 and PRO_8 diets were significantly higher than those of shrimp fed PRO_0 diet. But no significant difference between shrimp fed PRO_2 and PRO_8 diets. In the edible portion amino acid composition, Ser, Gly and Ala contents of shrimp fed PRO_0, PRO_2, PRO_4 and PRO_6 diets were significantly lower than those of shrimp fed PRO_8 diet. However, Val, Ile, Leu, Phe, His and Lys contents of shrimp fed PRO_0, PRO_2, PRO_4 and PRO_6 diets were significantly higher than those of shrimp fed PRO_8 diet (Table 4).

In conclusion, observed weight gain and specific growth rate trend indicating, PRO_4 was the most effective diet among different experimental diets. While, based on the feed conversion ratio and protein efficiency ratio, PRO_2 appeared to be the most appropriate diet. Protide supplementation clearly appeared to have potential impact in whiteleg shrimp nutrition and efficiency to replace the fish meal higher than 10%, but less than 20% (PRO2~PRO4) without

affecting the growth performance. Therefore, Protide supplement at 10~20% can be recommended to replace fish meal in juvenile whiteleg shrimp *Litopenaeus vannamei* nutrition, to make the shrimp farming more environmental friendly and a better economic enterprise.

Tables and Figures

Table 1. Composition and proximate analysis of the experimental diets (% of DM basis)[*1]

Ingredient (%)	Diets				
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8
Fish meal [*2]	20.0	18.0	16.0	14.0	12.0
PROTIDE® [*3]	0	2.0	4.0	6.0	8.0
Dehulled soybean meal [*2]	39.8	39.9	39.9	39.9	40.0
Squid liver powder [*2]	5.0	5.0	5.0	5.0	5.0
Wheat gluten meal [*2]	9.0	9.0	9.0	9.0	9.0
Wheat flour [*2]	14.9	14.8	14.9	14.9	14.8
Corn starch [*2]	4.5	4.5	4.5	4.5	4.5
Lecithin [*2]	1.0	1.0	1.0	1.0	1.0
Soybean oil [*2]	1.5	1.5	1.4	1.4	1.4
Fish oil [*4]	2.0	2.0	2.0	2.0	2.0
Vit. premix [*5]	1.0	1.0	1.0	1.0	1.0
Min. premix [*6]	1.0	1.0	1.0	1.0	1.0
Calcium Phosphate [*7]	0.3	0.3	0.3	0.3	0.3
Proximate analysis (% of dry matter basis)					
Moisture	7.27	7.23	7.05	7.25	7.03
Crude protein	48.8	49.0	49.5	49.3	49.2
Crude lipid	7.6	7.3	6.9	6.9	7.2
Crude ash	9.0	8.8	8.5	8.2	8.0

[*1]Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed companies are using currently.

[*2]Suhyup feed C. Uiryeong, Korea

[*3]CJ feed Co. Seoul, Korea

[*4]Jeil feed Co. Hamman, Korea

[*5]Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150; Choline bitate, 3000l Inositol, 150; Menadion, 6; Niacin, 150; Pyridoxine · HCl, 15; Rivoflavin, 30; Thiamine mononitrate, 15; dl-α-Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; Cobalamin, 0.06

[*6]Contains (as mg/kg in diets) : NaCl, 437.4; $MgSO_4 \cdot 7H_2O$, 1379.8; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calciumiodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025

[*7] Sigma-Aldrich Korea Yongin, Korea

Table 2. Percent weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth ratio (SGR) and survival rate in whiteleg shrimp fed 5 different PROTIDE levels for 9 weeks (% of DM basis)[*1]

	Diets[*2]					Pooled SEM[*4]
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8	
WG[*3]	638[a]	647[a]	580[ab]	551[b]	547[b]	14.83
FCR[*3]	1.68[b]	1.65[b]	1.91[a]	1.95[a]	2.05[a]	0.05
PER[*3]	1.24[a]	1.24[a]	1.06[b]	1.06[b]	1.00[b]	0.03
SGR[*3]	3.63[a]	3.65[a]	3.49[ab]	3.41[b]	3.39[b]	0.04
Survival[*3]	72.0[b]	73.0[b]	77.0[ab]	82.0[a]	84.0[a]	1.50

[*1] Values are means from triplicate groups of shrimp where the means in each row with a different superscripts are significantly different ($P<0.05$).

[*2] Refer to the table 1.

[*3] WG = (final weight - initial weight) x 100/initial weight

[*3] FCR = (dry feed intake/wet weight gain) x 100

[*3] PER = weight gain/dietary protein intake

[*3] SGR = (ln final weight - ln initial weight)/days

[*3] Survival rate = (total shrimp - dead shrimp)×100/total shrimp

[*4] Pooled SEM = Pooled standard error of mean ($SD/\sqrt{n}$)

Table 3. Whole body and Edible portion proximate composition of juvenile whiteleg shrimp fed 5 different PROTIDE levels for 9 weeks (% of DM basis)[*1]

		Diets[*2]					Pooled SEM[*3]
		PRO_0	PRO_2	PRO_4	PRO_6	PRO_8	
Moisture	Whole	78.07^{a}	77.03^{a}	77.40^{a}	78.08^{a}	77.37^{a}	0.19
	Edible	76.69^{a}	76.49^{a}	76.75^{a}	76.96^{a}	76.70^{a}	0.07
Crude lipid	Whole	2.86^{b}	2.64^{c}	2.70^{bc}	2.69^{c}	3.54^{a}	0.11
	Edible	2.20^{a}	1.57^{b}	0.99^{e}	1.08^{d}	1.30^{c}	0.15
Crude protein	Whole	73.76^{c}	73.94^{bc}	74.72^{ab}	75.22^{a}	74.42^{ab}	0.19
	Edible	85.36^{b}	85.70^{b}	86.95^{a}	87.26^{a}	87.94^{a}	0.34
Ash	Whole	13.83^{ab}	13.51^{c}	13.66^{bc}	14.00^{a}	14.08^{a}	0.07
	Edible	6.92^{ab}	6.96^{a}	6.95^{a}	6.81^{b}	6.81^{b}	0.03

[*1]Values are means from triplicate groups of shrimp where the means in each row with a different superscripts are significantly different ($P<0.05$).

[*2] Refer to the table 1.

[*3] Pooled SEM = Pooled standard error of mean ($SD/\sqrt{n}$)

Table 4. Whole body amino acid composition of juvenile white leg shrimp fed 5 different PROTIDE levels for 9 weeks(% of DM basis)[*1]

	Diets[*2]					Pooled
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8	SEM[*3]
Asp.	12.06^{ab}	12.47^{ab}	12.71^{a}	12.55^{ab}	11.88^{b}	0.12
Thr.	2.88^{a}	2.90^{a}	2.96^{a}	2.89^{a}	2.82^{a}	0.02
Ser.	2.73^{b}	2.65^{b}	2.69^{b}	2.61^{b}	3.00^{a}	0.05
Glu.	12.28^{ab}	12.40^{ab}	12.76^{a}	12.62^{a}	12.03^{b}	0.10
Pro.	5.73^{ab}	5.88^{ab}	6.11^{a}	5.97^{a}	5.36^{b}	0.10
Gly.	6.25^{b}	6.45^{b}	6.65^{b}	6.82^{b}	7.74^{a}	0.18
Ala	3.03^{b}	3.13^{b}	3.20^{b}	3.22^{b}	4.75^{a}	0.22
Val.	3.63^{a}	3.68^{a}	3.77^{a}	3.74^{a}	3.36^{b}	0.05
Ile.	3.55^{a}	3.53^{a}	3.63^{a}	3.64^{a}	3.23^{b}	0.05
Leu	6.16^{a}	6.18^{a}	6.27^{a}	6.28^{a}	5.78^{b}	0.07
Tyr.	1.53^{a}	2.66^{a}	2.72^{a}	2.73^{a}	2.51^{a}	0.24
Phe.	3.49^{a}	3.49^{a}	3.55^{a}	3.54^{a}	3.22^{b}	0.04
His.	2.11^{ab}	2.19^{a}	2.16^{ab}	2.08^{b}	1.93^{c}	0.03
Lys.	7.11^{a}	7.06^{a}	7.21^{a}	7.18^{a}	6.65^{b}	0.08
Arg.	6.23^{ab}	6.28^{ab}	6.41^{ab}	6.48^{a}	6.08^{b}	0.06

[*1]Values are means from triplicate groups of shrimp where the means in each row with a different superscripts are significantly different ($P<0.05$).

[*2] Refer to the table 1.

[*3] Pooled SEM = Pooled standard error of mean ($SD/\sqrt{n}$)

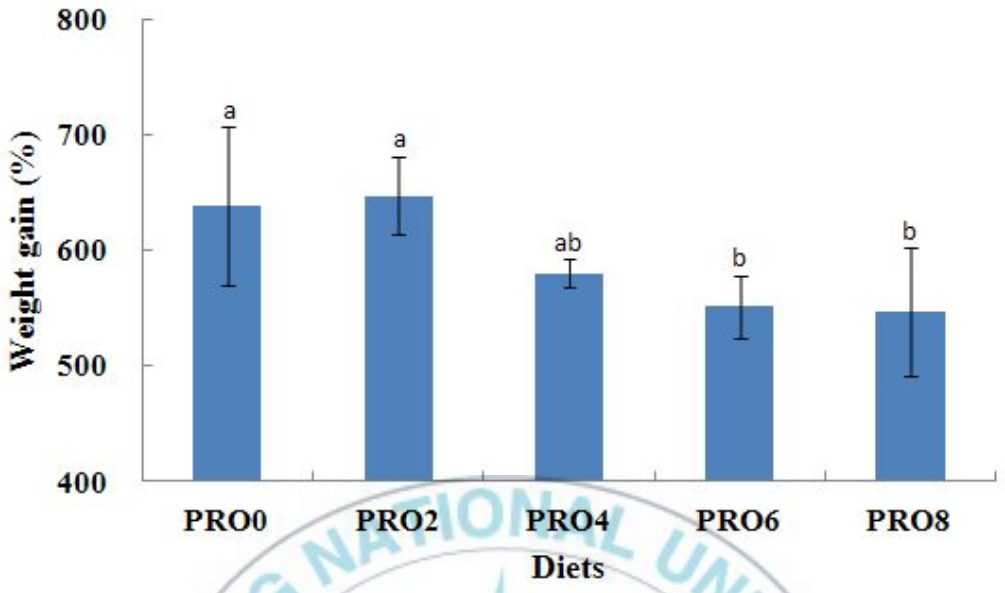

Fig. 1. Weight gain in juvenile whiteleg shrimp fed 5 different levels of dietary PROTIDE during 9 weeks.

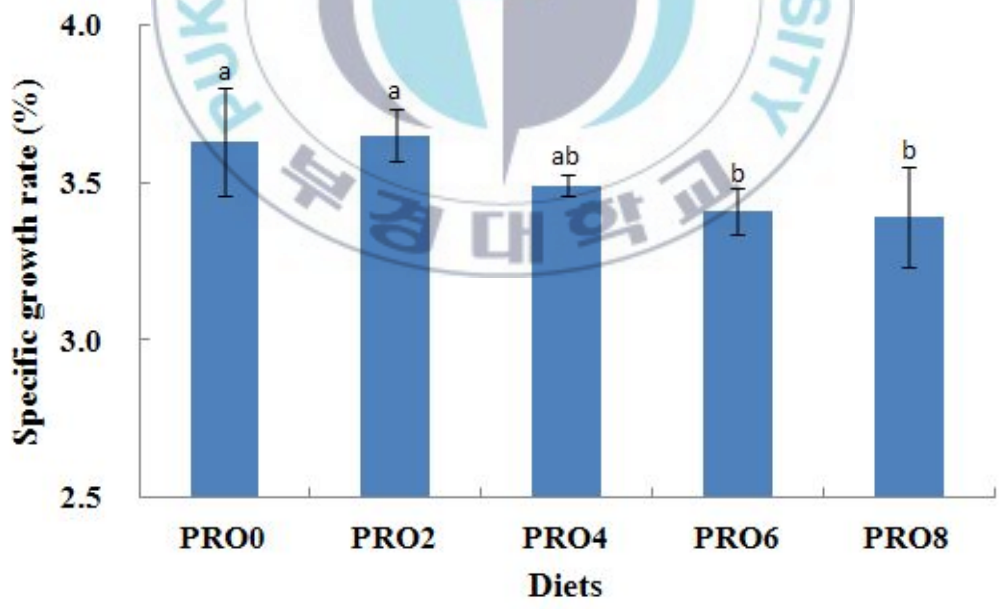

Fig. 2. Specific growth rate in juvenile whiteleg shrimp fed 5 different levels of dietary PROTIDE during 9 weeks.

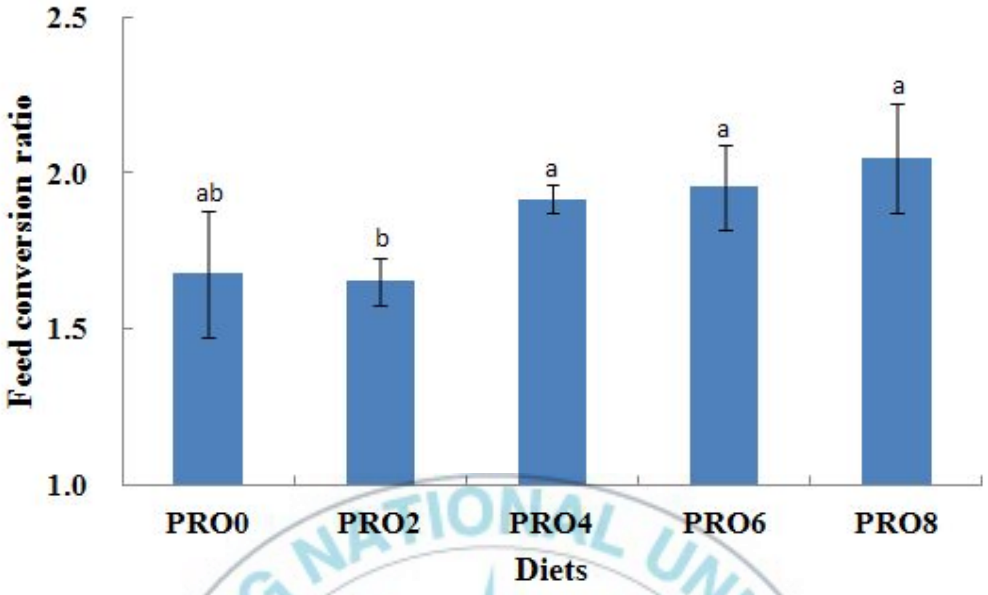

Fig. 3. Feed conversion ratio in juvenile whiteleg shrimp fed 5 different levels of dietary PROTIDE during 9 weeks.

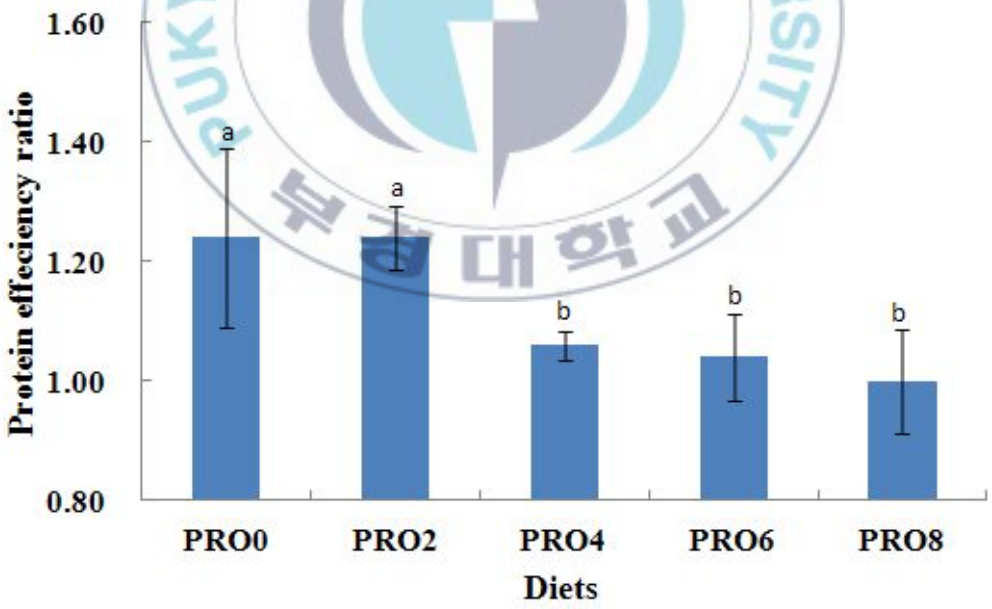

Fig. 4. Protein efficiency ratio in juvenile whiteleg shrimp fed 5 different levels of dietary PROTIDE during 9 weeks.

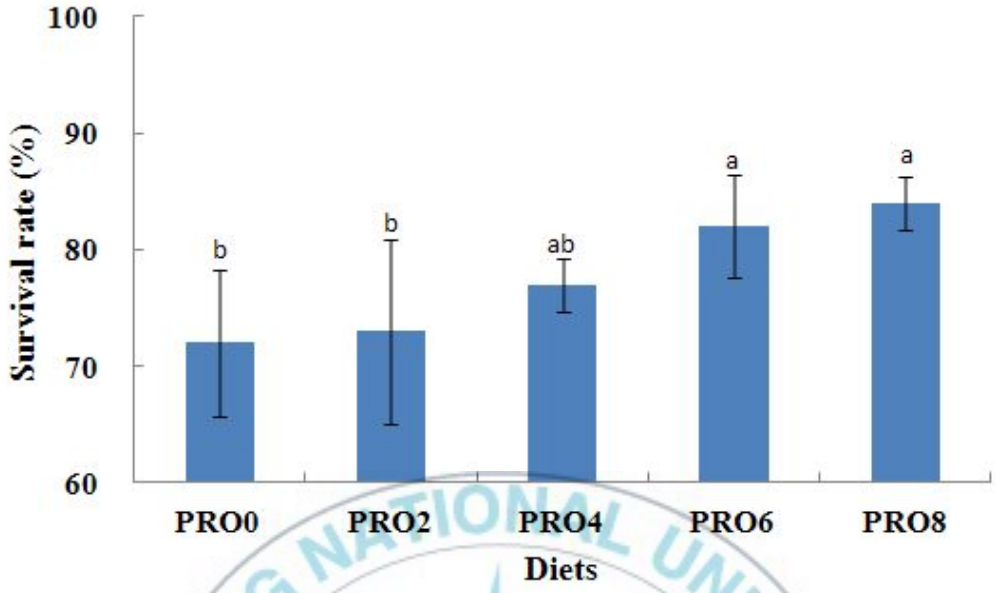

Fig. 5. Survival rate in juvenile whiteleg shrimp fed 5 different levels of dietary PROTIDE during 9 weeks.

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Ⅴ. Reference

Adamek et al., 1996 Z. Adamek, J. Hamackova, J. Kouril, R. Vachta and I. Stibranyiova, Effect of Ascogen probiotics supplementation on farming success in rainbow trout (*Oncorhynchus mykiss*) and wels (Silurus glais) under conditions of intensive culture. Krmiva (Zagreb), (1996), pp. 11–20.

Akiyama, D.M., Dominy, W.G. and Lawrence, A.L., 1991. Penaeid shrimp nutrition for the commercial feed industry - revised. Am. Soybean Assoc., AQ 32: 35.

Alam, M.S., Teshima, S., Yaniharto, D., Ishikawa, M., Koshio, S., 2002. Influence of different dietary amino acid pattern on growth and body composition of juvenile Japanese flounder *Paralichthys olivaceus*. Aquaculture 210, 359–369.

Alvarez, J.S., Hernandez-Liamas, A., Galindo, J., Fraga, L., Garcia, T., Villarreal, H., 2007. Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp. *Litopenaeus schmitti* (Perez-Farfante & Kensley 1997). Aquac. Res. 38, 689-659

Ansuman Hajra, Apurba Ghosh, Sanat Kumar Mandal Biochemical studies on the determination of optimum dietary protein to energy ratio for tiger prawn, *Penaeus monodon* (Fab.), juveniles. Aquaculture Volume 71, Issues 1-2, 15 June 1988, Pages 71-79

AOAC, 1995. Official methods of analysis. 16th ed. Association of Official Analytical Chemists. Arlington, Virginia, USA.

Aranyakananda P. & Lawrence A.L. (1993) Dietary protein and

energy requirements of the white-legged shrimp, *Penaeus vannamei* and the optimal protein to energy ratio. In: WAS'93 International Conference Torremolinos (Spain), 26 - 28 May 1993 from Discovery to Commercialization (ed. by M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig & J. Wyban), Aquatic Resources Research Institute, Chulalongkorn University, Bangkok, Thailand. Special Publication European Aquaculture Society, vol. 19, 21pp. Oostende (Belgium).

Association of Official Analytical Chemists (1995) Official Methods of Analysis. 16th edn. Association of Official Analytical Chemists. Arlington, VA.

Bai, S. C., Xiaojie Wang and Eunsun Cho (1999). Optimum dietary protein level for maximum growth of jnvenile yellow puffer, Fisheries Science, 65(3): 380-383.

Bai, S. C., H. K. Jang and E. S. Cho, 1998. Possible use of the animal by-product mixture as a dietary fish meal replacer in growing common carp (*Cyprinus carpio*), J. of Fish. Soci. Vol. 31(3): 380-385.

Bai, S. C., H. K. Jang and K. H. Kim, 1997. Evaluation of leather meal and meat and bone meal as the fish meal replacer in israeli carp diets. J. of Aquaculture Vol. 10(2): 153-161.

Bai, S. C. and Kim, K. W., 1997. Effects of dietary animal protein sources on growth and body composition in Korean rockfish, *Sebastes schlegeli*. Journal of Aquaculture Vol. 10: 77-85 (in Korean with English abstract).

Brito R., Rosas C., Chimal M.E. & Gaxiola G. (2001) Effect

ofdifferent diets on growth and digestive enzyme activity in *Litopenaeus vannamei* (Boone, 1931) early post-larvae. Aquaculture Research 32, 257-266.

Browdy C.L. & Moss S.M. (2005) Shrimp culture in urban, super intensive closed systems. In: Urban Aquaculture (ed. by B.A. Costa Pierce), pp.173-186. Blackwell Science, Oxford, UK.

Burford M., Thompson P., McIntosh R., Bauman R. & Pearson D. (2003) Nutrient and microbial dynamics in high intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219, 393-411.

C. Burrells, P.D. William and P.F. Forno, Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to diseases in salmonids. Aquaculture, (2001a), pp. 159–169.

C. Burrells, P.D. William, P.J. Southage and S.L. Wadsworth, Dietary nucleotides: a novel supplement in fish feeds 2. Effects on vaccination, salt water transfer, growth rate and physiology of Atlantic salmon. Aquaculture, (2001b), pp. 171–184.

Carver and Walker, 1995 J.D. Carver and W.A. Walker, The role of nucleotides in human nutrition. J. Nutr. Biochem, (1995), pp. 58–72.

Carter C. G. and R. C. Hauler, 2000. Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. Aquaculture Vol. 185: 299 -311

Cho C.Y., Hynes J.D., Wood K.R. & Yoshida H.K. (1994) Development of high-nutrient-dense, low-pollution diets and

prediction of aquaculture wastes using biological approaches. Aquaculture 124, 293-305.

Colvin L.B. & Brand C.W. (1977) The protein requirement of penaeid shrimp at various life-cycle stages in controlled environment systems. Proceedings of World Mariculture Society 8, 821-840.

Cosgrove, 1998 M. Cosgrove, Nucleotides. Nutrition, (1998), pp. 748 - 751

Dall, W., Smith, D.M., 1987. Changes in protein-bound and free amino acids in the muscle of the tiger prawn, *Penaeus esculentus* during starvation. Mar. Biol. 95, 509 - 520.

Eusebio, P., Coloso, R., 1998. Evaluation of leguminous seed meals and leaf meals as plant protein sources in diets for juvenile penaeus indicus. Isr. J. Aquac.-Bamidgeh 50, 47-54.

Food and Agriculture Organization of the United Nations 2011

Garling D.L. Jr. & Wilson R.P. (1976) Optimum dietary protein to energy ration for channel catfish fingering, *Ictalurus punctatus*. Journal of Nutrition 106, 1368-1375

Guillaume J. (1997) Protein and amino acids. In: Crustacean Nutrition (ed. by L.R. D'Abramo, D.E. Conklin & D.M. Akiyama), pp. 26-50. World Aquaculture Society, Baton Rouge, LA, USA.

Hedlund, S. 2007. Vannamei production to increase again this year. Market report. Sea food Business. January, p.18.

Hopkins J., Hamilton R. II, Sandifer P., Browdy C. & Stokes A. (1993) Effects of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. Journal of World Aquaculture Society 24, 304-320

Hu Y., Tan B., Mai K., Zheng S. & Cheng K. (2008) Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. Aquaculture Nutrition 10, 1-8.

Huang K., Wang W, & Lu J. (2003) Protein requirements in compounded diets for *Penaeus vannamei* juveniles. Journal of Fishery Sciences of China 10, 318-324

International Food Information Council. 2004

Kaushik, S.J., 1998. Whole body amino acid composition of European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. Aquat. Living Resour.11, 355–358.

Kaushik, S.J., Luquet, P., 1980. Influence of dietary amino acid patterns on the free amino acid contents of blood and muscle of rainbow trout (*Salmo gairdnerii* R.). Comp. Biochem. Physiol. 64B, 187–190.

Kiyohara, S., Hidaka, I., Tamura, T., 1975. Gustatory response in the puffer-II. Single fiber analysis. Bull. Jpn. Soc. Sci. Fish. 41, 383–391.

Kim K W, X J Wang and Bai, 2002 Optimum dietary protein level for maximum growth of juvenile olive flounder *Paralichthys olivceus*. Aquaculture research, Vol. 33:673-679.

KOREA STATISTICS , 2011

Kureshy N. & Davis D. A. (2002) Protein requirement for maintenance and maximum weight gain for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 204, 125-143.

Lane, D. E. 1985. A bibliography on the white sturgeon (*Acipensertrans montanus*) Richardson, 1836. Canadian man us cript report of fisheries and aquatic science, no. 1826. Fisheries and oceans. Government of Canada, Nanaimo, British Columbia, Canada

Lee, K. J., Dabrowski, K., Blom, J.H. and S. C. Bai, 2001 Replacement of fish meal by a mixture of animal by-products in juvenile rainbow trout diets. North American Journal of Aquaculture Vol. 63: 109-117.

Lee, K. J., Dabrowski, K., Blom, J.H. and S. C. Bai and Stromberg P. C. A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*). J. Anim. Physiol. a. Anim. Nutr. 86 201–213

Lee, K. J. and S. C. Bai, 1997. Haemoglobin powder as a dietary fish meal replacer in juvenile Japanese eel, *Anguilla japonica* (Temmincket Schlegel), Aquaculture Research, Vol. 28: 509-516.

Leonardi, M., Sandino, A.M., Klempau, A., 2003. Effect of a

nucleotide-enriched diet on the immune system, plasma cortisol levels and resistance to infectious pancreatic necrosis (IPN) in juvenile rainbow trout (*Oncorhynchus mykiss*). Bull. Eur. Assoc. Fish Pathol. 23, 52 – 59.

Li, P., Lewis, D.H., Gatlin III, D.M., 2004a. Dietary oligonucleotide from yeast RNA influences immune responses and resistance of hybrid striped bass (Morone chrysops_M. saxatilis) to Streptococcus iniae infection. Fish Shellfish Immunol. 16, 561 – 569.

Li, P., Wang, X., Gatlin III, D.M., 2004b. Excessive dietary levamisole suppresses growth performance of hybrid striped bass (Morone chrysops_M. saxatilis), and elevated levamisole in vitro impairs macrophage function. Aquac. Res. 35, 1380 – 1383.

Lim, C. and A. Persyn. 1989. Practical Feeding – Penaeid Shrimps. In, Editor, Tom Lovell. Nutrition and Feeding of Fish. Van Nostrand Reinhold. New York. pp. 205-222.

Lim, C., Dominy, W., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp *penaeus vannamei*. Aquaculture 87, 53-64

Liu D. H., He J. G., Liu Y. J. Zheng S. X. & Tian L. X. (2005) Effects of dietary protein levels on growth performance and immune condition of Pacific white shrimp *Litopenaeus vannamei* juvenile satvery low salinity. Acta Scientiarum Naturalium Universitatis Sunyatseni 44, 217-223.

Low, C., Wadsworth, S., Burrells, C., Secombes, C.J., 2003. Expression of immune genes in turbot (Scophthalmus maximus)

fed a nucleotide-supplemented diet. Aquaculture 221, 23 – 40.

Mackie, A.M., 1973. The chemical basis of food detection in the lobster Homarus gammarus. Mar. Biol. 21, 103 – 108.

Mackie, A.M., Adron, J.W., 1978. Identification of inosine and inosine 5V-monophosphate as the gustatory feeding stimulants for the turbot, Scophthalmus maximus. Comp. Biochem. Physiol. 60A, 79 – 83.

Marcel B Roberfroid PhD. A European consensus of scientific concepts of functional foods. Nutrition Volume 16, Issues 7-8, July-August 2000, Pages 689-691

McAbee B., Browdy C., Rhodes R. & StokesA. (2003) The use of green house enclosed raceway systems for the superintensive production of pacific white shrimp *Litopenaeus vannamei* in the United States. Global Aquaculture Advocate 6, 40-43.

Mohanty, S., Kaushik, S.J., 1991. Whole body amino acid composition of Indian major carps and its significance. Aquat. Living Resour. 4, 61 – 64.

NRC (National Research Council). (1997) Nutrients of Fish. National academy Press, Washington, DC.

Penaflorida, V. D., 1995. Growth and survival of juvenile tiger shrimp fed diets where fish meal is partially replaced with papaya (Coreca papaya) or camote (Ipomea batats lam) leaf meal. Isr. J. Aquac.-Bamidgeh 47, 25-33

Peng Li, Delbert M. Gatlin III. 2006. Nucleotide nutrition in fish:

Current knowledge and future applications. Aquaculture 251 (2006) 141–152

Piedad-Pascual, F., Cruz, E. M., Sumalangcay Jr., A., 1990. Supplemental feeding of Penaeus monodon juveniles with diets containing various levels of defatted soybean meal. Aquaculture 89, 183-191.

Ramadan and Atef, 1991 A. Ramadan and M. Atef, Effect of the biogenic performance enhancer (Ascogen "S") on growth rate of tilapia fish. ActaVet. Scand., (1991), pp. S304–S306.

Ramadan et al., 1994 A. Ramadan, N.A. Afifi, M. Moustafa and A.M. Samy, The effect of ascogen on the immune response of tilapia fish to Aeromonas hydrophila vaccine. Fish Shellfish Immunol., (1994), pp. 159–165.

Robinson K.R., Norton H.R. & Baker D.H. (1979) Estimation of nutrient requirements frim growth data. Jounal of Nutrition 109, 1710-1714.

Samocha, T., Davis, D.A., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by co-extruded soybean pourtry by-product meal in preactical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 231, 197-203.

Shiau S.Y. & Peng C.Y. (1992) Utilization of different carbohydrates and protein levels in grass prawn *Penaeus monodon*, reared in seawater. Aquaculture 101, 241-250

Smith L. L., Lawrence A. & Strawn K. (1984) Growth and digestibility by three sizes of *Penaeus vannamei* Boone: effects of

dietary protein level and protein source. Aquaculture 46, 85-96.

Sudong Xia, Yong Li, WenqiWang, Mayalagu Rajkumar, Kumaravel Paramasivam, Kumaraguru Vasagam & Hua Wang (2010) influence of dietary protein on growth, digestibility, digestive enzyme activity and stress tolerance in white leg shrimp, *Litopenaeus Vannamei* (Boone,1931), Reared in high-density tank trials. Aquaculture Research, 411, 1845-1854

Sudaryono, A., Tsvetnenko, E., Hutabarat. J., Evans, L., 1990. Lupin ingredients in shrimp Penaeus monodon diets: influence of lupin species and type of meals. Aquaculture 171, 121-133.

Swick, R.A., Akiyama, D.M., Boonyaratpalin, M., Creswell, D.C., 1995. Use of soybean meal and synthetic methionine in shrimp feed. American Soybean Association, Technical Bulletin, vol. AQ 43-1995.

Tabacheck, J. L. 1986. Influence of dietary protein and lipid levels on growth, body composition and utilization effeciencies of Arctic Char, *Salvelinus alpines*. Journal of Fish Biology 29, 139-151

Usha, Goswami and S.C. Goswami, 1982. Formulation of cheaper prawn diets and their biological ecaluation in penaeid prawns. Proc. Symp. Coastal Aquaculture, 1, 211-214

Zarain-Herzberg M., Fraga I. & Hernandez-Llamas A. (2010) Advances in intensifying the cultivation of the shrimp *Litopenaeus vannamei* in floating cages. Aquaculture 300, 87–92.