ผลของการเสริมแอสทาแซนทินและวิตามินเอที่มีต่อการเติบโต การรอด และความทนต่อความเค็มต่ำของกุ้งกุลาดำ *Penaeus monodon* วัยอ่อน

นางสาวรุ่งจิตร ยอดดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN 974-17-6211-9

EFFECTS OF ASTAXANTHIN AND VITAMIN A SUPPLEMENTATION ON GROWTH, SURVIVAL AND LOW SALINITY TOLERANCE OF *Penaeus monodon* LARVAE

MISS RUNGJIT YODDEE

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Biotechnology
Faculty of Science
Chulalongkorn University
Academic year 2004

ISBN 974-17-6211-9

Thesis Title	EFFECTS OF ASTAXANTHIN AND VITAMIN A SUPPLEMENTATION ON GROWTH, SURVIVAL AND LOW SALINITY TOLERANCE OF *Penaeus monodon* LARVAE
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รุ่งจิตร ยอดดี : ผลของการเสริมแอสทาแซนทินและวิตามินเอที่มีต่อการเติบโต การรอด และความทนต่อความเค็มต่ำของกุ้งกุลาดำ *Penaeus monodon* วัยอ่อน. (EFFECTS OF ASTAXANTHIN AND VITAMIN A SUPPLEMENTATION ON GROWTH, SURVIVAL AND LOW SALINITY TOLERANCE OF *Penaeus monodon* LARVAE) อ.ที่ปรึกษา : รศ.ดร.สมเกียรติ ปิยะธีรธิติวรกุล, อ.ที่ปรึกษาร่วม : ศ.ดร.เปี่ยมศักดิ์ เมนะเศวต, 118 หน้า. ISBN 974-17-6211-9.

เลี้ยงกุ้งกุลาดำวัยอ่อน 3 ระยะ (ซูเอี้ย ไมซิส และโพสลาร์วา) ด้วยอาหารทดลอง 9 สูตร มีแอสทาแซนทิน 3 ระดับ (0 200 และ 500 ส่วนในล้านส่วน) และวิตามิน เอ 3 ระดับ (0 20,000 และ 40,000 ไอยูต่อกิโลกรัม) วางแผนการทดลองแบบ CRD 3×3 factorials design ทำการทดลอง 3 ซ้ำ อาหารทดลองทุกสูตรมีระดับโปรตีนและไขมันใกล้เคียงกันคือ 50 และ 11 เปอร์เซ็นต์ ตามลำดับ พบว่าอาหารเสริมแอสทาแซนทินและวิตามินเอทุกระดับมีปฏิสัมพันธ์ร่วมกันต่อการเติบโต การรอดและความทนต่อความเครียดในรูปการตายสะสม (CM_{50}) ของกุ้งวัยอ่อนระยะซูเอี้ย ไมซิส และโพสลาร์วา ระยะซูเอี้ย ที่ระดับแอสทาแซนทิน 0 ส่วนในล้านส่วน กุ้งวัยอ่อนที่ได้รับอาหารที่ไม่มีการเสริมวิตามินเอ มีอัตรารอดและอัตราการเติบโตจำเพาะสูงกว่ากุ้งวัยอ่อนที่ได้รับการเสริมวิตามินเอที่ระดับต่างๆอย่างมีนัยสำคัญ ที่ระดับแอสทาแซนทิน 200 ส่วนในล้านส่วน พบว่ากุ้งวัยอ่อนที่ได้รับอาหารเสริมแอสทาแซนทิน 200 ส่วนในล้านส่วนร่วมกับวิตามินเอ 20,000 ไอยูต่อกิโลกรัม มีอัตรารอดสูงกว่ากุ้งวัยอ่อนที่ได้รับอาหารสูตรอื่นอย่างมีนัยสำคัญ ($P<0.05$) อย่างไรก็ตามไม่มีความแตกต่างของอัตราการเติบโตจำเพาะในกุ้งที่ได้รับอาหารสูตรต่างๆ ที่ระดับแอสทาแซนทิน 500 ส่วนในล้านส่วน กุ้งวัยอ่อนที่ได้รับอาหารเสริมแอสทาแซนทิน 500 ส่วนในล้านส่วนเพียงอย่างเดียว มีอัตรารอดและอัตราการเติบโตจำเพาะสูงสุด ระยะไมซิส ไม่พบความแตกต่างอย่างมีนัยสำคัญของอัตราการเติบโตจำเพาะของกุ้งวัยอ่อนที่ได้รับแอสทาแซนทินกับวิตามินเอในทุกระดับ ที่ระดับแอสทาแซนทิน 0 ส่วนในล้านส่วน กุ้งวัยอ่อนที่ไม่ได้รับการเสริมแอสทาแซนทินและวิตามินเอ มีอัตรารอดสูงสุด ที่ระดับแอสทาแซนทิน 200 ส่วนในล้านส่วน อัตรารอดของกุ้งวัยอ่อนที่ได้รับแอสทาแซนทิน 200 ส่วนในล้านส่วนร่วมกับวิตามินเอ 20,000 ไอยูต่อกิโลกรัมมีค่าสูงกว่ากุ้งวัยอ่อนที่ได้การเสริมวิตามินเอระดับอื่นอย่างมีนัยสำคัญ ที่ระดับแอสทาแซนทิน 500 ส่วนในล้านส่วน พบว่าการเสริมแอสทาแซนทินเพียงอย่างเดียวในระดับนี้ ส่งผลให้กุ้งวัยอ่อนมีอัตรารอดสูงกว่าการเสริมร่วมกับวิตามินเออย่างมีนัยสำคัญ ระยะโพสลาร์วา ที่ระดับแอสทาแซนทิน 0 ส่วนในล้านส่วน กุ้งวัยอ่อนที่ได้รับแอสทาแซนทินร่วมกับวิตามินเอ 20,000 ไอยูต่อกิโลกรัมมีอัตรารอดสูงสุด ในขณะที่อัตราการเติบโตจำเพาะและ CM_{50} สูงสุด พบในกุ้งวัยอ่อนที่ไม่มีการเสริมแอสทาแซนทินร่วมกับวิตามินเอ ที่ระดับแอสทาแซนทิน 200 ส่วนในล้านส่วน กุ้งวัยอ่อนที่ได้รับแอสทาแซนทินร่วมกับวิตามินเอ 20,000 ไอยูต่อกิโลกรัม มีอัตรารอดและอัตราการเติบโตจำเพาะสูงกว่ากุ้งวัยอ่อนที่ได้รับการเสริมวิตามินเอระดับอื่นอย่างมีนัยสำคัญ อย่างไรก็ตาม ค่า CM_{50} สูงสุด พบในกุ้งวัยอ่อนที่ไม่ได้รับการเสริมแอสทาแซนทินและวิตามินเอ ที่ระดับแอสทาแซนทิน 500 ส่วนในล้านส่วน กุ้งวัยอ่อนที่ไม่มีการเสริมแอสทาแซนทินร่วมกับวิตามินเอมีค่าอัตรารอด อัตราการเติบโตจำเพาะ และค่า CM_{50} สูงสุด ปริมาณแอสทาแซนทินและวิตามินเอที่สะสมในกุ้งระยะโพสลาร์วา 15 พบว่าการสะสมของสารเหล่านี้มีความสัมพันธ์โดยตรงกับปริมาณของสารที่กุ้งได้รับจากอาหาร

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต.....

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4572451823: MAJOR BIOTECHNOLOGY

KEY WORD: ASTAXANTHIN / VITAMIN A / PENAEUS MONODON LARVAE

RUNGJIT YODDEE: EFFECTS OF ASTAXANTHIN AND VITAMIN A SUPPLEMENTATION ON GROWTH, SURVIVAL AND LOW SALINITY TOLERANCE OF *Penaeus monodon* LARVAE. ASSOC. PROF. SOMKIAT PIYATIRATITIVORAKUL: THESIS ADVISOR, PROF. PIAMSAK MENASVETA: THESIS COADVISOR, 118 pp. ISBN 974-17-6211-9.

Penaeus monodon larvae (zoea, mysis, and postlarvae) were fed 9 different diets consisting three levels of astaxanthin (0, 200, and 500 ppm) and three levels of vitamin A (0, 20,000, and 40,000 IU/kg) combinations. The experiment was 3x3 factorials in completely randomized design with three replicates. The diets were formulated from raw natural materials with mean protein and lipid levels of 50 and 11%, respectively. The results showed that, there was a significant interaction between astaxanthin and vitamin A on survival and growth of zoea, mysis, and postlarval *P. monodon*. The interaction also found on 50%cumulative mortality of the postlarvae after a low salinity stress test. At 0 ppm astaxanthin, zoea shrimp fed diet without additional astaxanthin and vitamin A was significantly higher survival and specific growth rate than shrimp fed the other diets ($P<0.05$). At 200 ppm astaxanthin, zoea shrimp fed diet with additional 20,000 IU/kg vitamin A had higher significant survival rate than shrimp fed the other vitamin A levels ($P<0.05$). However, it was not difference significantly of specific growth rate of all treatments. At 500 ppm astaxanthin, the survival rate and specific growth rate of zoea shrimp fed diet with additional only 500 ppm astaxanthin were higher than shrimp fed the other diets. Survival rate of mysis shrimp fed diet without astaxanthin and vitamin A was higher than the shrimp fed other diets. At 200 ppm astaxanthin, the results showed that survival rate of mysis shrimp fed diet containing 200 ppm astaxanthin with additional 20,000 IU/kg vitamin A had higher than the other groups. At 500 ppm astaxanthin, shrimp fed diet only 500 ppm astaxanthin was the highest survival rate than shrimp fed the other diets ($P<0.05$). However, specific growth rates were no significant difference in all diets. At 0 ppm astaxanthin, postlarval shrimp received diet with additional 20,000 IU/kg vitamin A was significant higher survival rate than shrimp fed the other diets. However, the highest specific growth rate and CM_{50} were found on shrimp fed diet with no additional astaxanthin and vitamin A. At 200 ppm astaxanthin, postlarvae fed diet with additional 20,000 IU/kg vitamin A gave the better survival rate and specific growth rate than larvae fed the other diets. However, shrimp fed diet without additional astaxanthin and vitamin A had higher CM_{50} than the other diets. At 500 ppm astaxanthin, the results showed that shrimp fed diet with only 500 ppm astaxanthin had the best survival rate, specific growth rate, and CM_{50}. The astaxanthin and vitamin A content of shrimp after acquiring different astaxanthin and vitamin A levels, the results indicated that amount of astaxanthin and vitamin A accumulation positively related to astaxanthin and vitamin A in the diets.

Field of study Biotechnology Student's signature……

Academic year 2004 Advisor's signature……

Co-advisor's signature……

ACKNOWLEDGEMENTS

I wish to express my honest gratitude to Assoc. Prof. Somkiat Piyatiratitivorakul, advisor, Prof. Piamsak menasveta, Co-advisor , for their helpful suggestion and supports throughout this research and also to Dr. Voranop Viyakarn, Assist. Prof. Charoen Nitithamyong, Assist. Prof. Thaithaworn Lirdwitayaprasit for kindly serving on my committee and second reader of this thesis.

I wish to express my sincere thanks to Dr. Sorawit Powtongsook for giving a consultation for determined astaxanthin and vitamin A by HPLC method, Dr. Suchana Chonvanich for kind reviewing of this thesis manuscript, Mr. Seree Donnua, Ms Tipawan Khongjed, Ms Suangsuda Supasai, and friends for their contributions and supports to this work.

I wish to express my special thanks Mr Borriphat Kunpai, Miss Rujirin Aukaw, Miss Walaiphon Tiaprasitt, and friends at King Mongkut's Institute Technology of Ladkrabang for giving spirit, helpful, and supports to this work.

Lastly, the greatest, to my parents and sister for their supports, understanding, patience, and just being.

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CHAPTER I

INTRODUCTION

Penaeus monodon is an important shrimp species for Thai economics, and a popular culture organism in Thailand, because of it is bigger size, higher growth rate, and higher value compared to other shrimp species. In addition, its tissue can be frozen for a long period with no major texture change, which leads to high demand in the market. Thailand is the biggest exporter of black tiger shrimp in the world more than 250,000 metric tons in 2003. Currently, a factor influencing the success of shrimp culture is larval quality. Larviculture uses live organisms such as micro-algae (*Chaetoceros* sp. and *Skeletonema* sp.), rotifers and *Artemia* sp., as food source that need optimal lights and temperature to raise. As a result, the quality of larval shrimp is not stable all year round, and varies depend on seasons to seasons. Therefore, artificial diet is developed to improve the quality of larvae. The most important requirement for a successful aquaculture is to have healthy larvae that can withstand to the environmental stress.

In crustaceans, vitamin A (VA) and its derivatives can enhance immune response and protection against diseases by oxygen free radicals (Latscha, 1989). In black tiger shrimp, astaxanthin (AX), which is carotenoid pigments and precursor of vitamin A, was as high as 98% of total carotenoid contents (Augustin et al., 1985; Okada et al., 1994; Thompson et al., 1995). Astaxanthin can produce body pigment, increase the immune system, enlarge the efficiency of rejection to fluorescence bacteria, and assist of development in the earlier stage of shrimp (Dall, 1995; Hunter, 1996).

In this study, I hypothesized that when astaxanthin and vitamin A was added in the feed, they might influence survival, growth, and stress tolerance of shrimp larval stages.

The purposes of this study are:

1) To determine the effects of synthetic astaxanthin and vitamin A on growth and survival of *P. monodon* larvae; zoea, mysis, and early postlarval stages.
2) To examine the influences of synthetic astaxanthin and vitamin A on low salinity tolerance of *P. monodon* postlarvae (PL-15), and
3) To determine the relationship of synthetic astaxanthin and vitamin A contents in shrimp tissue and diets.

Benefits for this project:

1) To introduce an optimal artificial diet, which provides better growth and survival rate to shrimp's larval stages, and
2) To understand the interaction between astaxanthin and vitamin A on larval performances that can be benefit to better knowledge in shrimp nutrition.

CHAPTER II

LISTERATURE REVIEWS

Biology of *Penaeus monodon*

Presently, the black tiger prawn, *Penaeus monodon*, is widely cultivated in South East Asia. Production of them has increased steadily to meet market demand. Thailand is the world's largest shrimp producer based primarily on *P. monodon,* with a production of more than 250,000 metric tons in 2003. This shrimp is the fastest growing species and suitable for aquaculture in Asia (Lester, 1988; Pillay, 1990). *P. monodon* is the largest of the commercial species, reaching 330 mm or more in length (Bailey-Brock and Moss, 1992). Thai hatcheries are mostly located around the Gulf of Thailand coast (Forbes, 1992). The species can survive in low and high salinity, even though 10 to 25 ppt is considered to be an optimum salinity for them (Pillay, 1990). It also cannot tolerate temperatures below 10^{o}C and temperatures above 37.5^{o}C (Pillay, 1990).

The penaeid life cycle includes several distinct stages find in a variety of habitats (Bailey-Brock and Moss, 1992). Juveniles often prefer brackish waters of estuaries and coastal wetlands, while adults are usually found offshore at higher salinity and greater depths (Bailey-Brock and Moss, 1992). Larval stages inhabit plankton-rich surface waters off-shore, with an on-shore migration as they develop into the maturity stage (Figure 1).

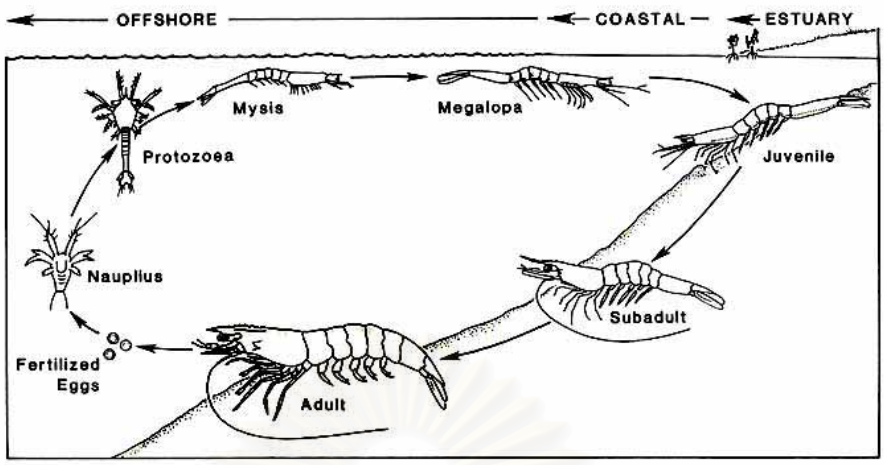

Figure 1 The life cycle of penaeid species with different stages in various habitats
Source: Bailey-Brock and Moss (1992)

The development of larval shrimp

During spawning, eggs and sperm are simultaneously released from the female while she is swimming. Fertilization is external, and egg development occurs in the water column (Bailey-Brock and Moss, 1992). The development of penaeid larvae is divided into 12 stages: 6 nauplii, 3 zoea, and 3 mysis stages before metamorphosing into a postlarva (Figure 2) (Cook and Murphy, 1969; Stickney, 1979; Motoh, 1981). Time of development for each stage is an up to 24 hours for egg, 2 to 3 days for nauplius, 3 to 4 days for zoea, 5 days for mysis, and 35 days for postlarva (Daniel and John, 2000). Typically, the nauplii feed on internal stores of yolk while the zoea stages filter unicellular algae and phytoplankton from the water (Stickney, 1979; Bailey-Brock and Moss, 1992). The examples of phytoplankton are *Chaetoceros* spp., *Skeltonema* spp., *Tetraselmis* spp., and *Isochrysis* spp. (Cook and Murphy, 1969; Yap, 1979; Pillay, 1990; Forbes, 1992; Liao, 1992). The mysis stages feed voraciously on a mixture phytoplankton and zooplankton such as rotifer (*Brachionus plicatilis*) and *Artemia salina* (Stickney, 1979; Pillay, 1990; Forbes, 1992; Liao, 1992). The postlarvae and juveiniles consume both animal, and plant matter, including microalgae, detrital aggregates, macrophytes, larva of mollusk larvae, and brachyuran larvae (Cook and Murphy, 1969; Yap, 1979; Chong

and Sasekumar, 1981; Gleason and Wellington, 1988; Kongkeo, 1991). These are all active-feeding stages (after nauplius), where the shrimp require an appropriate sized phytoplankton and zooplankton diet (Bailey-Brock and Moss, 1992). A primary problem in culturing larval shrimp is food (Leger and Sorgeloos, 1992). Ideally one would feed larvae with their natural diet, which consists of a wide variety and abundance of phytoplanktonic and zooplanktonic organisms (Leger and Sorgeloos, 1992). However, quality of both phytoplankton and zooplankton also depend on light, temperature, and season that could cause unstable health of larvae (Stickney, 1979; Pillay, 1990; Forbes, 1992; Leger and Sorgeloos, 1992). In addition, *Artemia salina*, a favorite mainstay of shrimp hatcheries, is high cost (Forbes, 1992). Therefore, artificial larval feed developments have been focused in order to solve the problem of unstable larval health.

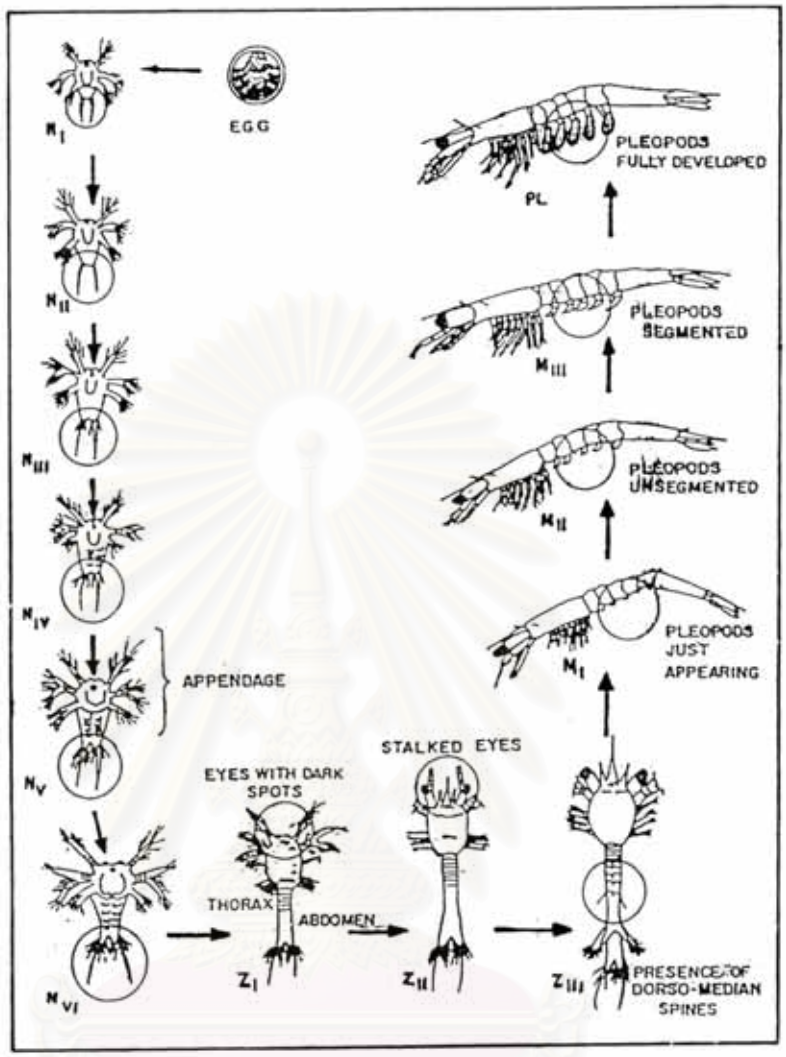

Figure 2 The life cycles of penaeid shrimp
N = Nauplius stage, Z = Zoea stage, M = Mysis stage, and
PL = Postlarval stage
Source: Motoh, 1981

Artificial diet is certainly nutrition, which is according to shrimp's desire. The ratio of the nutritional composition can be controlled, free from contaminants, and a good shelf life (Pillay, 1990). Benefits of artificial diet include consisted in size of diet, easy to use, and not depending on season (Darachai et al, 1996). In addition, feed

costs and maintenance may be reduced. The feed should provide nutrients to an animal for normal function, maintenance, and growth. Generally, artificial diets also include supplemented essential nutrients such as lecithin, cholesterol, astaxanthin, vitamin C (Darachai et al., 1996; Paibulkichakul et al., 1998). Several researches have been done on finding new additives for shrimp feeds. However, no study has been done on the role of certain micronutrients and vitamins, especially astaxanthin and vitamin A on aquatic animal health. The study showed that *P. monodon* fed on no vitamin diet had the poorest growth rate (Catacutan and Cruz, 1989). Therefore, the absence of vitamins in the diet has serious effects on growth and survival of prawn.

Astaxanthin

Astaxanthin (3,3'-dihydroxy-4,4'-diketo-β,β-carotene) (Figure 3) is considered to be a major carotenoid in crustacean, comprising about 86-98% of the total pigments in *P. monodon* (Okada et al., 1994). It accounts for 85% in head of *P.monodon* (Wu and Sun, 1993). Recently, astaxanthin is shown to be the predominant carotenoid associated with the red body color of *P. monodon* (Katayama et al., 1971; Tanaka et al., 1976; Howell and Matthews, 1991). The concentration and distribution of pigment in penaeids varies with species, stages, tissue, and organs of the animals and also depends on rearing conditions mainly the food sources (Katayama et al., 1971).

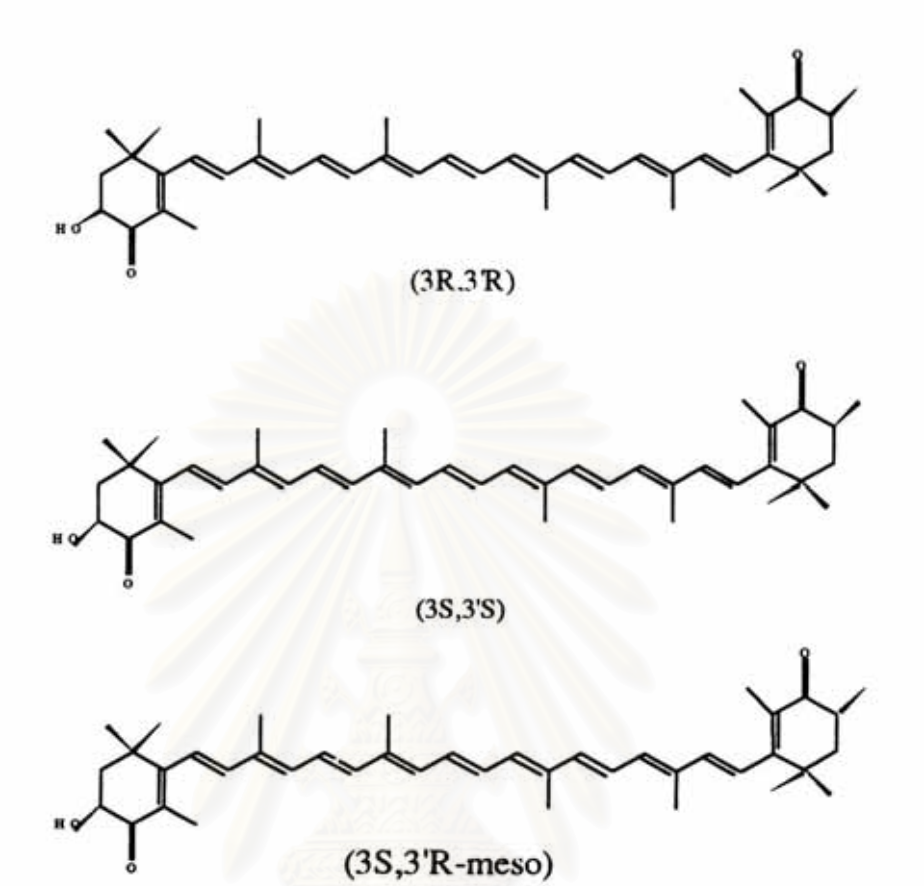

Figure 3 Three configurations of astaxanthin
Source: Johnson and An (1991)

In penaeids, astaxanthin is present in forms of free astaxanthin (F-AX), esterified astaxanthin: monoester astaxanthin (M-AX), diester astaxanthin (D-AX), and forms that bounds to protein called carotenoprotein (Chien, 1996). The concentration and distribution of these forms of astaxanthin also varies with species, stages, and organ or tissue of the animals (Dall, 1995). In *P. monodon,* when total carotenoid content increases, D-AX increase proportionally, and M-AX rises exponantially, but F-AX increases in a decaying exponential fusion until an upper limit reached (Okada et al., 1994). The average percentages were 24.5±2.3%, 24.5±14.8%, and 51.0±14.6% for D-AX, M-AX, and F-AX, respectively. It appears that the portion of D-AX remain relatively

constant, and the variation of M-AX copes with that of F-AX. In *P. monodon'* s head, the percentages of D-AX, M-AX, and F-AX were 20.6%, 37.0%, and 42.4%, respectively. The other forms of astaxanthin such as its optical isomers in penaeids are seldom studied. Astaxanthin optical isomers in of *P. monodon*'s head were: (3R, 3'R)-astaxanthin, 15%, (3R, 3'S)-astaxanthin, 43.7%, and (3S, 3'S)-astaxanthin, 41.3% (Wu and San, 1993).

Astaxanthin is naturally deposited by shrimp (Akiyama et al., 1992). Astaxanthin is the pigments commonly used to give attractive coloration to salmon, trout, and shrimp (Pillay, 1990). However, shrimp can not synthesize astaxanthin by themselves or appropriate precursors must be supplied in the diet (Goodwin, 1984; Latsha, 1990; Pan et al., 1999; Boonyaratpalin et al., 2001). Carotenoids ingested by animals in their food are commonly absorbed by the small intestine (duodenum) along with other lipids (Latsha, 1990). Astaxanthin differ fundamentally in their ability to synthesize this highly oxidated ketocarotenoid from precursors (Latsha, 1990). However, shrimp can transform β-carotene, lutein, echinenone, zeaxanthin, and canthaxanthin, and other intermediate carotenoids into astaxanthin (Katayama et al., 1972) (Figure 4).

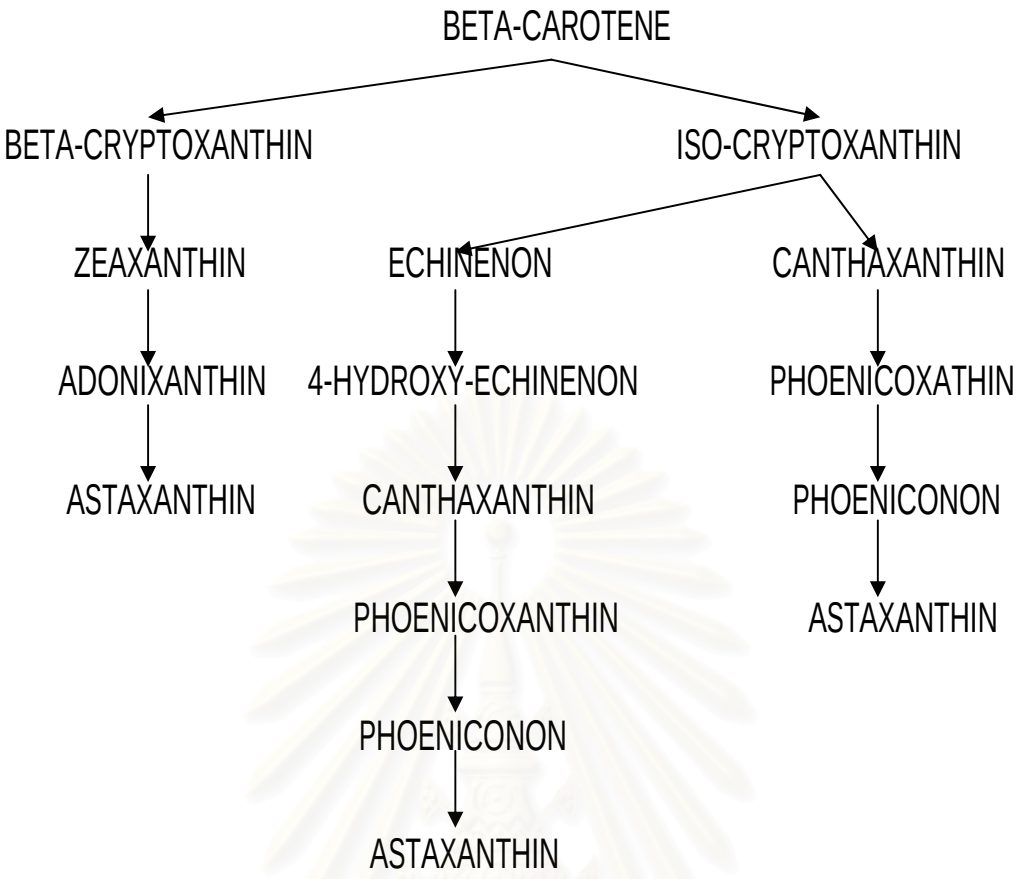

Figure 4 Pathway to synthesize astaxanthin from some intermediates
Sources: Katayama et al. (1971); Tanaka et al. (1975)

Positive functions of astaxanthin

Astaxanthin contains a long conjugated double bond system with relatively unstable electron orbital that may scavenge oxygen radicals in cells (Stanier et al., 1971). The antioxidant activity of astaxanthin is found to be approximately 10 times stronger than β–carotene and 100 times greater than that of α–tocopherol (Shimidzu et al., 1996). Astaxanthin also showed strong activity as an inhibitor of liquid peroxidation mediated by active forms of oxygen and was proposed to be a 'super vitamin E' (Miki, 1991). Dietary supplementation of carotenoids improved or corrected the color of shrimp, improved survival, reduce the mortality rate during embryonic development, and tolerance against environmental stresses (Torrissen, 1984; Chien and Jeng, 1992; Liao

et al., 1993; Menasveta et al., 1993; Thongrod et al., 1995; Kurmaly and Guo, 1996). In addition, feed containing astaxanthin at 50 ppm fed on to shrimp for 6 weeks improved coloration (Akiyama et al., 1992). This coloration is a positive selection criterion for the consumer and in consequence synthetic astaxanthin is routinely added to the diets of farmed salmonids (Thompson et al., 1995). Astaxanthin has also been reported to mask the effects of 'blue shrimp' (Akiyama et al., 1992). Astaxanthin was more efficiently used in pigmentation than either β–carotene or the algal meal (Chien and Jeng, 1992).

Sources of astaxanthin

According to Johnson and An (1991) and Darachai et al. (1996) astaxanthin for farmed animals came from different sources such as:

1) Synthetic astaxanthin

F. Hoffman-La Roche, Basal Switzerland accomplished to synthesize of trans-astaxanthin which is marketed as 'Carophyll pink' containing 8% astaxanthin (Latsha, 1989). Synthetic astaxanthin is presently the principle source used in feeds. An important precursor for the synthesis of astaxanthin is (S)-3-acetooxy-4-oxo-β-ionone, which can be obtained by asymmetric hydrolysis of the (R)-terpene alcohol acetates by various organisms.

2) Crustaceans, and crustacea byproducts

Shrimp (*Pandalus borealis*) wastes have been used traditionally as natural pigment sources for trout and salmon (Guillou et al., 1995). Carotenoid levels in most crutaceans are usually quite low and satisfactory pigmentation requires the addition of 10 to 25% by weight of the chitinous extract to the bulk diet. Crustacean wastes have high level of ash, chitin, moisture, and low levels of protein, and other nutrients that limit their usefulness in animal feed.

3) Algae

Certain green algae in the subphylum Chlorophyceae possess astaxanthin as their secondary carotenoid (Boussiba and Vonshak, 1991; Bubrick, 1991). Qualitative astaxanthin in algae depends on the method and control of culture. Astaxanthin accumulation in high level was found in *Haematococcus pluvialis* (0.5 to 2% astaxanthin in dry weight basis). Most of the astaxanthin (87%) is esterified, which may affect its deposition and metabolism in some animals (Kakizeno et al., 1992). Low deposition of astaxanthin in salmon fed on algae studied by Kvalheim and Knutsen (1985) suggested that astaxanthin was present principally as esters; however, poor deposition caused solely by esterification was not confirmed by others. The predominant configurational isomer in *H. pluvialis* was shown to be 3S, 3'S. Moreover, astaxanthin can be produced by *Chlamydomonas* sp. and *Neochloris wimeri* (Bubrick, 1991).

4) Microorganisms

The colonies of yeast *Phaffia rhodozyma* can produce carotenoid pigments mainly astaxanthin. Andrews and Starr (1976) determined configuration of yeast astaxanthin and made unexpected discovery that the predominant isomer was 3R, 3'R, the opposite of his earlier finding of 3S, 3'S in lobster. Wild strains of *P. rhodozyma* so far isolated containing up to 500 μg total carotenoid/g dry weight (or 0.02 to 0.03%) of which 40 to 95% is astaxanthin (Andrews and Starr 1976). The content of astaxanthin varies substantially depending on strain and method of culture. Other bacteria, including *Mycobacterium lacticola, Brevibacterium* sp., and fungi in the genus Peniophora have been reported to contain astaxanthin (Droop, 1955). Carotenoid levels of fungi are low and growth rates are slow. Fermentation development has not been pursued. The industrially important xanthophyll, canthaxanthin, and zeaxanthin are produced by *Brevibacterium* sp. and *Flavobacterium* sp. However, the productivity is too low for commercial.

As a rule, the pure carotenoids show poor stability. Astaxanthin is highly sensitive to a wide variety of factors such as light, heat, acids, alkaline, and oxygen so precautions should be taken to protect this carotenoid from oxidation (Latsha, 1990; Pillay, 1990; Lorenz, 2000). They can be stabilized somewhat by the use of antioxidants and carotenoids-protein complex (Latsha, 1990; Pillay, 1990). Pelleting of feeds offers the optimal conditions of low heat and oxygen exposure and losses of astaxanthin are as low as 1 to 4% (Lorenz, 2000). The extrusion process popular with many feed manufactures losses generally range from 4 to 12% (Lorenz, 2000).

Chien et al. (1999) studied oxygen depletion stress on mortality and lethal cause of *P. monodon* juvenile fed on astaxanthin 360 ppm or control diet for two weeks. Their results suggested that shrimp fed on astaxanthin supplement diet had better resistance to dissolved oxygen depletion stress and had higher survival than shrimp fed on diet without astaxanthin supplement. Lethal dissolved oxygen level of astaxanthin shrimp was lower than a control shrimp. It appeared that astaxanthin shrimp could tolerate lower dissolved oxygen than the control group. During the first two hours when dissolved oxygen greater than 2 mg/l, oxygen consumption rate (OCR) of astaxanthin shrimp was higher than the control shrimp. But after the first two hours, OCR of astaxanthin was lower than the control shrimp. It appeared that astaxanthin may play a buffer role on oxygen intake according to the environment dissolved oxygen conditions (Chien et al., 1999).

Yamada et al. (1990) conducted experiments related to carotenoids in shrimp. In experiment 1, four diets (control, astaxanthin, β-carotene, and canthaxanthin) were fed to *P. japonicus* (8.5 g) for eight weeks. Astaxanthin was incorporated into body tissue at the higher rate than the other groups. Astaxanthin, β-carotene, and canthaxanthin were deposited in prawn body tissue mainly as astaxanthin esters. In experiment 2, the same final survival rate, daily feed intake, percent gain, feed efficiency, and astaxanthin content (ester and free astaxanthin) of 3.7 g of shrimp were fed astaxanthin contents at 200 and 400 ppm for eight weeks.

Chien and Jeng (1992) experimented that *P. japonicus* fed diet contained astaxanthin 500, 1,000, and 2,000 mg/kg, β-carotene 500, 1,000, and 2,000 mg/kg, and algal (*Dunaliella salina)* meal 1,000 mg/kg for 1, 2, or 3 mouths. Their results showed that shrimp fed astaxanthin 500 mg/kg, which had the best survival rate than shrimp fed the other diets. However, there were no significant difference in weight gain of shrimp fed astaxanthin or β-carotene concentrations at 500, 1,000, and 2,000 mg/kg. For astaxanthin contents, the mainly accumulate of shrimp was found in heads, shells, and flesh, respectively. The higher astaxanthin contents in heads, shells, and flesh were found in shrimp fed astaxanthin 1,000 mg/kg in the diets. Shrimp fed algal meal at a concentration of 1,000 mg/kg showing the poor of survival rate. Of the three pigment sources fed in the experiment, astaxanthin was the most readily used by the shrimp and can be incorporated directly into the shrimp tissue without further conversion (Chien and Jeng, 1992).

Darachai et al. (1996) reported that zoeal *P. monodon* fed astaxanthin from *Haematococcus pluvialis*, *Chaetoceros* sp., and control diet survived significantly better than zoea fed synthetic astaxanthin (200 ppm). For mysis stage, shrimp fed *Chaetoceros* sp. and *Artemia salina* and astaxanthin from algae had better survival rates than the other diets. For postlarval stage, larvae fed astaxanthin from algae gave the better survival rate and higher tolerance than postlarvae fed other diets. This indicated that astaxanthin from *H. pluvialis* (mostly in esterified form) performed significantly better than free synthetic, astaxanthin (Darachai et al., 1996).

Petit et al. (1997) demonstrated that *P. japonicus* postlarva-18 fed synthetic astaxanthin (60 ppm) for 20 and 30 days, could absorb and metabolize dietary astaxanthin. The supply of this pigment modifies the exuviation frequency, shortens the moulting cycle, and postlarval development. However, astaxanthin intake had not a marked effect on the protein content.

Pan et al. (2001) found that *P. monodon* postlarva-8 fed astaxanthin supplementation at 80 ppm for 4 week, astaxanthin contents in shrimp decreased

significantly as prawn weight increased. The overall average (initial 67±16 ppm) astaxanthin contents were 47±13 ppm, 45±12 ppm, 31±15 ppm, and 20±8 ppm for the 1st to the 4th weeks, respectively. There was also no significant difference in the astaxanthin contents between shrimp in the 1st week and 2nd weeks, but those of shrimp in the 2nd, 3rd, and 4th weeks differed significantly. Partially, there was no difference in average individual weight between prawn in the 1st and 2nd weeks, but those of shrimp in the 2nd, 3rd, and 4th weeks differed significantly. Their results also showed that, the lack of change in astaxanthin contents between the 1st and 2nd was probably related to nonsignificant growth during this period. The concluded that fast growth from the 2nd weeks of shrimp produced a significant decrease in astaxanthin contents.

Pan et al. (2003) studied *P. monodon* postlarva-5 fed diets containing either 0 or 71.5 mg astaxanthin/kg body weight/day for eight weeks. Their results showed that juvenile contained 10.5 and 45.6 μg/g body astaxanthin. However, when shrimp were challenged of *Vibrio damsela* for 48 hours, the survival of control shrimp (36.5±3.2%) was significantly lower than that of shrimp fed astaxanthin (45.2±2.8%). Their results suggested that shrimp fed astaxanthin had higher total antioxidant status (TAS) and lower superoxide dismutase, and alanine aminotransferase than controls. They also concluded that shrimp fed astaxanthin could enhance antioxidant defense capability of *P. monodon* juveniles, consequently provide protection to some extent against *V. damsela* challenge in the early stage.

Vitamin A

β-carotene can be converted to vitamin A in the mucosa (Figure 5), which is already converted into vitamin A on absorption across the intestinal mucosa (Latsha, 1990). Vitamin A is required for the biochemical processes involved in vision (Latsha, 1990; Matsuno, 1991). Furthermore, vitamin A plays an important role in growth, development, and integrity of mucous surfaces. The utilization of provitamin A by fish depends on water temperature (should not be lower than 12°C to 14°C and β-carotene content in feed more than 2,500 IU/kg referred by Poston et al., 1977; NRC, 1981 and

1983). In addition, when freshwater fish are faced with a serious shortage of vitamin A in the diet, they could synthesize the vitamin A available carotenoid sources and thus compensate for any deficiency of vitamin A in the diet (AL-Khalifa and Simpson, 1988).

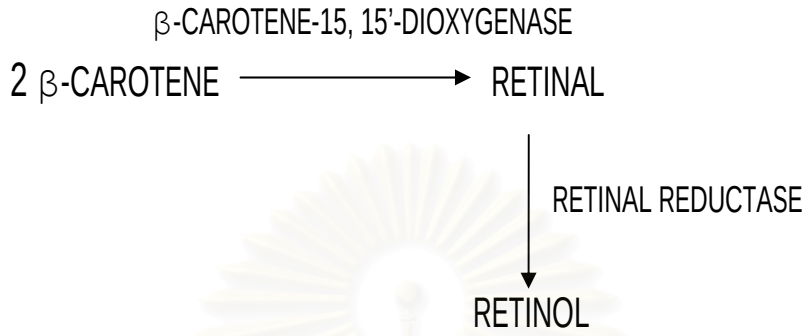

Figure 5 Conversion of β-carotene to vitamin A (retinol)
Source: Blomhoff et al. (1991)

Vitamins are complex organic compounds required in minute amounts for normal growth, health, metabolism, and reproduction (Stickney, 1979; Akiyama et al., 1992). In intensive, high density culture such as heavily stocked ponds and raceways, natural foods are limited, so vitamins must be supplied in diet to achieve normal growth (Akiyama et al., 1992). The amount of vitamin A required by shrimp depends on shrimp size, age, growth rate, environmental conditions, and nutrient interrelationships (Akiyama et al., 1992).

Vitamin A occurs in two forms, vitamin A_1 (retinol) found in mammals and marine fish and vitamin A_2 (retinol$_2$) found in freshwater fish (Akiyama et al., 1992). Vitamin A is fat-soluble vitamin and can be deposited in the liver while water-soluble vitamins are hardly deposited in the organism (Stickney, 1979; Pillay, 1990). Vitamin A_1 (retinol) has the chemical formula $C_{20}H_{29}OH$ and vitamin A_2 (retinol$_2$) has the formula $C_{20}H_{27}OH$ (John and Ronald, 2002) (Figure 6). The relationship of the vitamin A to naturally occurring β-carotene containing two symmetrical betaionone rings (John and Ronald, 2002). Retinene or retinal, the aldehyde form of vitamin A, forms the photoreactive prosthetic

group of opsins present in the retina (Rønnestad et al., 1998). Retinene has been isolated from the retina of dark-adapted eyes and is involved in vision in dim light (John and Ronald, 2002). Retinoic acid, which is the oxidized form of vitamin A alcohol, has been shown to have some vitamin A activity (John and Ronald, 2002). Vitamin A alcohol occurs as light-colored viscous oil which is heat labile and subject to air oxidation. Vitamin A is water insoluble but soluble in fat and organic solvent (Stickney, 1979).

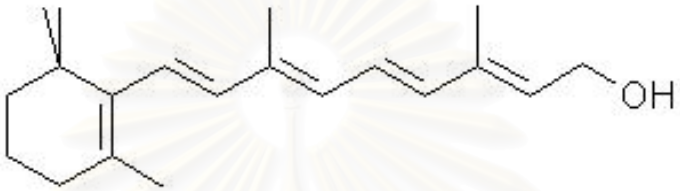

Figure 6 Structure of retinol (vitamin A)
Source: Parrish et al. (1985)

Positive functions of vitamin A

Vitamin A is essential in maintaining epithelial cells (He et al., 1992). Vitamin A is stimulates for new cell growth and aids in maintaining resistance to infection. It increases longevity under various conditions of senility in mammals (Akiyama et al., 1992). Vitamin A and retinene are essential for normal. It has been reported that the mechanism of action of retinoic acid (the active derivative of vitamin A) is closely similar to that of steroid hormones and thyroxin, involving activation of the expression of specific genes, and thus placing retinoids in the category of hormones regulating growth, differentiation, and embryonic development (Wolf, 1990; Akiyama et al., 1992). In addition, vitamin A is also involved in calcium transport across some membranes (Akiyama et al., 1992). Fish have variable abilities to hydrolyze β-carotene into retinol (Akiyama et al., 1992).

Requirements of vitamin A

Vitamin requirements vary from species to species (Stickney, 1979). The requirement for maximum growth and reproduction is related to exposure to light and reflects observations in other animals that near-normal growth will occur with a very low vitamin A in stress, infection, and ultraviolet radiation (John and Ronald, 2002). Recommended supplementation level of vitamin A in commercial feed is 10,000 IU/kg (Akiyama et al., 1992). Hoffman (1987) reported that vitamin A of formulated feed for marine shrimp is 4,000 to 100,000 IU/kg. However, NRC (1993) suggested that requirement vitamin A of Atlantic salmon was 2,500 IU/kg feed while 6,000 IU/kg feed for typical added levels in feed (Stickney, 2000). Generally, vitamin levels in prepared feed are sufficiently high that even with processing, storage, and leaching losses, the remaining levels meet requirements (Pillay, 1990).

Deficiency symptoms could be related to depigmentation, soft exoskeleton, poor growth, poor vision, keratinization of epithelial tissue, xerophthalmia, night blindness, hemorrhage in the anterior chamber of the eye, hemorrhage at the base of the fins, and abnormal bone formation (Stickney, 1979; Pillay, 1990; Akiyama et al., 1992). Nerve degeneration has been reported in pigs, chickens, rats, rabbits, and ducks but only occasionally observed in fish after long periods of deficiency.

Hypervitaminosis is rare in fish, although it is possible at very high levels (Stickney, 1979; Pillay, 1990). For example, excess vitamin A causes enlargement of liver and spleen, abnormal growth, bone formation, and epithelial keritonization, skin lesions, hyperplasia of head cartilage, resulting in ankylosis, and fusion of vertebrae (Stickney, 1979; Pillay, 1990). Hypervitaminosis A is reflected in a very high liver oil vitamin A content and elevated serum alkaline phosphatase (Pillay, 1990). Removal of excess vitamin A from the diet promotes rapid recovery (John and Ronald, 2002).

Sources of vitamin A

Animals cannot synthesize vitamin A de novo and a source must be available to them if normal retinal function is to be achieved retina (Rønnestad et al., 1998). Sources of vitamin A include fish liver oils and liver meals containing fish oil residues (Stickney, 1979; Akiyama et al., 1992). Cod liver oil is one typical standard reference oil, which contains relatively small amounts of vitamin A, whereas black sea bass, swordfish, and ling cod oils contain 100-fold more. Whale liver oil contains kitol, which has little or no biological activity until heated above 200°C. Then one molecule of biologically active vitamin A is generated per molecule of whale kitol. The biologically in active kitol may be deposited in the whale as a defense mechanism against hypervitaminosis A a during excessive vitamin A intake. When tuna, shark or lingcod viscera are used in the preparation of moist diets, the possibility of hypervitaminosis A occurs. Synthetic vitamin A preparations, such as vitamin A palmitates are available and are often used to supplement rations low in fishmeal, fish viscera, or carotenes. Droop (1955) reported that the source of vitamin A in shrimp was often, directly or indirectly, β-carotene from phytoplankton. Some fish species seem to be able to utilize β-carotene as a vitamin A source, whereas others are unable to split the β-carotene molecule and vitamin A must be added to the diet in the retinol, retinene, or retinoic acid form (Thompson et al., 1995). Several carotenoids can be converted into vitamin A in the liver of fish (Thompson et al., 1995).

He et al. (1992) reported effects of the deletion of individual fat-soluble vitamins (A, D, E, and K) from semi-purified diets of *Penaeus vannamei* (PL-6) for 8 weeks. Their results showed that the best growth, 7283% weight gain, was observed on shrimp fed a control diet with supplementation of vitamin A, D_3, E, and K_3. Significantly lower growth occurred in shrimp fed diets deficient in vitamin A (6242%), D_3 (5588%), and E (4821%). Shrimp growth was not affected by feeding the vitamin K_3-deficient diet. They suggested that vitamin A, D, and E are essential nutrients in shrimp diets.

Alava et al. (1993) found that the weight gain of *P. japonicus* fed the control diet (included vitamin A, E, and C were 15 mg, 50 mg, and 50 mg, respectively) was significantly higher than of those fed with diet not supplemented with vitamin A or E. Their experiments demonstrated that the control and initial prawn tissues contained higher vitamin A, E, and C than those of prawns fed with diets unsupplemented with vitamin A, E, or C. In addition, the significantly highest gonadosomatic index (GSI) was observed in prawns fed with the control diet. Their study indicated the importance of supplemental vitamins A, E, and C in diets of broodstock prawns for enhancing ovarian development.

Thompson et al. (1995) experimented a rainbow trout, *Oncorhynchus mykiss*, which was maintained for 4 months on diets with supplementary vitamin A (18 mg/kg dry diet) and astaxanthin (100 mg/kg dry diet), astaxanthin alone, vitamin A alone, and neither vitamin A nor astaxanthin. Their results found that fish had the similar specific growth rates achieved by each of 4 groups. However, fish fed diet supplemental vitamin A and astaxanthin gave lower trend of specific growth rate. For food conversion efficiencies (FCR), vitamin A intake seemed to have no effect on FCR in trout. In case of immune system, vitamin A and/or astaxanthin had a significant effect on total serum antiprotease activity, serum complement activity, and mean serum lysozyme activity, being lower in the group fed depletive diet. However, the effect was not quite significant statistically. They concluded that vitamin A and astaxanthin appear to have little potential as an immunostimulant in aquaculture.

Triño and Sarroza (1995) studied *P. monodon* (PL-18), which was reared in a modified extensive culture system where natural food organisms are available for 120 days. Two diets were tested, one with vitamin and mineral supplements and the other without. They found that mean final weight, net production, and net cost of production of shrimp fed the diet without vitamin and mineral supplements were lower and survival rate higher than other shrimp. However, no significant differences were observed among treatment means ($P>0.05$). The food conversion efficiencies were the same for

both treatments. In addition, histological analysis showed no difference in the cellular structure of the hepatopancreas.

Rønnestad et al. (1998) studied halibut (*Hippoglossus hippoglossus*) larvae were fed excess *Artemia* or natural copepods (mainly *Temora longicornis*) for 14 days. The result showed that the content of retinol and retinal were 50 to 80% lower in halibut larvae fed *Artemia* compared with larvae fed mix *Temora*. *Artemia* and *Temora* were major differences in the vitamin A and carotenoid composition. Cryptoxanthin /canthaxanthin were the major carotenoid in *Artemia* which was not in *Temora*. *Temora* did not contain any form of vitamin A: all-*trans* retinal and all-*trans* retinol. In *Artemia*; however, large amounts of an unknown retinoid component were present (70 μg/g dry weight). This component was at first identified as 13-*cis* retinol. This suggests that halibut larvae are not able to efficientcy convert the available carotenoids or the unknown retinoid component into retinal and retinol.

CHAPTER III

MATERIALS AND METHODS

The study was carried out at the Marine Biotechnology Research Unit (MBRU), Department of Marine Science, Chulalongkorn University and Angsila Marine Animals Research Station, Chonburi Province.

Preparations before the experiment

1) Experimental units. A plastic container with a volume of 22 liters was used as a rearing unit for shrimp larvae. Ten liters of filtered seawater was used for the experiment. Inside of the container was coated with epoxy to prevent toxic leakage from the surface of the rearing unit. Pictures of experimental unit are shown in Figure 7. All of experimental units were incubated in a temperature-controlling unit, which kept the temperature quite constant at 29.0±1°C.

2) Seawater. Seawater was transferred from Bangpra District, Chonburi Province. The salinity of the seawater was 30 ppt, temperature on 28 to 30°C, and ammonia less than 0.1 ppm. Before using the seawater, the seawater was filtered with 1 μ (Smith et al., 1992) and sterilized by ultraviolet and ozone system.

Figure 7 Experimental units of *P. monodon* larvae fed on different diets.

Experimental diets

Experimental diets consisted of the combinations of three levels of astaxanthin (AX; 0, 200, and 500 ppm) and three levels of vitamin A (VA; 0, 20,000, and 40,000 IU/kg) (Table 1). The compositions of experimental diets are shown in Table 2. Astaxanthin used Carophyll Pink, which had 8% active astaxanthin. Vitamin A used premix, which had 10,000,000 IU/kg of vitamin A. The basal ingredient of this experiment was practical diet. The experimental diets were produced by procedure as follows;

1) Mixed ingredients (particle size <20 μ) gently for 15 minutes.
2) Added 50% of 70°C water, mixed, and then cooled to 40°C.
3) Affixed mineral mix, vitamin mix, and astaxanthin or/and vitamin A.
4) Mixed to homogenize the feed mixture.
5) Dried the mixture by freeze dryer at -48°C for 48 hours.
6) Ground and sieved to sort the feed into three sizes; <63 μ for zoea, 63-125 μ for mysis, and >125 μ for postlarvae (Kanazawa et al., 1985).

All artificial diets were stored with nitrogen gas in dark containers at -20°C until used.

Table 1 The feeding experiments combination between astaxanthin and vitamin A

Diets		g/100 g of feed	
AX (ppm)	VA (IU/kg)	AX	VA
0	0	0	0
0	20,000	0	2
0	40,000	0	4
200	0	2.50	0
200	20,000	2.50	2
200	40,000	2.50	4
500	0	6.25	0
500	20,000	6.25	2
500	40,000	6.25	4

Table 2 Percent composition of the experimental diets.

Ingredients	g/100 g of feed
Fish meal	56.5
Wheat flour	10.0
Soy bean meal	5.0
Tuna oil	2.0
Mineral mix[1]	4.0
Vitamin mix[2]	4.0
Vitamin C	0.1
Lecithin	2.0
Cholesterol[3]	1.5
Wheat gluten	6.4
Head shrimp meal	2.0
Cellulose (make to 100 g feed)	6.5
Astaxanthin	(0-4)
Vitamin A	(0-6.25)

[1]Mineral mix 1 kg consists of: Calcium 147 g ;Phosphorus 147 g ;Manganese 10,062 mg ;Iron 2,010 mg ;Copper 3,621 mg ;Zinc 6,424 mg ;Cobolt 105 mg ;Iodine 1,000 mg ;Selenium 60 mg.

[2]Vitamin mix 100 g consists of: Vitamin A 10,000,000 IU ;Vitamin K3 1,000 mg ;Vitamin B6 1,500 mg ;Vitamin D3 1,000,000 IU ;Vitamin B1 500 mg ;Vitamin C 10,000 mg ;Vitamin E 10,000 mg ;Vitamin B2 5,000 mg ;Folate 1,000 mg ;D,L-methionine 16,038 mg.

[3]95% Cholesterol, Laboratory grade.

Experimental animals and design

P. monodon nauplii were used in the experiment obtained from a commercial hatchery in Chonburi Province. The feeding experiments were started at the beginning of zoea I, mysis I, and postlarva I, respectively. The initial larval density for was 100

larvae/l for zoea, 80 larvae/l for mysis, and 30 larvae/l for postlarvae. The experiment was 3x3 factorials involved completely randomized design with three replicates. At the beginning of each larval stage, a natural food was fed once to the larval as following; *Chaetoceros* sp. for zoea shrimp and *Artemia* sp. for mysis and postlarvae stages. During the experiment, shrimp were reared in 10 litres of seawater at 30 ppt and 2/3-water exchange daily. Larvae were fed on five times daily (6:00, 10:00, 14:00, 16:00, and 22:00 hours). The experiment in each stage was terminated at the larvae reaching mysis I for zoea, postlarva I for mysis, and postlarva 15 for early postlarval experiment.

Data collection

1) Proximate analysis of diets for each treatment was done according to AOAC (1984). Aataxanthin and vitamin A contents of diets were analyzed by High performance liquid chromatography (HPLC) using methods described by Weber (1988) and Manz and Philipp (1988), respectively. All methods for analyzing was shown in Appendixes 1-5.

2) Survival rate and specific growth rate (%increment of length) of the larvae for each dietary treatment were determined when they reached the early stage of the next developmental stage.

3) For the low salinity stress tests, ten individuals of postlarval 15 from each dietary treatment were placed in 300 ml of diluted seawater at salinity 2 ppt. Then, the number of dead shrimp was recorded every 10 minutes for a period of 2 hours. Data was analyzed using a probit analysis to determine time of 50% cumulative mortality (CM_{50}).

4) At the end of postlarval experimental trials, postlarval 15 from each dietary treatment was sacrificed to find astaxanthin and vitamin A in the whole body tissue using a method described by Boonyaratparin et al. (2001) and White et al. (2003).

5) In all experiments, water quality was monitored daily for:

- Salinity (ppt) measured by salinityrefractometer.
- Temperature (^{o}C) detected by thermometer.
- Dissolved oxygen (mg/l) determined by YSI model 57.
- pH measured by pH meter
- Ammonia and nitrite tested by using VBC test kit

6) All experimental data were analyzed using Analysis of Variance and Duncan's new multiple-range test (SAS, 1985).

CHAPTER IV

RESULTS AND DISCUSSIONS

Diet quality and characteristics

The result showed that there were 50.01 to 52.15% of protein, 10.54 to 11.69% fat, 15.02 to 18.03% ash, 2.59 to 3.91% fiber, 6.03 to 7.03% moisture, and 10.04 to 13.28% carbohydrate (Table 3). Carbohydrate content of the diets was determined by subtraction of others.

Table 3 Proximate analysis of experimental diets

Diets		%Protein	%Fat	%Ash	%Fiber	%Moisture	%Carbohydrate
AX	VA						
0	0	51.22	11.69	15.02	3.91	6.53	11.63
0	20,000	52.15	11.25	16.75	3.04	6.22	10.59
0	40,000	50.04	11.38	15.98	3.77	5.98	12.85
200	0	51.40	11.66	18.03	2.59	6.28	10.04
200	20,000	51.41	10.54	16.98	2.94	6.25	11.88
200	40,000	50.15	11.11	15.59	3.58	7.03	12.54
500	0	49.93	11.31	17.75	3.26	6.86	10.89
500	20,000	50.79	11.05	16.61	3.14	6.03	12.38
500	40,000	50.01	10.73	15.83	3.69	6.47	13.28

Experimental diets were dried by freezed dry method, which was the best method in order to keep nutrients in high quality. Thamrujikul (1990) demonstrated that freezed dry method could maintain vitamin C in feed better than cabinets dry and vacuum dry method. As larvae undergo metamorphosis very often, their nutrient requirements differ from adults or juveniles (Liao, 1992). For this study, the amounts of

protein levels estimated 50.01 to 52.15% were appropriate for marine shrimp larvae. Protein requirements are influenced by water temperature, body size, stocking density, oxygen levels and the presence of toxins (Pillay, 1990). The optimal protein level in a shrimp diet depends on other components of the diet. Teshima and Kanazawa (1984) showed that the protein requirement in *P. japonicus* larvae decreases from 55 to 45% when carbohydrate concentration in the diet increases from 5 to 25%. Shrimp feed was formulated to contain a high protein level, which was suitable to culture larval shrimp because postlarval shrimp require a higher protein level than larger shrimp. Recommended protein level in feed for shrimp (weight 0 to 5 g) was 45% (Akiyama et al., 1992). On the other hand, if the culture system is not totally dependent on feed, i.e. if natural foods are available, these protein levels may be reduced. Lee (1971) concluded that *Penaeus monodon* weighed 0.5 g desired 45% to 50% protein level while 1.3 g shrimp, required 40% protein level. Leger and Sorgeloos (1992) reported dietary protein requirements (in %dry diet) for *P. monodon* was 34 to 55%. In addition, in this study preparation of practical diet used wheat gluten, which was 70% of protein contents as binder causing the experimental diet, with higher protein. Recommended lipid levels for commercial feeds range from 6 to 7.5% (Akiyama et al., 1992). Lipid contents of most commercial aquaculture diets are less than 10% because higher levels do not appear to result in higher growth (Pillay, 1990). However, fat levels in this experiment were 10.54 to 11.69%, which were a little high levels. Since this study, astaxanthin and vitamin A in shrimp, which can be dissolved in fat, it is importance to maintain high fat levels. A high dietary lipid content of the diet is known to improve the utilization of carotenoids (Pillay, 1990). Fiber levels, in the experimental diets were 2.59 to 3.91%. In the same way, total fiber level of commercial feed should not exceed 4% (Akiyama, 1992). In addition, the amount of ash, moisture, and carbohydrate level in the practical diets were 15.02 to 18.03%, 6.03 to 7.03%, and 10.04 to 13.28%, respectively, which were in a suitable range for shrimp larvae.

The amount of astaxanthin contents in diet was closed to the expected formulated diet. However, the amount of vitamin A contents in the diet was less than that of the proposed diet (Table 4).

Table 4 Astaxanthin and vitamin A contents in the experimental diets (MEAN±SD)

Diets		AX (ppm)	A (IU/kg)
AX	VA		
0	0	0.46±0.04	290±18.35
0	20,000	5.70±2.25	12,287±237.15
0	40,000	2.86±0.21	33,726±436.15
200	0	189.64±11.54	304±26.41
200	20,000	268.03±17.98	18,745±524.82
200	40,000	232.17±36.76	32,468±713.52
500	0	460.52±20.59	456±61.15
500	20,000	604.75±79.95	15,650±341.26
500	40,000	535.22±52.18	35,692±342.67

The amount of astaxanthin contents in diet was closed to the expected content. However, the amount of vitamin A contents in the diet was less than that of the formulated diet. Difference in vitamin A content are probably due to its loss during feed preparation process, because astaxanthin is sensitive to light, heat, and oxygen (Stickney, 1979; Lorenz, 2000). Pelleting of feeds at low heat, and low oxygen exposure, could minimize a loss of astaxanthin as low as 1 to 4% (Lorenz, 2000).

The characteristics of diet in water is shown in Figures 8-10. The larval diets could stay uniformly more than 3 hours. The appearance of diet dissolved in water was done to measure suspension of diet by microscope at 4 hours. The results showed that zoea diet could suspend in the water more than 4 hours while mysis diet sank within 4 hours. Postlarval diet had the shortest suspended time. It sank within 2 hours.

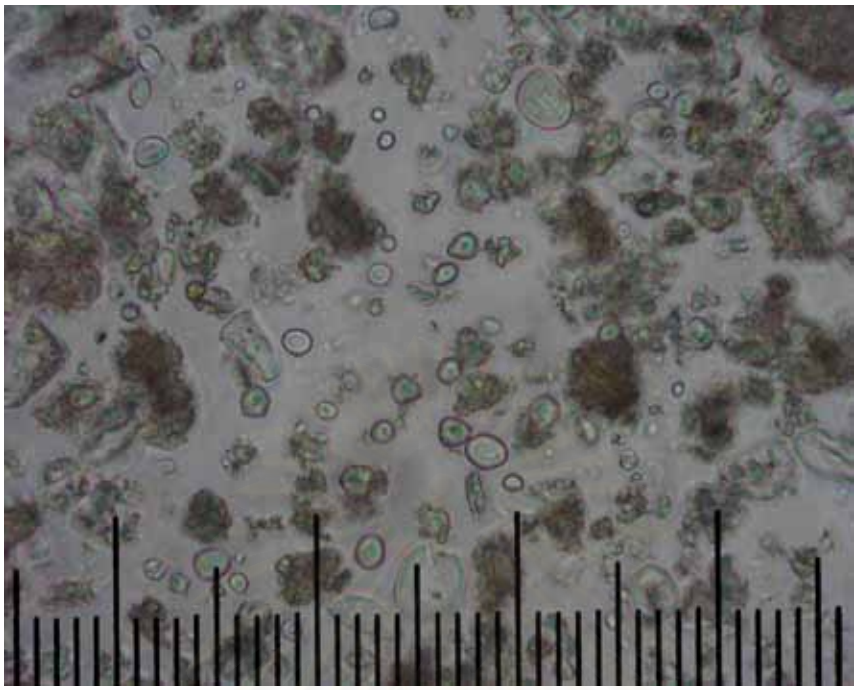

Figure 8 Characteristics of zoea diet in water (20x)

Note: 1 scale = 10 μ

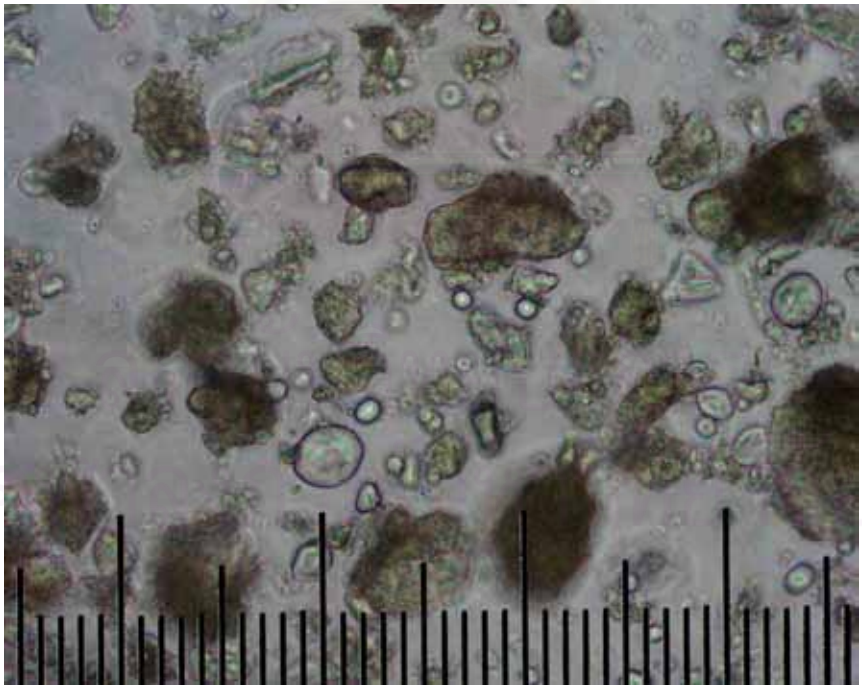

Figure 9 Characteristics of mysis diet in water (20x)

Note: 1 scale = 10 μ

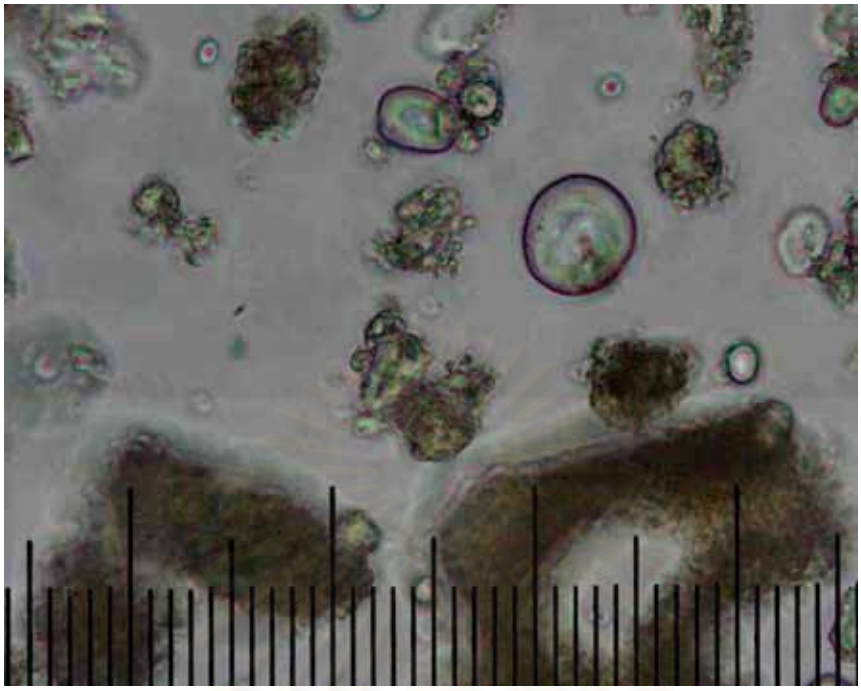

Figure 10 Characteristics of postlarval diet in water (20x)
Note: 1 scale = 10 μ

The color of particulate diets varied from yellowish to reddish depending on the amount of the astaxanthin and vitamin A contents (Figure 11). Astaxanthin are responsible for the broad varieties of colors in nature; however, most notable are the brilliant yellow, orange, and red colors of fruits, leaves, and aquatic animals (Simpson et al., 1985; Meyers, 1994; Lorenz, 2000).

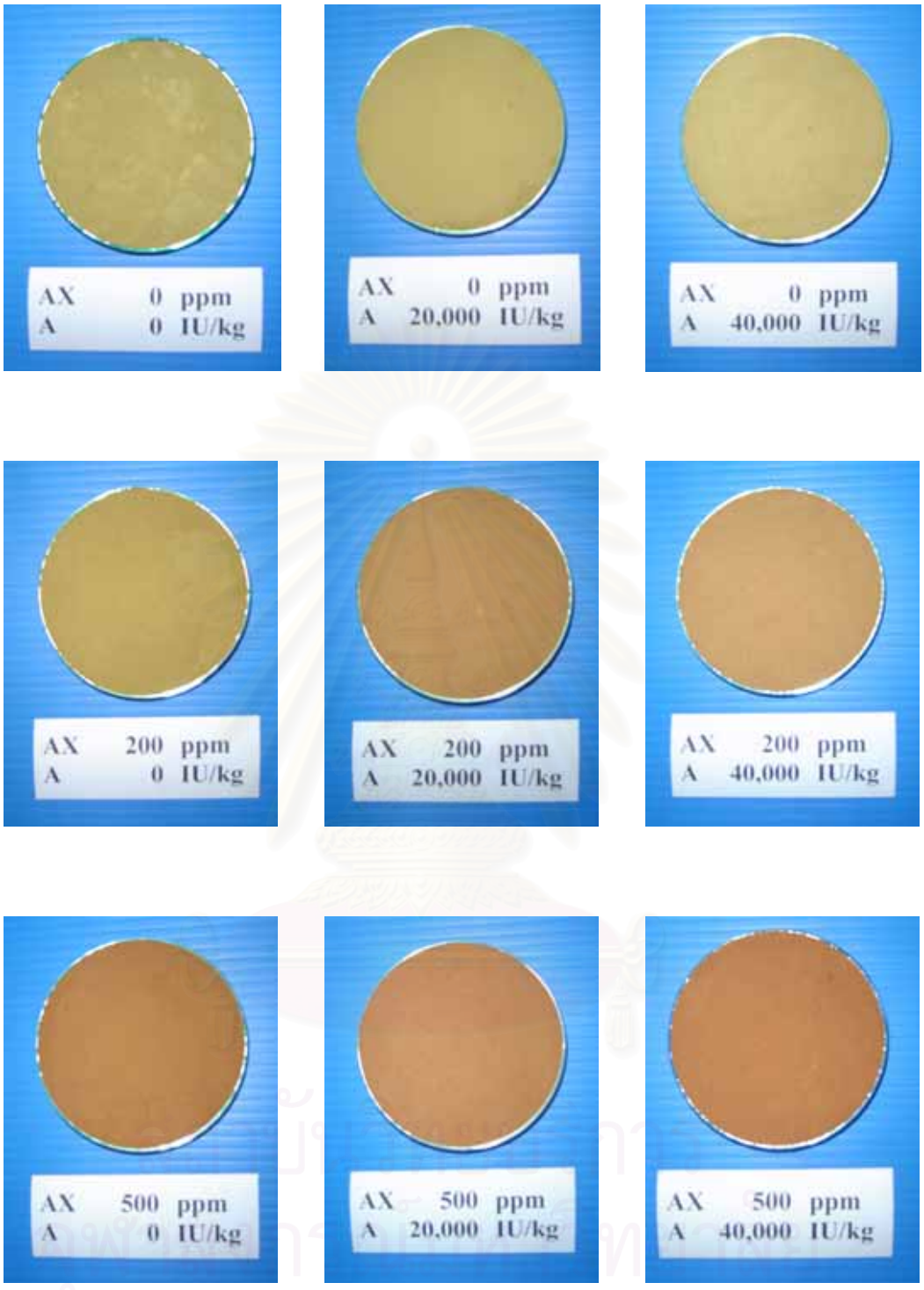

Figure 11 Appearance and color of practical diet in different levels of astaxanthin and vitamin A contents.

Water quality

The water quality of *P. monodon* larvae rearing units for all treatment groups was exhibited in Table 5. The data of water quality were similar for the whole dietary treatments, which were in the normal range for shrimp cultivation. Water quality criteria are primarily based on single-factor laboratory experiments with selected life stages and species (Colt and Huguenin, 1992). In general, water quality criteria for larvae are more stringent than for juveniles and adults (Colt and Huguenin, 1992). Ammonia concentrations in all experimental units were in a safe ammonia concentration for juvenile *P. monodon* and lower than 3.7 mg/L (Chen and Lei, 1990). Chin and Chen (1987) suggested ammonia concentration (μg/l NH_3-N) of penaeid shrimp was 17 for zoea stage, 48 for mysis stage, and 100 for postlarval stage. For nitrite concentration of penaeid shrimp hatcheries was 0.29 mg/l NO_2-N for zoea stage, 0.45 mg/l NO_2-N for mysis stage, and 1.36 mg/l NO_2-N for postlarval stage (Chen and Chin, 1988).

Temperature and salinity are important to distinguish among the environmental responses of the larvae, which are adapted to oceanic salinity and surface temperatures (James and Pante, 1992). Water temperature of about 30°C and 30 ppt of salinity are optimal for the larvae of *P. semisulcatus* (Kumlu et al., 2000). Relatively low temperature (22°C) decreased rate of larval development and growth but did not cause high mortality (Kumlu et al., 2000). Ideally, best production of penaeid larvae is obtained in oceanic quality water having physical parameters in the range of 28 to 34 ppt salinity, 27 to 30°C temperature, and pH of 7.8 to 8.2 (Smith et al., 1992).

Table 5 Water quality of the rearing units at different stages and dietary treatments of larvae cultivation.

Diet	Stage	Salinity (ppt)	Temp (^{0}C)	DO (mg/l)	pH	NH_3 (ppm)	NO_2^- (ppm)
1	Z1-Z3	30	29.2-30.1	5.99-6.04	8.1-8.2	0-0.25	0
	M1-M3	30	29.2-30.1	5.82-6.33	8.3-8.4	0-0.50	0-0.10
	PL1-PL15	25	29.2-30.1	5.92-6.04	7.7-8.5	0-1.0	0-0.25
2	Z1-Z3	30	29.8-30.0	6.00-6.28	8.1-8.4	0-0.50	0
	M1-M3	30	29.9-30.0	5.92-6.15	8.0-8.2	0-0.50	0-0.10
	PL1-PL15	25	28.9-30.1	5.84-6.12	7.8-8.5	0-1.00	0-0.25
3	Z1-Z3	30	29.2-30.1	5.86-6.04	8.2-8.4	0-0.50	0
	M1-M3	30	29.2-30.0	6.01-6.19	8.0-8.2	0-0.50	0-0.10
	PL1-PL15	25	29.1-30.0	5.53-5.84	7.8-8.5	0-1.00	0-0.25
4	Z1-Z3	30	29.8-30.0	5.89-6.02	8.2-8.3	0-0.25	0
	M1-M3	30	29.8-30.0	5.99-6.12	8.0-8.1	0-0.25	0-0.10
	PL1-PL15	25	29.2-30.1	5.99-6.10	7.7-8.5	0-1.0	0-0.10
5	Z1-Z3	30	29.2-30.1	5.83-6.14	8.1-8.4	0-0.50	0
	M1-M3	30	29.2-30.0	5.99-6.04	8.1-8.2	0-0.50	0-0.10
	PL1-PL15	25	29.8-30.0	6.00-6.28	8.1-8.4	0-1.00	0-0.10
6	Z1-Z3	30	29.2-30.0	5.94-6.21	8.0-8.4	0-0.50	0
	M1-M3	30	29.2-30.1	5.86-6.04	8.2-8.4	0-0.50	0-0.10
	PL1-PL15	25	29.2-30.1	5.97-6.21	8.2-8.3	0-1.00	0-0.25
7	Z1-Z3	30	29.4-30.0	6.05-6.45	8.2-8.3	0-0.25	0
	M1-M3	30	29.2-30.0	5.83-6.14	8.1-8.4	0-0.25	0-0.10
	PL1-PL15	25	29.4-30.0	5.94-6.21	8.0-8.4	0-1.0	0-0.25
8	Z1-Z3	30	29.4-30.1	5.78-6.04	8.1-8.3	0-0.25	0
	M1-M3	30	29.4-30.1	6.05-6.45	8.2-8.3	0-0.25	0-0.10
	PL1-PL15	25	29.2-30.1	5.78-6.05	8.2-8.3	0-1.0	0-0.25
9	Z1-Z3	30	29.2-30.1	5.88-5.96	8.2-8.3	0-0.50	0
	M1-M3	30	29.8-30.0	5.89-6.02	8.2-8.4	0-0.50	0-0.10
	PL1-PL15	25	29.2-30.1	5.97-6.20	8.2-8.3	0-1.00	0-0.10

Effects of astaxanthin and vitamin A on zoea stage of *P. monodon*.

Times to metamorphosis of zoea shrimp to mysis shrimp was 4 days and 14 hours supporting Støttrup and McEvory (2003) report that the duration of the zoea was around 5 days at 28°C. Times to metamorphosis of zoea to mysis was 3 to 4 days (Daniel and John, 2000). The results of survival rate and specific growth rate of zoea shrimp are shown in Table 6 and Figures 12-14. There was a significant interaction between astaxanthin and vitamin A on survival and growth of zoea *P. monodon* ($P<0.05$) (Appendic 11).

Table 6 Survival rate and specific growth rate of *P. monodon* zoea fed different astaxanthin and vitamin A levels in diets (MEAN±SD).

Diet		%Survival rate*	Specific growth rate*
AX	VA		
0	0	60.20±0.35^{a}	56.50±0.09^{a}
0	20,000	56.70±2.90^{a}	56.34±0.08^{b}
0	40,000	42.02±11.52^{b}	56.42±0.06^{ab}
200	0	46.87±2.85^{ab}	56.44±0.07
200	20,000	60.77±6.07^{a}	56.47±0.04
200	40,000	45.56±10.72^{b}	56.36±0.07
500	0	70.74±3.57^{a}	56.58±0.03^{a}
500	20,000	60.77±6.07^{ab}	56.34±0.04^{b}
500	40,000	59.21±6.53^{b}	56.48±0.12^{ab}

*Mean with different superscripts represents significant difference at $p<0.05$ in a same colume of the same value of astaxanthin.

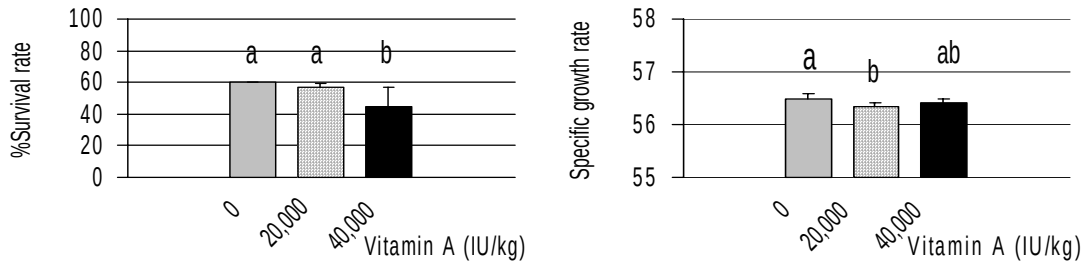

Figure 12 Survival rate and specific growth rate of *P. monodon* zoea fed 0 ppm astaxanthin with vitamin A difference levels of diets

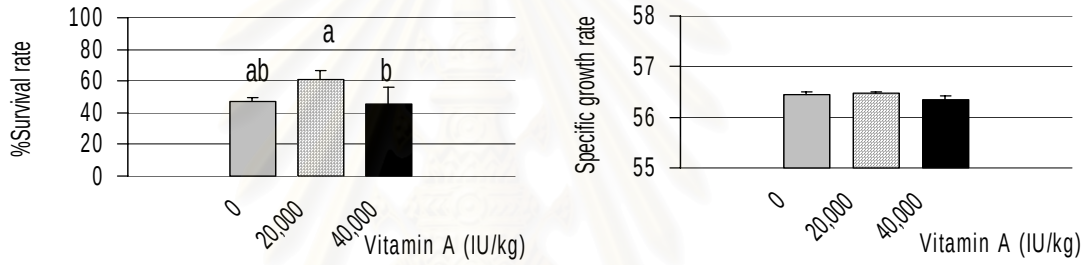

Figure 13 Survival rate and specific growth rate of *P. monodon* zoea fed 200 ppm astaxanthin with vitamin A difference levels of diets

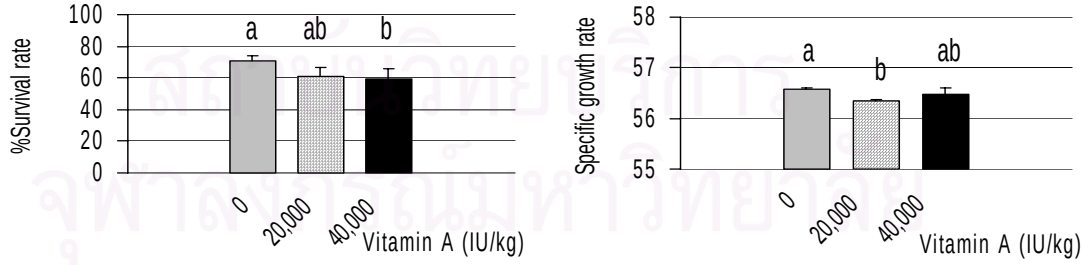

Figure 14 Survival rate and specific growth rate of *P. monodon* zoea fed 500 ppm astaxanthin with vitamin A difference levels of diets

At 0 ppm astaxanthin, survival rate of zoea shrimp fed without additional vitamin A was significantly higher than shrimp fed the other diets ($P<0.05$) (60.20±0.35%). However, there was no difference significantly with shrimp fed with additional 20,000 IU/kg vitamin A (56.70±2.90%). For specific growth rate, shrimp fed without additional vitamin A was significantly higher than shrimp fed with additional 40,000 IU/kg and 20,000 IU/kg vitamin A.

At 200 ppm astaxanthin, the results indicated that shrimp fed with additional 20,000 IU/kg vitamin A was higher than shrimp fed other additional levels significantly. Survival rates of shrimp fed with additional A 20,000 IU/kg, 0 IU/kg, and 40,000 IU/kg vitamin A (60.77±6.07%, 46.87±2.85%, and 45.56±10.72%, respectively). There is no difference significance of specific growth rate of all tested diets. Previous studies on trout by Hilton (1983) and Atlantic salmon by Storebakken et al. (1993) also showed no significant effect of vitamin A concentrations on growth rate. It appears that fat-soluble vitamin A and E contained in lipids in the basal ingredients were sufficient to maintain excellent survival; however, for significantly better weight gain and gonadal development, supplementation of both vitamin A and E is necessary (Alava et al., 1993).

At 500 ppm astaxanthin, the interaction demonstrated that on the survival rate and specific growth rate of shrimp fed without additional vitamin A were higher than shrimp fed the other vitamin A diets (70.74±3.57% and 56.58±0.03, respectively). Practical diet, vitamin A may obtain from raw materials such as fishmeal and shrimp head meal. These vitamin A contents in diet were enough for normal growth of shrimp. The results also showed likely that zoea shrimp require more astaxanthin with increase of vitamin A. Wyban et al. (1995) referred by Chien, (1996) reported that adding paprika, a source of carotenoid pigment, on *P. vannamei* broodstock diet would improve quality of nauplii and increase their survival to zoea-II from 25% to 82%. At this stage the larva is light sensitive; therefore, the experimental unit should be properly covered to ensure darkness (Pillay, 1990; Liao, 1992). While astaxanthin can help in protection against UV light and help in stimulation of growth (Meyers, 1994). In the case of Atlantic salmon, it has been reported that diets supplemented with astaxanthin and

canthaxanthin promoted growth rates during the early start feeding period (Torrissen, 1984).

Effects of astaxanthin and vitamin A on mysis stage of *P. monodon*.

The duration of mysis to postlarval stage in this experiment was 5 days and 11 hours. The same result also showed in a study of Daniel and John (2000). The results of dietary effects on survival rate and growth rate on mysis stage are shown in Table 7 and Figures 15-17. There was an interaction between astaxanthin and vitamin A on survival rate of shrimp mysis stage ($P<0.05$) (Appendic 11). However, specific growth rates were not significant different in all treatments. In addition, survival rate of shrimp fed 200 ppm astaxanthin with 40,000 IU/kg vitamin A was 0%, since could not detected growth of this groups.

Table 7 Survival rate and specific growth rate of *P. monodon* mysis were fed different astaxanthin and vitamin A levels in diets (MEAN±SD).

Diet		%Survival rate*	Specific growth rate*
AX	VA		
0	0	59.09±7.35^{a}	17.27±0.07
0	20,000	49.75±9.78^{ab}	17.27±0.05
0	40,000	35.73±5.57^{b}	17.24±0.07
200	0	54.42±1.74^{a}	17.28±0.09
200	20,000	59.31±6.58^{a}	17.27±0.05
200	40,000	0^{b}	nd
500	0	72.84±7.76^{a}	17.36±0.05
500	20,000	20.64±4.36^{b}	17.29±0.07
500	40,000	9.62±2.37^{c}	17.28±0.02

nd = not detectable.

*Mean with different superscripts represents significant difference at $p<0.05$ in a same colume of the same value of astaxanthin

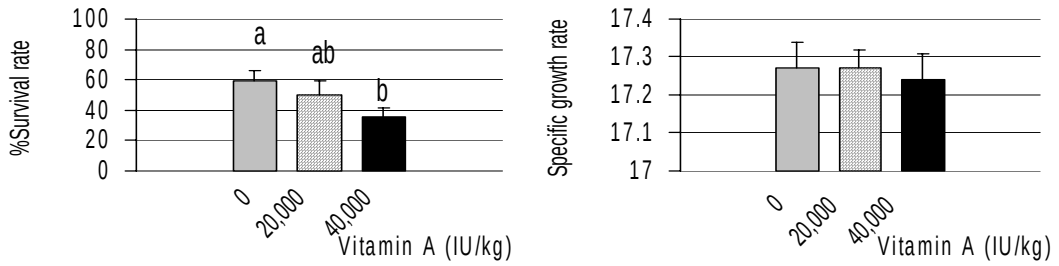

Figure 15 Survival rate and specific growth rate of *P. monodon* mysis fed 0 ppm astaxanthin with vitamin A difference levels of diets

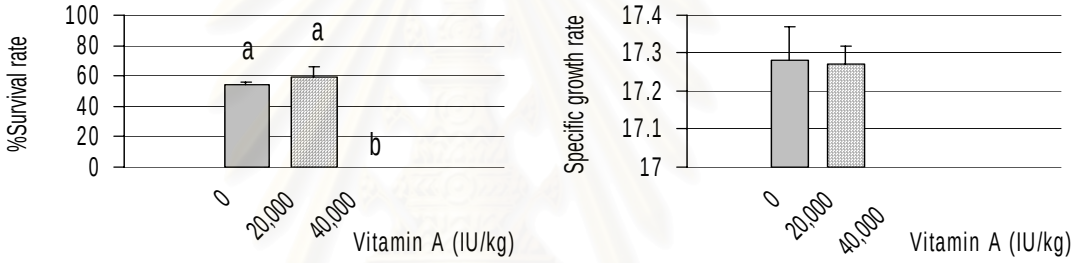

Figure 16 Survival rate and specific growth rate of *P. monodon* mysis fed 200 ppm astaxanthin with vitamin A difference levels of diets

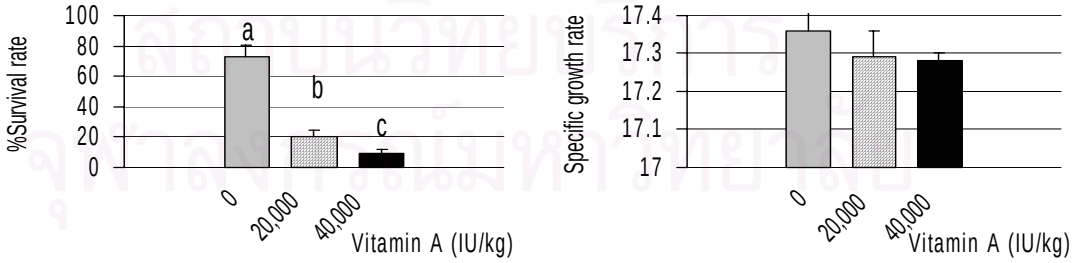

Figure 17 Survival rate and specific growth rate of *P. monodon* mysis fed 500 ppm astaxanthin with vitamin A difference levels of diets

At 0 ppm astaxanthin, survival rate of shrimp fed not vitamin A was higher than the shrimp fed only 20,000 IU/kg and 40,000 IU/kg vitamin A (59.09±7.35%, 49.75±9.78%, and 35.73±5.57%, respectively).

At 200 ppm astaxanthin, the results showed that survival rate of shrimp fed with additional 20,000 IU/kg vitamin A had higher than the other groups (59.31±6.58%). However, there had no significance difference with shrimp fed only 200 ppm astaxanthin (54.42±1.74%). Survival of shrimp fed without additional 40,000 IU/kg vitamin A was 0% and could not growth of this group. It implies that supplemented vitamin A too high determined toxic to mysis especially when astaxanthin was also high. Since astaxanthin could converted into vitamin A (Latsha, 1990).

At 500 ppm astaxanthin, shrimp fed only 500 ppm astaxanthin gave the best of survival rate than shrimp fed 500 ppm astaxanthin with the other vitamin A (72.84±7.76%). It has been observed that prawns supplemented with astaxanthin have a higher rate of survival compared to prawns fed β,β-carotene or algal meal (Chien and Jeng, 1992). By the way, specific growth rates were no difference significantly on mysis shrimp fed with/without astaxanthin and vitamin A. The results suggested that astaxanthin has no effect on growth rate, in agreement with findings of Yamada et al. (1990) and Nègre-Sadargues et al. (1993). Similarly, Boonyaratpalin et al (2001) concluded that supplement with β–carotene or astaxanthin in diets had no significant effect on juvenile shrimp growth, average final weight, survival rate, feed conversion ratio, and on the immune response in production of haemocytes. Thongrod et al. (1995) found no significant effect of shrimp weight gain on total carotenoid or astaxanthin concentration in the diet treatments. The results of significantly reduced growth (85.7% of the growth of shrimp in the control treatment) in vitamin A-deficiency treatment demonstrated that vitamin A is an essential nutrient for shrimp (*Penaeus vannamei*) (He et al., 1992).

Effects of astaxanthin and vitamin A on postlarvae stage of *P. monodon*.

The survival rates, specific growth rates and time of 50% cumulative mortality(CM_{50}) displayed in Table 8 and Figures 18-23. There were significant differences on the interaction between astaxanthin and vitamin A in the survival, growth and stress test of postlarvae *P. monodon* ($P<0.05$). For tolerance of postlarva 15, the larvae fed all groups of diets started to die at 10 minutes due to salinity stress. After 120 minutes, some shrimp in all treatments fed diets resisted to 2 ppm salinity such as diet without additional astaxanthin and vitamin A and diet with additional only 500 ppm astaxanthin.

Table 8 Survival rate, specific growth rate, and time to 50% cumulative mortality (CM_{50}) of *P. monodon* postlarvae were fed different astaxanthin and vitamin A levels in diets (MEAN±SD).

Diet		%Survival rate*	Specific growth rate*	CM_{50} (min)*
AX	VA			
0	0	54.00±3.71^{a}	4.17±0.01^{a}	26.47±8.04^{a}
0	20,000	60.78±13.93^{a}	4.16^{ab}	15.00±2.61^{b}
0	40,000	20.89±3.24^{b}	4.12±0.04^{b}	11.64±2.30^{b}
200	0	40.89±4.29^{a}	4.09±0.02^{b}	21.84±7.39^{a}
200	20,000	43.11±11.20^{a}	4.15±0.03^{a}	9.63±1.21^{b}
200	40,000	0.44±0.77^{b}	nd	nd
500	0	80.67±7.64^{a}	4.17±0.03^{a}	40.05±26.57^{a}
500	20,000	0.44±0.51^{b}	4.11±0.02^{b}	nd
500	40,000	0^{b}	nd	nd

nd = not detectable

*Mean with different superscripts represents significant difference at $p<0.05$ in a same colume of the same value of astaxanthin

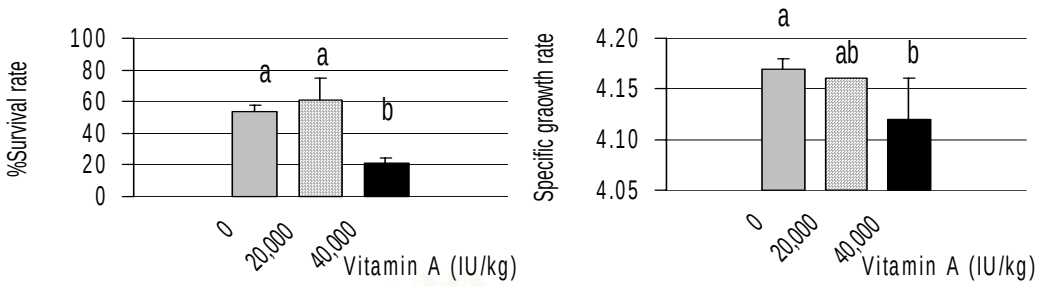

Figure 18 Survival rate and specific growth rate of *P. monodon* postlarvae fed 0 ppm astaxanthin with vitamin A difference levels of diets

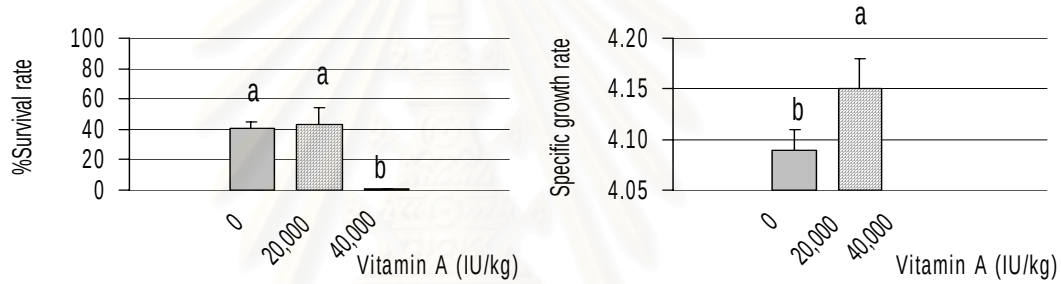

Figure 19 Survival rate and specific growth rate of *P. monodon* postlarvae fed 200 ppm astaxanthin with vitamin A difference levels of diets

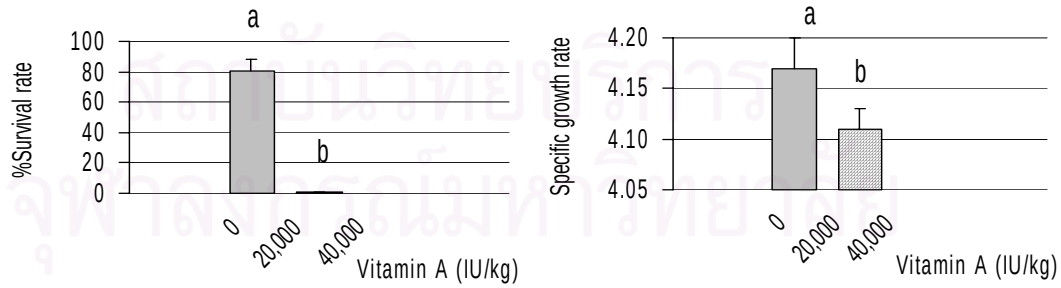

Figure 20 Survival rate and specific growth rate of *P. monodon* postlarvae fed 500 ppm astaxanthin with vitamin A difference levels of diets

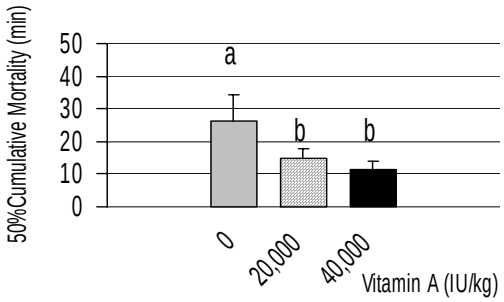

Figure 21 Time of 50% cumulative mortality (min) on salinity stress of *P. monodon* postlarvae fed 0 ppm astaxanthin and vitamin A levels in diets

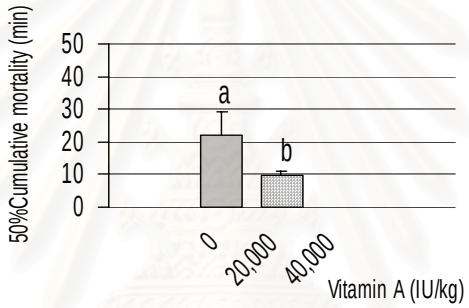

Figure 22 Time of 50% cumulative mortality (min) on salinity stress of *P. monodon* postlarvae fed 200 ppm astaxanthin and vitamin A levels in diets

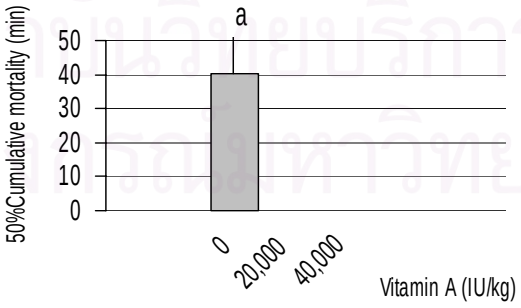

Figure 23 Time of 50% cumulative mortality (min) on salinity stress of *P. monodon* postlarvae fed 500 ppm astaxanthin and vitamin A levels in diets

At 0 ppm astaxanthin, shrimp larvae received with additional 20,000 IU/kg vitamin A were healthy and had higher survival rate (60.78±13.93%) than shrimp fed other diets. However, the highest specific growth rate was found on shrimp fed without additional astaxanthin and vitamin A, which were 4.17±0.01. He et al. (1992) reported that survival of shrimp fed diets deficient in vitamin A, D_3, and K_3 did not differ from that of shrimp fed the control diet. However, the highest specific growth rate was found on shrimp fed without additional astaxanthin and vitamin A, which were 4.17±0.01. For stress test, shrimp fed without additional vitamin A could more withstand salinity stress than shrimp fed only 20,000 and 40,000 IU/kg vitamin A, which were 26.47±8.04, 15.00±2.61, and 11.64±2.3 minutes, respectively.

At 200 ppm astaxanthin, larvae fed with additional 20,000 IU/kg vitamin A gave the best survival rate and specific growth rate than larvae fed other diets, which were 43.11±11.20% and 4.15±0.03, respectively. However, larvae fed without additional vitamin A were found higher tolerance than shrimp fed the other groups (21.84±7.39 minutes). Postlarval shrimp fed 40,000 IU/kg vitamin A with/without additional astaxanthin showed low survival rate since experimental diets had higher vitamin A concentration than the requirement of shrimp. The results of vitamin A contents in natural diets of mysis shrimp, which were *Chaetoceros* sp. and *Artemia* sp. There found that vitamin A content of *Chaetoceros* sp. and *Artemia* sp. were 98±16.21 μg/g and 201±14.5 μg/g, respectively, which was less than vitamin A content in experimental diets. Maybe too high vitamin A was toxic for larval shrimp. It is important to emphasize that excess vitamin A is toxic to organisms, including fish larvae, and that an additional supply of vitamin A (ester or aldehyde) through enrichment emulsions of *Artemia* might be harmful to the fish larvae (Takeuchi et al., 1995). Hypervitaminosis is rare in fish, although it is possible at very high levels (Stickney, 1979). For example, excess vitamin A causes enlargement of liver, and spleen, abnormal growth, bone formation, and epithelial keritonization, skin lesions, and hyperplasia of head cartilage, resulting in ankylosis, and fusion of vertebrae (Stickney, 1979; Pillay, 1990). Removal of excess vitamin A from the diet promotes rapid recovery (John and Ronald, 2002). Carotenoids

are generally not toxic since they are converted into active vitamin A by a process probably regulated by demand (Rønnestad et al., 1998).

At 500 ppm astaxanthin, the results showed that shrimp fed only 500 ppm astaxanthin were found the best of survival rate, specific growth rate, and CM_{50}, which were 80.67±7.64, 4.17±0.03, and 40.05±26.57 minutes, respectively. Survival rate on postlarval shrimp fed 500 ppm astaxanthin without additional vitamin A was higher than mysis and zoea shrimp, respectively. Darachai et al. (1996) reported that utilization of free astaxanthin (major group in synthetic astaxanthin) might be poor for zoea and mysis but may be better for postlarvae. Similarly, *P. japonicus* postlarva was able to absorb and metabolize dietary astaxanthin (Petit et al., 1997). Thongrod et al. (1995) found that after *P. monodon* postlarvae fed diets supplemented with astaxanthin, the survival rate increased as dietary astaxanthin levels increased. They concluded that astaxanthin was required for survival of *P. monodon*. Chien and Jeng (1992) reported a positive correlation between pigment concentration in the tissue and survival of kuruma prawn *Marsupenaeus japonicus*.

In addition, the specific growth rate of zoea to postlarvae stages decreased. It is well known that crustacean growth rate decreases concomitantly with increasing age and consequently with increasing body weight (Choe, 1970). However, the postlarva-15 fed astaxanthin supplemented had no significant difference in growth rate (Darachai et al., 1996). Similarly previous studies on trout (Hilton, 1983) showed no significant effect of vitamin A on growth rate. Likewise, there was no marked effect of astaxanthin on either food conversion efficiency or growth rate (Hilton, 1983). He et al. (1992) found a significant reduction of growth (85.7% of growth of shrimp in the control treatment, 4,800 IU/kg vitamin A) in vitamin deficiency treatment. It demonstrates that vitamin A is an essential nutrition for the shrimp (*P. vannamei*) (He et al., 1992).

By the way, good quality postlarval shrimp could easily withstand the rigors of harvest and transport and readily adapt to experimental change in rearing conditions (Olin and Fast, 1992). The resistance to osmotic stress has been used to evaluate

shrimp postlarval quality and postlarval nutritional status (Olin and Fast, 1992; Rees et al., 1994; Chien et al., 2003). In those studies, a close relationship between the strong antioxidant (of astaxanthin) and the stress resistance was indicated by an increase in shrimp survival. However, there is no any biochemical evidence provided yet (Chein et al. 1992). While an organism is subjected to stresses such as chemical, physical, biological upon sudden shortage of oxygen, abnormal oxidative reactions in the aerobic metabolic pathway resulting in formation of excess amounts of single oxygen (Ranby and Rabek, 1978 referred by Chien, 2002). The enhancement of antioxidation capacity by dietary astaxanthin and consequently, the improvement of recovery from thermal and osmotic stress suggests that astaxanthin is a 'semi-essential- nutrient for tiger prawn, particularly when the animal is under physiological stress (Chien et al., 2003). Vitamin A is an essential nutrient for all species of mammals, birds, and fishes and also is required by many lower forms of life. Its physiological functions in vertebrates are involved in regeneration of light-sensitive compound rhodopsin in the retina, transportation of calcium across some membranes, reproduction and embryonic development, and integrity of cellular and subcellular membranes (NRC, 1983, 1987).

P. japonicus juvenile fed astaxanthin (360 ppm) supplemented diet had better resistance (tolerance) to dissolved oxygen (DO) depletion (DO<1 mg/l, for 4 hours) and had higher survival than shrimp fed diet without astaxanthin supplemented (Chien et al., 1999). Lethal DO level of shrimp fed astaxanthin was lower than that of the control group, but not significant difference. Darachai et al. (1996) determined CM_{50} upon low salinity challenge, and the results showed that larvae fed astaxanthin endured better than larvae fed no astaxanthin supplemented and natural food. Astaxanthin seemed to be helpful to the postlarvae and to prolong their life upon this acute environmental stress (Darachai et al., 1996). Enhancement of resistance to salinity stress in penaeid shrimp postlarvae was associated with an increase in dietary and body astaxanthin (Merchie et al. 1998). In previous studied, after 1-2 hour exposure to salinity stress, postlarvae maintained on diets supplemented with high levels of astaxanthin had better survival than those of postlarvae fed diets containing lower or no astaxanthin supplemented (Stanier et al., 1971). Because astaxanthin contains a long conjugated double bond

system, they are relatively unstable and usually scavenge oxygen radicals in cell (Stanier et al., 1971). When shrimp were transferred from high to low salinity, they are exposed to stress and needed to utilize more energy to maintain osmotic stability.

Astaxanthin and vitamin A contents of postlarva 15 *P. monodon*.

The results of astaxanthin and vitamin A contents of postlarva 15 *P. monodon* were determined by HPLC method demonstrated on Table 9 and Figures 24-26. Astaxanthin and vitamin A contents for initial shrimp (postlarva 1) were 217.31±16.21 μg/g and 180.01±17.37 μg/g, respectively. In addition, astaxanthin contents in *Chaetoceros* sp. and *Artemia* sp. were 98±16.21 μg/g and 201±14.5 μg/g, respectively. Vitamin A content in *Artemia* sp. was 5,967±101.56 μg/g while *Cheatoceros* sp. not found vitamin A. Shrimp fed experimental diets were interaction between astaxanthin and vitamin A contents in postlarvae 15 shrimp.

Table 9 Astaxanthin and vitamin A contents in whole tissue shrimp fed different astaxanthin and vitamin A levels of diets.

Diet		Astaxanthin (μg/g)*	Vitamin A (μg/g)*
AX	A		
0	0	110.41±15.07^{c}	186.94±18.26^{c}
0	20,000	161.09±20.01^{b}	424.09±24.55^{b}
0	40,000	247.31±21.96^{a}	550.47±50.29^{a}
200	0	292.57±11.05^{a}	213.03±24.15^{a}
200	20,000	212.35±28.49^{b}	388.49±30.30^{b}
200	40,000	nd	nd
500	0	456.12±44.54^{a}	164.74±21.24^{a}
500	20,000	nd	nd
500	40,000	nd	nd

nd = not detectable

*Mean with different superscripts represents significant difference at $p<0.05$ in a same colume of the same value of astaxanthin

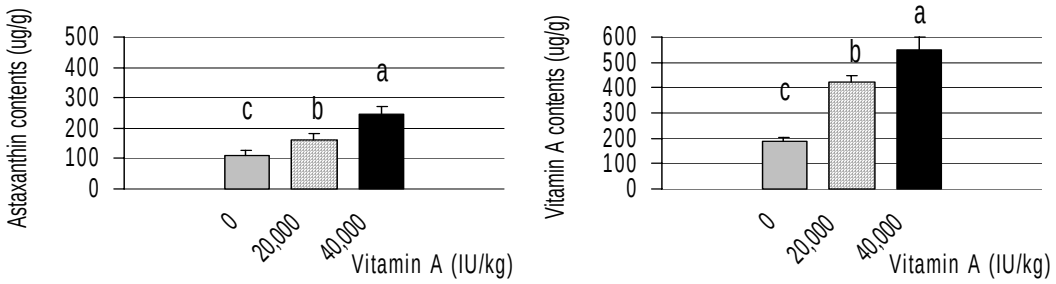

Figure 24 Astaxanthin content (μg/g) and vitamin A in whole tissue of *P. monodon* postlarvae fed 0 ppm astaxanthin and vitamin A levels in diets

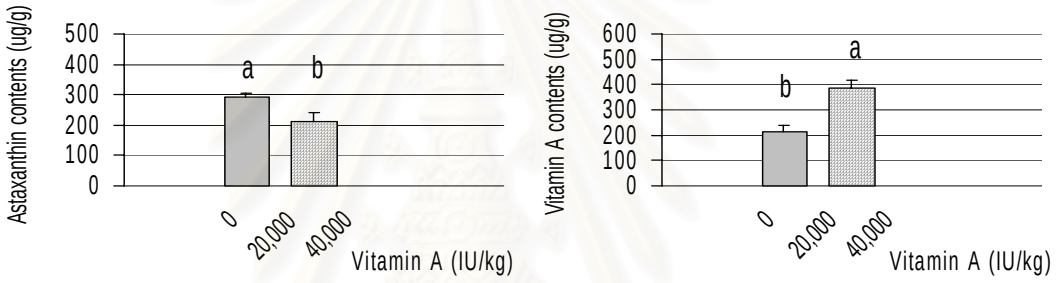

Figure 25 Astaxanthin content (μg/g) and vitamin A in whole tissue of *P. monodon* postlarvae fed 200 ppm astaxanthin and vitamin A levels in diets

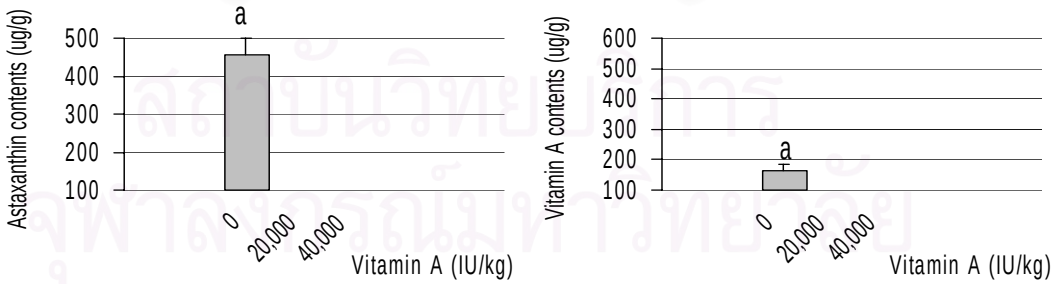

Figure 26 Astaxanthin content (μg/g) and vitamin A in whole tissue of *P. monodon* postlarvae fed 500 ppm astaxanthin and vitamin A levels in diets

At 0 ppm astaxanthin, the highest accumulate astaxanthin and vitamin A were found on shrimp fed only 40,000 IU/kg vitamin A (247.31±21.96 μg/g and 550.47±50.29 μg/g, respectively).

At 200 ppm astaxanthin, the results showed that shrimp fed only 200 ppm astaxanthin was higher astaxanthin and vitamin A accumulative in shrimp than shrimp fed astaxanthin with vitamin A (292.57+11.05 μg/g and 213+24.15 μg/g, respectively).

At 500 ppm astaxanthin, astaxanthin and vitamin A contents in shrimp fed without additional vitamin A (456.12±44.54 μg/g and 164.74±21.24 μg/g, respectively). After shrimp acquired different astaxanthin and vitamin A levels in diets, the results showed that shrimp with supplemental astaxanthin in diet had increased accumulative astaxanthin content in shrimp as well as collected vitamin A content. Increase in pigmentation could be accomplished by increasing carotenoid in the feed to the level at which the carotenoid uptake is higher than the increase in the surface area of prawn (Menasveta et al., 1993). The pigment gain from dietary sources can vary with species, sizes or age of animals, growth rates, rearing conditions and periods, dietary astaxanthin levels, pigmented tissue, and other unknown factors (Pan et al., 2001). Shrimp can directly deposit astaxanthin in their tissue (Nègre-Sadargues et al., 1993). Dietary astaxanthin was incorporated into body tissue at a higher rate than the other dietary carotenoids (Yamada et al., 1990). Hata and Hata (1972) suggested that the occurrence of hydroxyl groups within the molecular configuration of the carotenoid enhance its absorption by the digestive epithelium. A comparison of dietary astaxanthin, canthaxanthin, and β–carotene showed astaxanthin is the most effective for shrimp pigmentation (Yamada et al., 1990; Nègre-Sadargues et al., 1993). Dietary carotenoid concentration and duration of feeding may influent it's deposition rate (AL-Khalifa and Simpson, 1988). Supplement of astaxanthin in pellets is more efficient in maintaining pigmentation than that of nature canthaxanthin according to Petit et al. (1998). They concluded that the carotenoid pattern of postlarval stages consists mainly free and esterified astaxanthin, which do not depend on astaxanthin source (*Artemia salina* or synthetic astaxanthin). D'Abramo et al. (1983) found that major pigments

accumulated in the lobster were astaxanthin. They concluded that the level of pigmentation achieved at a particular dietary concentration was directly related to the proximity of the carotenoid to the astaxanthin end product in the biosynthetic scheme.

In crustaceans, up to 90% of vitamin A is concentrated in the eyes and high level of vitamin A is usually correlated with free-swimming species existing in deep water (Droop, 1955). There were no significant differences in vitamin A content of liver and blood of rainbow trout, indicating that astaxanthin was not converted to vitamin A in the vitamin A saturated fish, in which dietary retinol in dehydrogenerated to vitamin A_2 in the intestinal wall (AL-Khalifa and Simpson, 1988). In halibut larvae fed ly *Artemia* for 13 days was 1,413 μg of vitamin A/individual dry weight, which increased from initial, 750 μg of vitamin A/individual dry weight (Rønnestad et al., 1998). In addition, vitamin A levels in *Artemia* nauplii could be raised from 1.3 to 1,283 IU/g dry weight over an 18-h period through the addition of vitamin A palmitate to an egg-yolk-based emulsion (Dedi et al., 1995).

CHAPTER V

SUMMARY

SUMMARY

Upon the results of the present study, we may summarize that:

1. There is an interactive effect of astaxanthin and vitamin A supplemented diets on survival and growth of *Penaeus monodon* zoea, mysis, and postlarvae. Their interaction also found in postlarval stress to low salinity.

2. On zoea stage;

- Astaxanthin 0 ppm, the highest survival rate and specific growth rate were found in shrimp fed without additional and vitamin A (60.20±0.35% and 56.50±0.09, respectively).
- Astaxanthin 200 ppm, larvae fed astaxanthin 200 ppm with additional vitamin A 20,000 IU/kg was the highest survival rate (60.77±6.07%). However, no difference significantly on specific growth rate of shrimp was found in all treatments.
- Astaxanthin 500 ppm, shrimp fed additional only astaxanthin 500 ppm was the highest survival and specific growth rate, which were 70.74±3.57% and 56.58±0.03, respectively.

3. On mysis stage;

- Astaxanthin 0 ppm, survival rate of shrimp fed not supplemented astaxanthin and vitamin A was higher (59.09±7.35%) than the shrimp fed additional vitamin A (20,000 IU/kg or 40,000 IU/kg). There were differences significantly on specific growth rate in mysis shrimp.

- Astaxanthin 200 ppm, the results showed that survival rate of shrimp fed with additional vitamin A 20,000 IU/kg was higher than the other groups (59.31±6.58%). However, there was no significance difference with shrimp fed only astaxanthin 200 ppm (54.42±1.74%).

- Astaxanthin 500 ppm, the interaction showed that shrimp fed additional only astaxanthin 500 ppm was higher survival rate than shrimp fed astaxanthin 500 ppm with vitamin A 20,000 IU/kg and 40,000 IU/kg. Specific growth rate was no difference in all treatments.

4. On postlarval stage;

- Astaxanthin 0 ppm, shrimp larvae received additional only vitamin A 20,000 IU/kg were higher survival rate (60.78±13.93%) than shrimp fed the other diets. However, the highest specific growth rate was found on shrimp fed without additional astaxanthin and vitamin A (4.17±0.01) and could more withstand salinity stress than shrimp fed other diets (26.47±8.04 minutes).

- Astaxanthin 200 ppm, larvae fed with additional vitamin A 20,000 IU/kg gave the best survival rate and specific growth rate than larvae fed the other diets, which were 43.11±11.20% and 4.15±0.03, respectively. However, larvae fed without additional vitamin A were higher tolerance than shrimp fed the other diets (21.84±7.39 minutes).

- Astaxanthin 500 ppm, the results showed that shrimp fed only astaxanthin 500 ppm were found the best of survival rate, specific growth rate, and CM_{50}, which were 80.67±7.64, 4.17±0.03, and 40.05±26.57 minutes, respectively.

5. The higher survival rates, specific growth rate and time to 50% cumulative mortality (CM_{50}) were found in shrimp fed with additional astaxanthin. This experimented demonstrated that vitamin A 20,000 to 40,000 IU/kg were too large for normal growth of larval shrimp.

6. Astaxanthin and vitamin A contents in whole postlarva-15 shrimp, increase with their increment in the feed.

7. It is not necessary to supplement vitamin A in the diets that formulated form raw natural materials.

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APPENDICES

Appendic 1 Determinations of astaxanthin in feed (Weber, 1990 and Borowitzka, 1991)

Reagents:
1. n-heaxane
2. Acetone
3. Phosphoric acid
4. Silica gel 60, particle size 0.2 to 0.5 mm
5. Papain
6. Ethanol
7. Dichloromethane
8. Diethyl ether

Apparatus:
1. Sonicator
2. Rotary evaporator

Method:

1. Weighed 0.1 to 0.2 g of sample.
2. Added 20 mg of papain and 5 ml of distilled water.
3. Sonicated at 50^{o}C for 30 minutes and cooled to ambient temperature.
4. Added 4 ml of ethanol and shook.
5. Added 8 ml of dichloromethane and shook.
6. Added 10 ml of n-hexane, shook, and allowed the solvents separated.
7. Pipette upper layer into a new tube and extracted sample with 5 ml of n-hexane two times.
8. Filtered with open chromatography by silica gel [Packed column with 10 g of silica gel and 10 ml of n-hexane : diethyl ether (1:1)], then eluted with acetone until colorless.
9. Evaporated and dissolved the residue with 5 ml of acetone. This solution is ready for injection into the HPLC column

Appendic 1 (continued)

Specifications for HPLC (reverse phase):

Column: Stainless steel, length 25 cm, inner diameter 4 mm

Stationary phase: LiChrosorb RP-8

Mobile phase: Acetonitrile : Dichloromethane : Methanol : Distillated water (79.9:10:10:0.1)

Flow rate: 1.0 ml/min

Pressure: approx. 80 bar

Temperature: ambient

Injection volume: 20 μl

Detection: VIS-detection at 470 nm

Retention time: astaxanthin : about 5 minutes;

Run time: 10 minutes

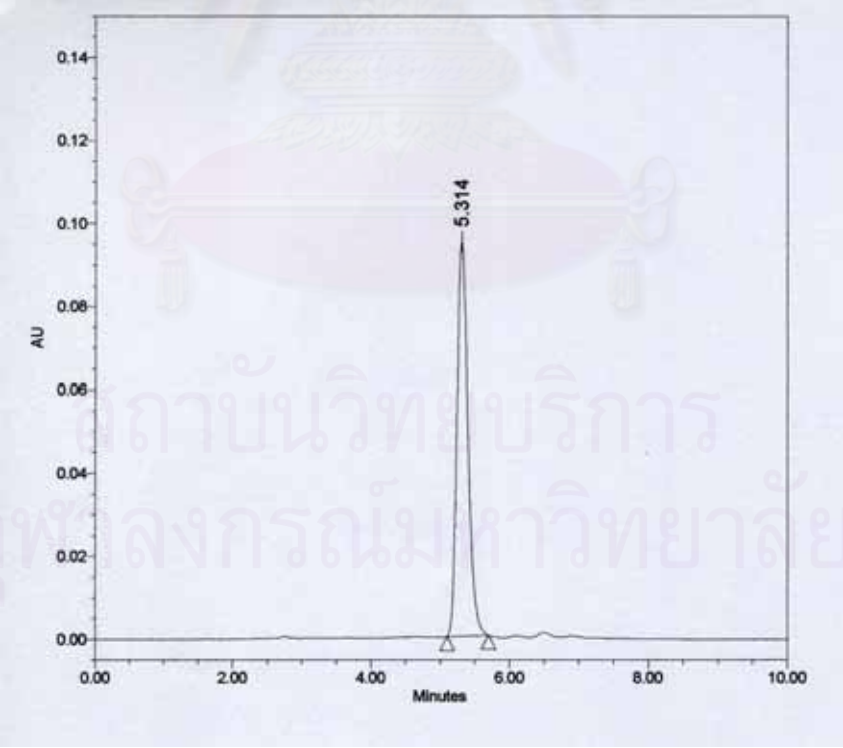

Figure 27 HPLC chromatogram of standard astaxanthin in reverse phase

Appendic 2 Determination of astaxanthin in shrimp (Boonyaratparin et al., 2001)

Reagents:
1. Tetrahydrofuran
2. n-hexane : acetone (86 : 14).

Apparatus:
1. Sonicator
2. Centrifuge
3. Freeze dry
4. Rotary evaporator

Method:

1. Dried shrimp to powder.
2. Accurately weighted 0.5 g of dried shrimp powder.
3. Moistened with 1 ml of water and allowed to swell for a few minutes.
4. Added 4 ml of Tetrahydrofuran and sonicated for 15 seconds.
5. Pipetted clear liquid into a centrifuge tube.
6. Repeated extraction three times.
7. Centrifuged at 4,500 rpm. for 5 minutes.
8. Evaporated clear supernatant and dissolved the residue with 5 ml of n-hexane : acetone (86:14). This solution is ready for injection into the HPLC column.

Appendic 2 (continued)

Specifications for HPLC (normal phase):

Column: Stainless steel, length 25 cm, and inner diameter 4 mm
Stationary phase: Silica gel pretreated with phosphoric acid
Mobile phase: n-hexane containing 14 percent acetone (86:14), isocratic
Flow rate: 1.5 ml/min
Pressure: approx. 80 bar
Temperature: ambient
Injection volume: 10 to 50 μl
Detection: VIS-detection at 470 nm
Retention time: astaxanthin about 12 minutes
Run time: 15 minutes

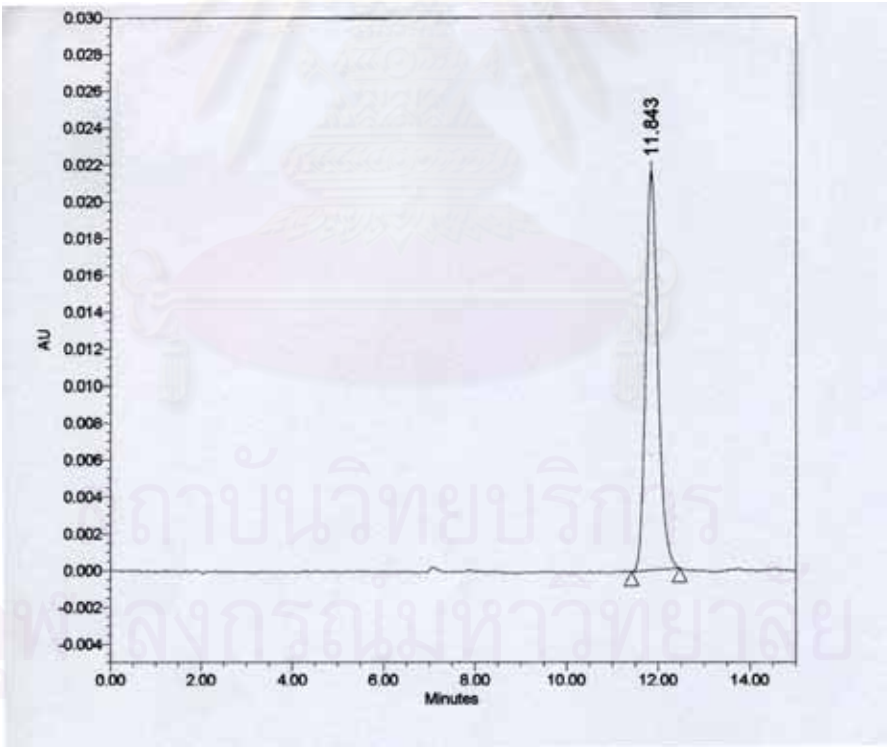

Figure 28 HPLC chromatogram of standard astaxanthin in normal phase

Appendic 3 Determination of astaxanthin in *Chaetoceros* sp. (Borowitzka, 1991)

Method:

1. Filtered 10 to 20 ml of *Chaetoceros* sp. Culture.
2. Added 10 ml 90%(v/v) cold acetone.
3. Stored filter in 90% acetone in the dark and on ice until assayed
4. Ground filter, transferred to centrifuge tube, and then centrifuge at 5,000 rpm for 3 minutes.
5. Pipetted clear liquid into a centrifuge tube.
6. Repeated extraction three times.
7. Centrifuged at 5,000 rpm. for 5 minutes.
8. Evaporated clear supernatant and dissolved the residue with 5 ml of n-hexane : acetone (86:14). This solution is ready for injection into the HPLC column.
9. Injected to HPLC with specifications for HPLC (normal phase) as same as determination of astaxanthin in shrimp

Appendic 4 Determination of vitamin A in feed (Manz and Philipp, 1988)

Reagents:
1. Ethanol
2. 50% Potassium hydroxide
3. Ethyl ether
4. Butylated hydroxytoluene

Apparatus:
1. Separating funnel
2. Reflux

Method:

1. Accurately weighed 40 g of sample into a 500 ml round-bottom flask
2. Added 120 ml of ethanol and 40 ml of 50% potassium hydroxide
3. Mixed and for 30 minutes.
4. Shook from time to time to prevent the material adhering to the sides of the flask. At the end of refluxed, cooled to 40^{o}C
5. Made up these solutions to 300 g, first with 20 ml of distilled water and then with ethanol in the preweighed round-bottom flask used.
6. Immediately weighed 50 g into a beaker and transferred into a separating funnel containing 100 ml of ethyl ether.
7. Shook for 1 minute and allowed the solvents to separate.
8. Transferred the aqueous layer into a second separating funnel and repeated the extraction once with 100 ml of ethyl ether.
9. Washed the combined ether phases with distilled water until neutral and transferred them to a 500 ml volumetric flask.
10. Added and dissolved 100 mg Butylated hydroxytoluene and made up to volume with ether.
11. Evaporated to dryness and dissolved the residue in 10ml of n-hexane. This solution is ready for injection into the HPLC column.

Appendic 5 Determination of vitamin A in shrimp (White et al., 2003)

Reagents: 1. Potassium hydroxide
3. n-hexane
4. Butylated hydroxytoluene

Method:

1. Dissolved sample with potassium hydroxide and added 100 mg of Butylated Hydroxytoluene as antioxidants.
2. Saponified for 20 minutes at 100°C.
3. Extracted three times with n-hexane.
4. Evaporated and dissolved residue with 5 ml of n-hexane. This solution is ready for injection into the HPLC column.

Specifications for HPLC (normal phase):

Column: Stainless steel, length 12.5 cm, and inner diameter 4 mm
Stationary phase: Silica gel pretreated with phosphoric acid
Mobile phase: n-hexane containing 2 percent isopropanol, isocratic
Flow rate: 1 ml/min
Pressure: approx. 40 bar
Temperature: ambient
Injection volume: 20 to 50 μl
Detection: VIS-detection at 326 nm
Retention time: (all-E)-retinol: about 6 minutes;
(13Z)-retinol: about 5 minutes
Run time: 10 minutes

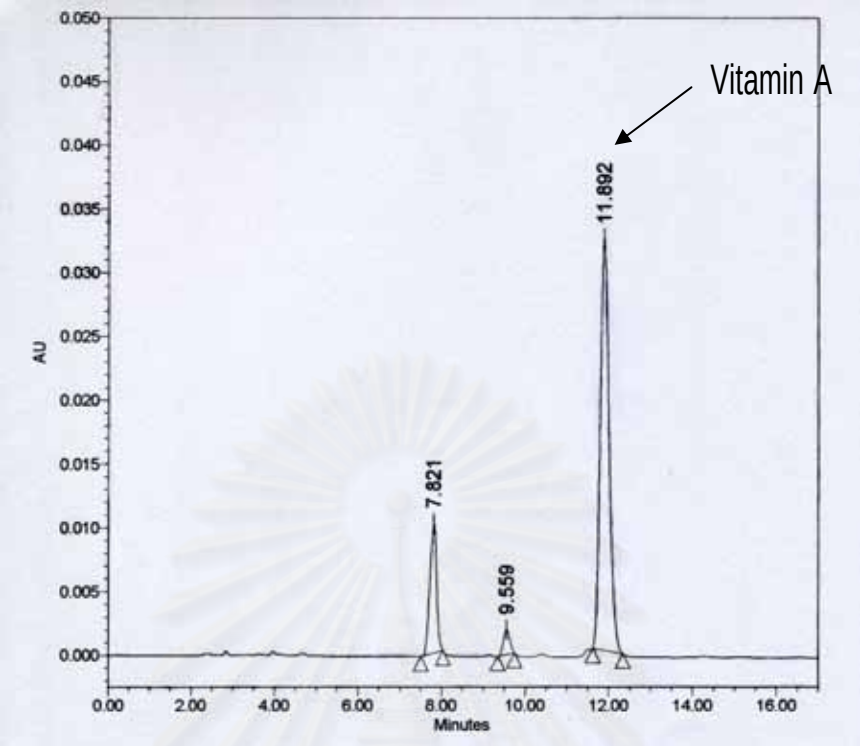

Figure 29 Chromatogram of vitamin A in normal phase

Appendic 6 Protein determination (AOAC, 1984)

Reagents:
1. Conc. Sulfuric acid solution
2. 0.1 N Sulfuric acid solution
3. 50% Sodium hydroxide solution
4. 4% Boric acid solution
5. Catalyst (7 g of Copper sulfate + 100 g of Potassium sulfate)
6. Indicator (Methyl Red : Methylene Blue; 3:2)

Apparatus:
Gerhardt Kjeldatherm Digestion Unit
Gerhardt Vapodest 1

Method:

1. Weighed 2 g of dty sample and put in a digestive tube.
2. Added 2 g of catalyst.
3. Set the Kjeldatherm and digested until the sample became black.
4. Digested completely for 4 hours (set the beginning temperature at 100^{o}C and increased 20^{o}C every 15 to 20 minutes until controlled at 380^{o}C).
5. Cooled until room temperature then added 40 ml distilled water.
6. Set the Vapodest 1 for distilling the digested sample, added 50% Sodium hydroxide and then kept the distillated in boric acid which added indicator 5-6 drops.
7. Titrated the solution with 0.5 N Sulfuric acid.

Protein content (%) = (A x B x 6.25 x 1.4)/ C

A = normality of Sulfuric acid used in titration

B = volume of sulfuric acid used in titration (ml)

C = weight of sample (g)

Appendic 7 Fat determination (AOAC, 1984)

Reagent: Petrolium ether

Apparatus: 1. Soxtherm automatic S-11, Garhardt
2. Whatman No.1 filter

Method:

1. Weighed 2 g of dry sample and covered with dry Whatman No.1 filter.
2. Put the cover into the thimble, which placed in the accurately weighed soxhlet bottle then added petrolium ether 80 ml into the bottle.
3. Set the Soxtherm automatic, which controlled silicone oil at 150°C.
4. Left for the extraction for 4 to 6 hours then evaporated petrolium ether from the extract.
5. Dried the bottle at 100°C for 1 hour and transferred to the desiccator.
6. Weighed and calculated according to the follows:

Fat content (%) = (A x 100)/ B

A = weight of extracted fat (g)

B = weight of sample (g)

Appendic 8 Ash determination (AOAC, 1984)

Apparatus: Muffle furnace

Method:

1. Weighed 2 g of dry sample into porcelain crucible and placed in temperature controlled furnace preheated to 600^{o}C.
2. Hold on this temperature 4 hours.
3. Transfer crucible directly to desiccator, cooled and weighed immediately.

Ash content (%) = (A / B) x 100

A = weight of remained ash (g)

B = Weight of dry sample (g)

Appendic 9 Fiber determination (AOAC, 1984)

Reagents: 1. 0.255 N Sulfuric acid solution
2. 0.313 N Sodium hydroxide solution

Apparatus: 1. Crude fiber digestion apparatus
2. Filtered fiber Whatman No.41
3. Muffle furnace

Method:

1. Boiled 200 ml of Sulfuric acid solution in 600 ml beaker and placed on hot plate.
2. Poured the weight sample (2 g out of fat) into the beaker and digested for 30 minutes (during the digestion, should be maintained the volume of Sulfuric acid by covering the beaker with condenser).
3. Filtered the digested sample with filtered paper (dry exactly at 105^{o}C for 2 hours) and washed the residue with distilled water until the digested was neutral.
4. Filled the residue into the beaker and added 200 ml of Sodium hydroxide, and then boiled the extract for 30 minutes.
5. Filtered the digest with the same filtered paper and washed the residue with distilled water until the digested was neutral.
6. Washed the residue again with 30 ml of 95% ethanol then put the filtered paper in the porcelain crucible and dried at 100^{o}C for 2 hours.
7. Transferred porcelain crucible directly to desiccator, cooled and weighed.
8. Placed the porcelain crucible in temperature controlled Muffle furnace preheated to 600^{o}C for 3 hours.
9. Transferred the porcelain crucible directly to desicator, cooled and weigh again.

Fiber content (%) = (A + B) – (C – D)/ weight of sample

A = weight of dry residue (g)
B = weight of dry filtered paper (g)
C = weight of filtered paper before used (g)
D = weight of sample

Appendic 10 Moisture determination (AOAC, 1984)

Apparatus: Hot air oven

Method:

1. Weighed 2 g of sample and placed in the dried extractly porcelain crucible.
2. Put the porcelain crucible in the hot air oven, which controlled temperature at 110^{o}C.for 2 hours.
3. Transferred porcelain crucible directly to desiccator, cooled and weighed.
4. Done 2 to 3 again until weight of dry sample was stable.

Moisture content (%) = [(A – B) x 100]/ A

A = weight of sample (g)

B = weight of dry sample (g)

Appendic 11 Statistical analysis (Factorial design)
Survival rate of zoea stage

General Linear Models Procedure

Dependent Variable: Survival Rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2038.09187407	254.76148426	5.83	0.0009
Error	18	786.27226667	43.68179259		
Corrected Total	26	2824.36414074			

R-Square	C.V.	Root MSE	DATA Mean
0.721611	11.82933	6.60922027	55.87148148

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	651.21236296	325.60618148	7.45	0.0044
AX	2	817.67867407	408.83933704	9.36	0.0016
VA*AX	4	569.20083704	142.30020926	3.26	0.0355

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	651.21236296	325.60618148	7.45	0.0044
AX	2	817.67867407	408.83933704	9.36	0.0016
VA*AX	4	569.20083704	142.30020926	3.26	0.0355

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 18 MSE= 43.68179

Number of Means 2 3

Critical Range 6.546 6.868

Duncan Grouping	Mean	N	VitaminA
A	59.419	9	20000
A			
A	59.269	9	0
B	48.927	9	40000

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 18 MSE= 43.68179

Number of Means 2 3

Critical Range 6.546 6.868

Duncan Grouping	Mean	N	Astaxanthin
A	63.576	9	500
B	52.974	9	0
B			
B	51.064	9	200

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	859.07215556	429.53607778	61.46	0.0001
Error	6	41.93153333	6.98858889		
Corrected Total	8	901.00368889			

R-Square	C.V.	Root MSE	DATA Mean
0.953461	4.460340	2.64359393	59.26888889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	859.07215556	429.53607778	61.46	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	859.07215556	429.53607778	61.46	0.0001

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 6.988589

Number of Means 2 3

Critical Range 5.282 5.474

Duncan Grouping	Mean	N	Astaxanthin
A	70.740	3	500
B	60.203	3	0
C	46.863	3	200

---------- VA=20000 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	33.18408889	16.59204444	0.61	0.5754
Error	6	164.03980000	27.33996667		
Corrected Total	8	197.22388889			

R-Square	C.V.	Root MSE	DATA Mean
0.168256	8.799834	5.22876340	59.41888889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	33.18408889	16.59204444	0.61	0.5754

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	33.18408889	16.59204444	0.61	0.5754

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 27.33997

Number of Means 2 3

Critical Range 10.45 10.83

Duncan Grouping	Mean	N	Astaxanthin
A	60.777	3	500
A			

Appendic 11 (continued)

	A	60.777	3	200
	A			
	A	56.703	3	0

---------- VA=40000 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	494.62326667	247.31163333	2.56	0.1573
Error	6	580.30093333	96.71682222		
Corrected Total	8	1074.92420000			

R-Square	C.V.	Root MSE	DATA Mean
0.460147	20.10043	9.83447112	48.92666667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	494.62326667	247.31163333	2.56	0.1573

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	494.62326667	247.31163333	2.56	0.1573

---------- VA=40000 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 96.71682

Number of Means 2 3

Critical Range 19.65 20.36

Duncan Grouping	Mean	N	Astaxanthin
A	59.210	3	500
A			
A	45.553	3	200
A			
A	42.017	3	0

---------- AX=0 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	558.70302222	279.35151111	5.93	0.0379
Error	6	282.49460000	47.08243333		
Corrected Total	8	841.19762222			

R-Square	C.V.	Root MSE	DATA Mean
0.664176	12.95278	6.86166404	52.97444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	558.70302222	279.35151111	5.93	0.0379

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	558.70302222	279.35151111	5.93	0.0379

---------- AX=0 ----------

Appendic 11 (continued)

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 47.08243

Number of Means 2 3

Critical Range 13.71 14.21

Duncan Grouping	Mean	N	Vitamin A
A	60.203	3	0
A			
A	56.703	3	20000
B	42.017	3	40000

---------- AX=200 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	427.04682222	213.52341111	4.01	0.0784
Error	6	319.49320000	53.24886667		
Corrected Total	8	746.54002222			

R-Square	C.V.	Root MSE	DATA Mean
0.572035	14.29014	7.29718210	51.06444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	427.04682222	213.52341111	4.01	0.0784

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	427.04682222	213.52341111	4.01	0.0784

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 53.24887

Number of Means 2 3

Critical Range 14.58 15.11

Duncan Grouping	Mean	N	Vitamin A
A	60.777	3	20000
A			
B A	46.863	3	0
B			
B	45.553	3	40000

---------- AX=500 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	234.66335556	117.33167778	3.82	0.0851
Error	6	184.28446667	30.71407778		
Corrected Total	8	418.94782222			

R-Square	C.V.	Root MSE	DATA Mean
0.560125	8.717231	5.54202831	63.57555556

Appendic 11 (continued)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	234.66335556	117.33167778	3.82	0.0851
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	234.66335556	117.33167778	3.82	0.0851

---------- AX=500 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 30.71408

Number of Means 2 3

Critical Range 11.07 11.48

Duncan Grouping		Mean	N	Vitamin A
	A	70.740	3	0
	A			
B	A	60.777	3	20000
B				
B		59.210	3	40000

Appendic 11 (continued)
Specific growth rate of zoea stage

General Linear Models Procedure

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.16434074	0.02054259	3.92	0.0077
Error	18	0.09440000	0.00524444		
Corrected Total	26	0.25874074			

R-Square	C.V.	Root MSE	DATA Mean
0.635156	0.128314	0.07241854	56.43851852

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	0.07916296	0.03958148	7.55	0.0042
AX	2	0.01071852	0.00535926	1.02	0.3799
A*AX	4	0.07445926	0.01861481	3.55	0.0265
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	0.07916296	0.03958148	7.55	0.0042
AX	2	0.01071852	0.00535926	1.02	0.3799
A*AX	4	0.07445926	0.01861481	3.55	0.0265

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 18 MSE= 0.005244

Number of Means 2 3

Critical Range .07172 .07525

Duncan Grouping	Mean	N	Vitamin A
A	56.51111	9	0
B	56.42333	9	40000
B			
B	56.38111	9	20000

Duncan's Multiple Range Test for variable: Secific growth rate

Alpha= 0.05 df= 18 MSE= 0.005244

Number of Means 2 3

Critical Range .07172 .07525

Duncan Grouping	Mean	N	ASTAXANTHIN
A	56.46667	9	500
A			
A	56.42556	9	200
A			
A	56.42333	9	0

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: SPECIFIC GROWTH RATE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02675556	0.01337778	2.81	0.1374
Error	6	0.02853333	0.00475556		
Corrected Total	8	0.05528889			

R-Square	C.V.	Root MSE	DATA Mean
0.483923	0.122030	0.06896054	56.51111111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	0.02675556	0.01337778	2.81	0.1374

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	0.02675556	0.01337778	2.81	0.1374

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.004756

Number of Means 2 3

Critical Range .1378 .1428

Duncan Grouping	Mean	N	ASTAXANTHIN
A	56.58000	3	500
A			
A	56.50667	3	0
A			
A	56.44667	3	200

---------- VA=20000 ----------

Dependent Variable: SPECIFIC GROWTH RATE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.03555556	0.01777778	5.69	0.0411
Error	6	0.01873333	0.00312222		
Corrected Total	8	0.05428889			

R-Square	C.V.	Root MSE	DATA Mean
0.654932	0.099106	0.05587685	56.38111111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	0.03555556	0.01777778	5.69	0.0411

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	0.03555556	0.01777778	5.69	0.0411

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.003122

Number of Means 2 3

Critical Range .1116 .1157

Appendic 11 (continued)

Duncan Grouping	Mean	N	ASTAXANTHIN
A	56.47000	3	200
B	56.33667	3	0
B			
B	56.33667	3	500

---------- VA=40000 ----------

Dependent Variable: SPECIFIC GROWTH RATE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02286667	0.01143333	1.46	0.3053
Error	6	0.04713333	0.00785556		
Corrected Total	8	0.07000000			

R-Square	C.V.	Root MSE	DATA Mean
0.326667	0.157083	0.08863157	56.42333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	0.02286667	0.01143333	1.46	0.3053

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	0.02286667	0.01143333	1.46	0.3053

---------- VA=40000 ----------

Duncan's Multiple Range Test for variable: SPECIFIC GROWTH RATE

Alpha= 0.05 df= 6 MSE= 0.007856

Number of Means 2 3

Critical Range .1771 .1835

Duncan Grouping	Mean	N	ASTAXANTHIN
A	56.48333	3	500
A			
A	56.42667	3	0
A			
A	56.36000	3	200

---------- AX=0 ----------

Dependent Variable: SPECIFIC GROWTH RATE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.04340000	0.02170000	3.58	0.0949
Error	6	0.03640000	0.00606667		
Corrected Total	8	0.07980000			

R-Square	C.V.	Root MSE	DATA Mean
0.543860	0.138044	0.07788881	56.42333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	0.04340000	0.02170000	3.58	0.0949

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	0.04340000	0.02170000	3.58	0.0949

Appendic 11 (continued)

---------- AX=0 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.006067

Number of Means 2 3

Critical Range .1556 .1613

Duncan Grouping	Mean	N	Vitamin A
A	56.50667	3	0
A			
B A	56.42667	3	40000
B			
B	56.33667	3	20000

---------- AX=200 ----------

Dependent Variable: SPECIFIC GROWTH RATE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.02015556	0.01007778	2.58	0.1557
Error	6	0.02346667	0.00391111		
Corrected Total	8	0.04362222			

R-Square	C.V.	Root MSE	DATA Mean
0.462048	0.110834	0.06253888	56.42555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	0.02015556	0.01007778	2.58	0.1557

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	0.02015556	0.01007778	2.58	0.1557

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.003911

Number of Means 2 3

Critical Range .1249 .1295

Duncan Grouping	Mean	N	Vitamin A
A	56.47000	3	20000
A			
A	56.44667	3	0
A			
A	56.36000	3 4	0000

---------- AX=500 ----------

Dependent Variable: SPECIFIC GROWTH RATE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.09006667	0.04503333	7.82	0.0213
Error	6	0.03453333	0.00575556		
Corrected Total	8	0.12460000			

Appendic 11 (continued)

R-Square	C.V.	Root MSE	DATA Mean
0.722846	0.134354	0.07586538	56.46666667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	0.09006667	0.04503333	7.82	0.0213
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	0.09006667	0.04503333	7.82	0.0213

---------- AX=500 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.005756

Number of Means 2 3

Critical Range .1516 .1571

Duncan Grouping	Mean	N	Vitamin A
A	56.58000	3	0
A			
B A	56.48333	3	40000
B			
B	56.33667	3	20000

Appendic 11 (continued)
Survival rate of mysis stage

General Linear Models Procedure

Dependent Variable: Surival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	15102.56546667	1887.82068333	54.47	0.0001
Error	18	623.88880000	34.66048889		
Corrected Total	26	15726.45426667			

R-Square	C.V.	Root MSE	DATA Mean
0.960329	14.66168	5.88731593	40.15444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	10067.25708889	5033.62854444	145.23	0.0001
AX	2	928.71315556	464.35657778	13.40	0.0003
VA*AX	4	4106.59522222	1026.64880556	29.62	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	10067.25708889	5033.62854444	145.23	0.0001
AX	2	928.71315556	464.35657778	13.40	0.0003
VA*AX	4	4106.59522222	1026.64880556	29.62	0.0001

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 18 MSE= 34.66049

Number of Means 2 3

Critical Range 5.831 6.118

Duncan Grouping	Mean	N	Vitamin A
A	62.116	9	0
B	43.230	9	20000
C	15.118	9	40000

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 18 MSE= 34.66049

Number of Means 2 3

Critical Range 5.831 6.118

Duncan Grouping	Mean	N	ASTAXANTHIN
A	48.192	9	0
B	37.908	9	200
B			
B	34.363	9	500

---------- A=0 ----------

Appendic 11 (continued)

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	549.77202222	274.88601111	7.03	0.0268
Error	6	234.68580000	39.11430000		
Corrected Total	8	784.45782222			

R-Square	C.V.	Root MSE	DATA Mean
0.700831	10.06856	6.25414263	62.11555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	549.77202222	274.88601111	7.03	0.0268

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	549.77202222	274.88601111	7.03	0.0268

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 39.1143

Number of Means 2 3

Critical Range 12.50 12.95

Duncan Grouping	Mean	N	ASTAXANTHIN
A	72.833	3	500
B	59.097	3	0
B			
B	54.417	3	200

---------- VA=20000 ----------

General Linear Models Procedure

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2434.73686667	1217.36843333	23.12	0.0015
Error	6	315.91233333	52.65205556		
Corrected Total	8	2750.64920000			

R-Square	C.V.	Root MSE	DATA Mean
0.885150	16.78504	7.25617362	43.23000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	2434.73686667	1217.36843333	23.12	0.0015

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	2434.73686667	1217.36843333	23.12	0.0015

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 52.65206

Number of Means 2 3

Critical Range 14.50 15.03

Appendic 11 (continued)

Duncan Grouping	Mean	N	ASTAXANTHIN
A	59.307	3	200
A			
A	49.750	3	0
B	20.633	3	500

---------- VA=40000 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2050.79948889	1025.39974444	83.95	0.0001
Error	6	73.29066667	12.21511111		
Corrected Total	8	2124.09015556			

R-Square	C.V.	Root MSE	DATA Mean
0.965496	23.11856	3.49501232	15.11777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	2050.79948889	1025.39974444	83.95	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	2050.79948889	1025.39974444	83.95	0.0001

---------- VA=40000 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 12.21511

Number of Means 2 3

Critical Range 6.983 7.237

Duncan Grouping	Mean	N	ASTAXANTHIN
A	35.730	3	0
B	9.623	3	500
C	0.000	3	200

---------- AX=0 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	829.92168889	414.96084444	6.89	0.0279
Error	6	361.45166667	60.24194444		
Corrected Total	8	1191.37335556			

R-Square	C.V.	Root MSE	DATA Mean
0.696609	16.10544	7.76156843	48.19222222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	829.92168889	414.96084444	6.89	0.0279

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	829.92168889	414.96084444	6.89	0.0279

---------- AX=0 ----------

Appendic 11 (continued)

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 60.24194

Number of Means 2 3

Critical Range 15.51 16.07

Duncan Grouping		Mean	N	Vitamin A
	A	59.097	3	0
	A			
B	A	49.750	3	20000
B				
B		35.730	3	40000

---------- AX=200 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	6502.36642222	3251.18321111	210.47	0.0001
Error	6	92.68373333	15.44728889		
Corrected Total	8	6595.05015556			

R-Square	C.V.	Root MSE	DATA Mean
0.985946	10.36807	3.93030392	37.90777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	6502.36642222	3251.18321111	210.47	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	6502.36642222	3251.18321111	210.47	0.0001

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 15.44729

Number of Means 2 3

Critical Range 7.852 8.138

Duncan Grouping		Mean	N	Vitamin A
	A	59.307	3	20000
	A			
	A	54.417	3	0
	B	0.000	3	40000

---------- AX=500 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	6841.56420000	3420.78210000	120.91	0.0001
Error	6	169.75340000	28.29223333		
Corrected Total	8	7011.31760000			

R-Square	C.V.	Root MSE	DATA Mean
0.975789	15.47884	5.31904440	34.36333333

Appendic 11 (continued)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	6841.56420000	3420.78210000	120.91	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	6841.56420000	3420.78210000	120.91	0.0001

---------- AX=500 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 28.29223

Number of Means 2 3

Critical Range 10.63 11.01

Duncan Grouping	Mean	N	Vitamin A
A	72.833	3	0
B	20.633	3	20000
C	9.623	3	40000

Appendic 11 (continued)
Specific growth rate of mysis stage

General Linear Models Procedure

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	796.74880000	99.59360000	29165.15	0.0001
Error	18	0.06146667	0.00341481		
Corrected Total	26	796.81026667			

R-Square	C.V.	Root MSE	DATA Mean
0.999923	0.380335	0.05843642	15.36444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	201.03228889	100.51614444	29435.31	0.0001
AX	2	199.42068889	99.71034444	29199.34	0.0001
VA*AX	4	396.29582222	99.07395556	29012.98	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	201.03228889	100.51614444	29435.31	0.0001
AX	2	199.42068889	99.71034444	29199.34	0.0001
VA*AX	4	396.29582222	99.07395556	29012.98	0.0001

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 18 MSE= 0.003415

Number of Means 2 3

Critical Range .05787 .06072

Duncan Grouping	Mean	N	Vitamin A
A	17.30778	9	0
A			
A	17.28000	9	20000
B	11.50556	9	40000

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 18 MSE= 0.003415

Number of Means 2 3

Critical Range .05787 .06072

Duncan Grouping	Mean	N	ASTAXANTHIN
A	17.30889	9	500
A			
A	17.26333	9	0
B	11.52111	9	200

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.01415556	0.00707778	1.37	0.3236
Error	6	0.03100000	0.00516667		
Corrected Total	8	0.04515556			

R-Square	C.V.	Root MSE	DATA Mean
0.313484	0.415302	0.07187953	17.30777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	0.01415556	0.00707778	1.37	0.3236

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	0.01415556	0.00707778	1.37	0.3236

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.005167

Number of Means 2 3

Critical Range .1436 .1488

Duncan Grouping	Mean	N	ASTAXANTHIN
A	17.36333	3	500
A			
A	17.28667	3	200
A			
A	17.27333	3	0

---------- VA=20000 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00020000	0.00010000	0.03	0.9691
Error	6	0.01900000	0.00316667		
Corrected Total	8	0.01920000			

R-Square	C.V.	Root MSE	DATA Mean
0.010417	0.325655	0.05627314	17.28000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	0.00020000	0.00010000	0.03	0.9691

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	0.00020000	0.00010000	0.03	0.9691

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.003167

Number of Means 2 3

Critical Range .1124 .1165

Appendic 11 (continued)

Duncan Grouping	Mean	N	ASTAXANTHIN
A	17.28667	3	500
A			
A	17.27667	3	200
A			
A	17.27667	3	0

---------- VA=40000 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	595.70215556	297.85107778	99999.99	0.0001
Error	6	0.01146667	0.00191111		
Corrected Total	8	595.71362222			

R-Square	C.V.	Root MSE	DATA Mean
0.999981	0.379958	0.04371626	11.50555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	595.70215556	297.85107778	99999.99	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	595.70215556	297.85107778	99999.99	0.0001

---------- VA=40000 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.001911

Number of Means 2 3

Critical Range .08734 .09052

Duncan Grouping	Mean	N	ASTAXANTHIN
A	17.27667	3	500
A			
A	17.24000	3	0
B	0.00000	3	200

---------- AX=0 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00246667	0.00123333	0.30	0.7535
Error	6	0.02493333	0.00415556		
Corrected Total	8	0.02740000			

R-Square	C.V.	Root MSE	DATA Mean
0.090024	0.373413	0.06446360	17.26333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	0.00246667	0.00123333	0.30	0.7535

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	0.00246667	0.00123333	0.30	0.7535

Appendic 11 (continued)

---------- AX=0 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.004156

Number of Means 2 3

Critical Range .1288 .1335

Duncan Grouping	Mean	N	Vitamin A
A	17.27667	3	20000
A			
A	17.27333	3	0
A			
A	17.24000	3	40000

---------- AX=200 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	597.31215556	298.65607778	82451.06	0.0001
Error	6	0.02173333	0.00362222		
Corrected Total	8	597.33388889			

R-Square	C.V.	Root MSE	DATA Mean
0.999964	0.522388	0.06018490	11.52111111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	597.31215556	298.65607778	82451.06	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	597.31215556	298.65607778	82451.06	0.0001

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.003622

Number of Means 2 3

Critical Range .1202 .1246

Duncan Grouping	Mean	N	Vitamin A
A	17.28667	3	0
A			
A	17.27667	3	20000
B	0.00000	3	40000

---------- AX=500 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.01348889	0.00674444	2.73	0.1432
Error	6	0.01480000	0.00246667		
Corrected Total	8	0.02828889			

Appendic 11 (continued)

R-Square	C.V.	Root MSE	DATA Mean
0.476826	0.286937	0.04966555	17.30888889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	0.01348889	0.00674444	2.73	0.1432
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	0.01348889	0.00674444	2.73	0.1432

---------- AX=500 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.002467

Number of Means 2 3

Critical Range .0992 .1028

Duncan Grouping	Mean	N	Vitamin A
A	17.36333	3	0
A			
A	17.28667	3	20000
A			
A	17.27667	3	40000

Appendic 11 (continued)

Survival rate of postlarval stage

General Linear Models Procedure

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2465.16642222	1232.58321111	41.22	0.0003
Error	6	179.41926667	29.90321111		
Corrected Total	8	2644.58568889			

R-Square	C.V.	Root MSE	DATA Mean
0.932156	9.344646	5.46838286	58.51888889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	2465.16642222	1232.58321111	41.22	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	2465.16642222	1232.58321111	41.22	0.0003

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 29.90321

Number of Means 2 3

Critical Range 10.93 11.32

Duncan Grouping	Mean	N	ASTAXANTHIN
A	80.667	3	500
B	54.000	3	0
C	40.890	3	200

---------- VA=20000 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	5773.18668889	2886.59334444	27.09	0.0010
Error	6	639.42226667	106.57037778		
Corrected Total	8	6412.60895556			

R-Square	C.V.	Root MSE	DATA Mean
0.900287	29.68359	10.32329297	34.77777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	5773.18668889	2886.59334444	27.09	0.0010

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	5773.18668889	2886.59334444	27.09	0.0010

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 106.5704

Appendic 11 (continued)

Number of Means 2 3

Critical Range 20.62 21.38

Duncan Grouping	Mean	N	ASTAXANTHIN
A	60.780	3	0
A			
A	43.110	3	200
B	0.443	3	500

---------- VA=40000 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	854.65482222	427.32741111	115.55	0.0001
Error	6	22.18966667	3.69827778		
Corrected Total	8	876.84448889			

R-Square	C.V.	Root MSE	DATA Mean
0.974694	27.04346	1.92309068	7.11111111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	854.65482222	427.32741111	115.55	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	854.65482222	427.32741111	115.55	0.0001

---------- VA=40000 ----------

Duncan's Multiple Range Test for variable: SURVIVAL RATE

Alpha= 0.05 df= 6 MSE= 3.698278

Number of Means 2 3

Critical Range 3.842 3.982

Duncan Grouping	Mean	N	ASTAXANTHIN
A	20.890	3	0
B	0.443	3	200
B			
B	0.000	3	500

---------- AX=0 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2733.45260000	1366.72630000	18.85	0.0026
Error	6	434.98300000	72.49716667		
Corrected Total	8	3168.43560000			

R-Square	C.V.	Root MSE	DATA Mean
0.862714	18.82773	8.51452680	45.22333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	2733.45260000	1366.72630000	18.85	0.0026

Source	DF	Type III SS	Mean Square	F Value	Pr > F

Appendic 11 (continued)

VA	2	2733.45260000	1366.72630000	18.85	0.0026

---------- AX=0 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 72.49717

Number of Means 2 3

Critical Range 17.01 17.63

Duncan Grouping	Mean	N	Vitamin A
A	60.780	3	20000
A			
A	54.000	3	0
B	20.890	3	40000

---------- AX=200 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3461.30568889	1730.65284444	35.95	0.0005
Error	6	288.86226667	48.14371111		
Corrected Total	8	3750.16795556			

R-Square	C.V.	Root MSE	DATA Mean
0.922974	24.65050	6.93856693	28.14777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	3461.30568889	1730.65284444	35.95	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	3461.30568889	1730.65284444	35.95	0.0005

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 48.14371

Number of Means 2 3

Critical Range 13.86 14.37

Duncan Grouping	Mean	N	Vitanmin A
A	43.110	3	20000
A			
A	40.890	3	0
B	0.443	3	40000

---------- AX=500 ----------

Dependent Variable: Survival rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	12943.09086667	6471.54543333	331.35	0.0001
Error	6	117.18593333	19.53098889		
Corrected Total	8	13060.27680000			

Appendic 11 (continued)

R-Square	C.V.	Root MSE	DATA Mean
0.991027	16.34590	4.41938784	27.03666667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	12943.09086667	6471.54543333	331.35	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	12943.09086667	6471.54543333	331.35	0.0001

---------- AX=500 ----------

Duncan's Multiple Range Test for variable: Survival rate

Alpha= 0.05 df= 6 MSE= 19.53099

Number of Means 2 3

Critical Range 8.829 9.151

Duncan Grouping	Mean	N	Vitamin A
A	80.667	3	0
B	0.443	3	20000
B			
B	0.000	3	40000

Appendic 11 (continued)

Specific growth rate of postlarval stage

General Linear Models Procedure

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	103.04651852	12.88081481	31331.71	0.0001
Error	18	0.00740000	0.00041111		
Corrected Total	26	103.05391852			

R-Square	C.V.	Root MSE	DATA Mean
0.999928	0.733944	0.02027588	2.76259259

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	34.52902963	17.26451481	41994.77	0.0001
AX	2	34.33738519	17.16869259	41761.68	0.0001
VA*AX	4	34.18010370	8.54502593	20785.20	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	34.52902963	17.26451481	41994.77	0.0001
AX	2	34.33738519	17.16869259	41761.68	0.0001
VA*AX	4	34.18010370	8.54502593	20785.20	0.0001

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 18 MSE= 0.000411

Number of Means 2 3

Critical Range .02008 .02107

Duncan Grouping	Mean	N	Vitamin A
A	4.143333	9	0
B	2.771111	9	20000
C	1.373333	9	40000

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 18 MSE= 0.000411

Number of Means 2 3

Critical Range .02008 .02107

Duncan Grouping	Mean	N	ASTAXANTHIN
A	4.151111	9	0
B	2.747778	9	200
C	1.388889	9	500

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.01286667	0.00643333	13.16	0.0064
Error	6	0.00293333	0.00048889		
Corrected Total	8	0.01580000			

R-Square	C.V.	Root MSE	DATA Mean
0.814346	0.533648	0.02211083	4.14333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	0.01286667	0.00643333	13.16	0.0064

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	0.01286667	0.00643333	13.16	0.0064

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.000489

Number of Means 2 3

Critical Range .04418 .04578

Duncan Grouping	Mean	N	ASTAXANTHIN
A	4.17333	3	0
A			
A	4.16667	3	500
B	4.09000	3	200

---------- A=20000 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	34.55582222	17.27791111	50161.68	0.0001
Error	6	0.00206667	0.00034444		
Corrected Total	8	34.55788889			

R-Square	C.V.	Root MSE	DATA Mean
0.999940	0.669739	0.01855921	2.77111111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	34.55582222	17.27791111	50161.68	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	34.55582222	17.27791111	50161.68	0.0001

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.000344

Number of Means 2 3

Critical Range .03708 .03843

Appendic 11 (continued)

Duncan Grouping	Mean	N	ASTAXANTHIN
A	4.16000	3	0
A			
A	4.15333	3	200
B	0.00000	3	500

---------- VA=40000 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	33.94880000	16.97440000	42436.00	0.0001
Error	6	0.00240000	0.00040000		
Corrected Total	8	33.95120000			

R-Square	C.V.	Root MSE	DATA Mean
0.999929	1.456311	0.02000000	1.37333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	33.94880000	16.97440000	42436.00	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	33.94880000	16.97440000	42436.00	0.0001

---------- VA=40000 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.0004

Number of Means 2 3

Critical Range .03996 .04141

Duncan Grouping	Mean	N	ASTAXANTHIN
A	4.12000	3	0
B	0.00000	3	200
B			
B	0.00000	3	500

---------- AX=0 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00462222	0.00231111	4.84	0.0561
Error	6	0.00286667	0.00047778		
Corrected Total	8	0.00748889			

R-Square	C.V.	Root MSE	DATA Mean
0.617211	0.526561	0.02185813	4.15111111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
A	2	0.00462222	0.00231111	4.84	0.0561

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A	2	0.00462222	0.00231111	4.84	0.0561

---------- AX=0 ----------

Appendic 11 (continued)

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.000478

Number of Means 2 3

Critical Range .04367 .04526

Duncan Grouping		Mean	N	Vitamin A
	A	4.17333	3	0
	A			
B	A	4.16000	3	20000
B				
B		4.12000	3	40000

---------- AX=200 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	33.98228889	16.99114444	38230.07	0.0001
Error	6	0.00266667	0.00044444		
Corrected Total	8	33.98495556			

R-Square	C.V.	Root MSE	DATA Mean
0.999922	0.767233	0.02108185	2.74777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
A	2	33.98228889	16.99114444	38230.07	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A	2	33.98228889	16.99114444	38230.07	0.0001

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.000444

Number of Means 2 3

Critical Range .04212 .04365

Duncan Grouping	Mean	N	Vitamin A
A	4.15333	3	20000
B	4.09000	3	0
C	0.00000	3	40000

---------- AX=500 ----------

Dependent Variable: Specific growth rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	34.72222222	17.36111111	55803.57	0.0001
Error	6	0.00186667	0.00031111		
Corrected Total	8	34.72408889			

R-Square	C.V.	Root MSE	DATA Mean
0.999946	1.269961	0.01763834	1.38888889

Appendic 11 (continued)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	34.72222222	17.36111111	55803.57	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	34.72222222	17.36111111	55803.57	0.0001

---------- AX=500 ----------

Duncan's Multiple Range Test for variable: Specific growth rate

Alpha= 0.05 df= 6 MSE= 0.000311

Number of Means 2 3

Critical Range .03524 .03652

Duncan Grouping	Mean	N	Vitamin A
A	4.16667	3	0
B	0.00000	3	20000
B			
B	0.00000	3	40000

Appendic 11 (continued)

Stress test of postlarval stage

Dependent Variable: Stress test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5816.99867346	1163.39973469	8.33	0.0001
Error	48	6707.88929436	139.74769363		
Corrected Total	53	12524.88796781			

R-Square	C.V.	Root MSE	DATA Mean
0.464435	56.90416	11.82149287	20.77439278

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	4074.39037829	2037.19518914	14.58	0.0001
AX	2	1741.34844607	870.67422303	6.23	0.0039
VA*AX	1	1.25984910	1.25984910	0.01	0.9248

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	1758.88761424	879.44380712	6.29	0.0037
AX	2	1741.34844607	870.67422303	6.23	0.0039
VA*AX	1	1.25984910	1.25984910	0.01	0.9248

Duncan's Multiple Range Test for variable: Stress test

Alpha= 0.05 df= 48 MSE= 139.7477

Harmonic Mean of cell sizes= 14.72727

Number of Means 2 3

Critical Range 8.759 9.212

Duncan Grouping	Mean	N	VITAMIN A
A	29.458	27	0
B	12.315	18	20000
B			
B	11.642	9	40000

Duncan's Multiple Range Test for variable: Stress test

Alpha= 0.05 df= 48 MSE= 139.7477

Harmonic Mean of cell sizes= 14.72727

Number of Means 2 3

Critical Range 8.759 9.212

Duncan Grouping	Mean	N	ASTAXANTHIN
A	40.055	9	500
B	17.706	27	0
B			
B	15.736	18	200

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: Stress test

	Mean Square	F Value	Pr > F		
Model	2	1612.52963052	806.26481526	2.93	0.0726
Error	24	6599.26767734	274.96948656		
Corrected Total	26	8211.79730786			

R-Square	C.V.	Root MSE	DATA Mean
0.196367	56.29141	16.58220391	29.45778778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	1612.52963052	806.26481526	2.93	0.0726

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	1612.52963052	806.26481526	2.93	0.0726

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Stress test

Alpha= 0.05 df= 24 MSE= 274.9695

Number of Means 2 3

Critical Range 16.13 16.94

Duncan Grouping	Mean	N	ASTAXANTHIN
A	40.055	9	500
A			
B A	26.473	9	0
B			
B	21.845	9	200

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---------- VA=20000 ----------

Dependent Variable: Stress test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	130.07866465	130.07866465	31.43	0.0001
Error	16	66.21406098	4.13837881		
Corrected Total	17	196.29272563			

R-Square	C.V.	Root MSE	DATA Mean
0.662677	16.51844	2.03430057	12.31533000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	1	130.07866465	130.07866465	31.43	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	1	130.07866465	130.07866465	31.43	0.0001

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Stress test

Alpha= 0.05 df= 16 MSE= 4.138379

Number of Means 2

Critical Range 2.033

Appendic 11 (continued)

Duncan Grouping	Mean	N	ASTAXANTHIN
A	15.0036	9	0
B	9.6271	9	200

---------- AX=0 ----------

Dependent Variable: Stress test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1088.40499994	544.20249997	21.27	0.0001
Error	24	614.19036323	25.59126513		
Corrected Total	26	1702.59536317			

R-Square	C.V.	Root MSE	DATA Mean
0.639262	28.57044	5.05878099	17.70634370

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	1088.40499994	544.20249997	21.27	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	1088.40499994	544.20249997	21.27	0.0001

---------- AX=0 ----------

Duncan's Multiple Range Test for variable: Stress test

Alpha= 0.05 df= 24 MSE= 25.59127

Number of Means 2 3

Critical Range 4.922 5.169

Duncan Grouping	Mean	N	VITAMIN A
A	26.473	9	0
B	15.004	9	20000
B			
B	11.642	9	40000

---------- AX=200 ----------

Dependent Variable: Stress test

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	671.74246341	671.74246341	23.99	0.0002
Error	16	448.05296530	28.00331033		
Corrected Total	17	1119.79542871			

R-Square	C.V.	Root MSE	DATA Mean
0.599880	33.62866	5.29181541	15.73602778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	1	671.74246341	671.74246341	23.99	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	1	671.74246341	671.74246341	23.99	0.0002

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Stress test

Alpha= 0.05 df= 16 MSE= 28.00331

Appendic 11 (continued)

Number of Means 2
Critical Range 5.288

Duncan Grouping	Mean	N	VITAMIN A
A	21.845	9	0
B	9.627	9	20000

Appendic 11 (continued)
Vitamin A contents in shrimp

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	365654.16162778	73130.83232556	80.98	0.0001
Error	12	10837.33580000	903.11131667		
Corrected Total	17	376491.49742778			

R-Square	C.V.	Root MSE	DATA Mean
0.971215	9.353371	30.05181054	321.29388889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	360246.46147222	180123.23073611	199.45	0.0001
AX	2	2552.53265556	1276.26632778	1.41	0.2811
VA*AX	1	2855.16750000	2855.16750000	3.16	0.1007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	247687.46705556	123843.73352778	137.13	0.0001
AX	2	2552.53265556	1276.26632778	1.41	0.2811
VA*AX	1	2855.16750000	2855.16750000	3.16	0.1007

Duncan's Multiple Range Test for variable: Vitamin A content

Alpha= 0.05 df= 12 MSE= 903.1113

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 4.909091

Number of Means 2 3

Critical Range 41.79 43.75

Duncan Grouping	Mean	N	VA
A	550.47	3	40000
B	406.29	6	20000
C	188.24	9	0

Duncan's Multiple Range Test for variable: Vitamin A content

Alpha= 0.05 df= 12 MSE= 903.1113

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 4.909091

Number of Means 2 3

Critical Range 41.79 43.75

Duncan Grouping	Mean	N	AX
A	387.17	9	0
B	300.76	6	200
C	164.74	3	500

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3505.94808889	1752.97404444	3.84	0.0843
Error	6	2736.77466667	456.12911111		
Corrected Total	8	6242.72275556			

R-Square	C.V.	Root MSE	DATA Mean
0.561606	11.34585	21.35717938	188.23777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	3505.94808889	1752.97404444	3.84	0.0843

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	3505.94808889	1752.97404444	3.84	0.0843

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Vitamin A content

Alpha= 0.05 df= 6 MSE= 456.1291

Number of Means 2 3

Critical Range 42.67 44.22

Duncan Grouping	Mean	N	AX
A	213.03	3	200
A			
B A	186.94	3	0
B			
B	164.74	3	500

---------- VA=20000 ----------

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1901.75206667	1901.75206667	2.50	0.1889
Error	4	3041.23133333	760.30783333		
Corrected Total	5	4942.98340000			

R-Square	C.V.	Root MSE	DATA Mean
0.384738	6.786699	27.57368008	406.29000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	1	1901.75206667	1901.75206667	2.50	0.1889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	1	1901.75206667	1901.75206667	2.50	0.1889

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Vitamin A content

Alpha= 0.05 df= 4 MSE= 760.3078

Number of Means 2

Critical Range 62.51

Appendic 11 (continued)

Duncan Grouping	Mean	N	AX
A	424.09	3	0
A			
A	388.49	3	200

---------- VA=40000 ----------

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	0	0.00000000	.	.	.
Error	2	5059.32980000	2529.66490000		
Corrected Total	2	5059.32980000			

R-Square	C.V.	Root MSE	DATA Mean
-.000000	9.136878	50.29577418	550.47000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	0	0	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	0	0	.	.	.

---------- AX=0 ----------

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	204366.82628889	102183.41314444	88.45	0.0001
Error	6	6931.62226667	1155.27037778		
Corrected Total	8	211298.44855556			

R-Square	C.V.	Root MSE	DATA Mean
0.967195	8.778951	33.98926857	387.16777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	204366.82628889	102183.41314444	88.45	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	204366.82628889	102183.41314444	88.45	0.0001

---------- AX=0 ----------

Duncan's Multiple Range Test for variable: Vitamin A content

Alpha= 0.05 df= 6 MSE= 1155.27

Number of Means 2 3

Critical Range 67.91 70.38

Duncan Grouping	Mean	N	VA
A	550.47	3	40000
B	424.09	3	20000
C	186.94	3	0

---------- AX=200 ----------

Appendic 11 (continued)

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	46175.80826667	46175.80826667	61.51	0.0014
Error	4	3002.96233333	750.74058333		
Corrected Total	5	49178.77060000			

R-Square	C.V.	Root MSE	DATA Mean
0.938938	9.110136	27.39964568	300.76000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	1	46175.80826667	46175.80826667	61.51	0.0014

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	1	46175.80826667	46175.80826667	61.51	0.0014

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Vitamin A content

Alpha= 0.05 df= 4 MSE= 750.7406

Number of Means 2

Critical Range 62.11

Duncan Grouping	Mean	N	VA
A	388.49	3	20000
B	213.03	3	0

---------- AX=500 ----------

Dependent Variable: Vitamin A content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	0	0.00000000	.	.	.
Error	2	902.75120000	451.37560000		
Corrected Total	2	902.75120000			

R-Square	C.V.	Root MSE	DATA Mean
0.000000	12.89644	21.24560190	164.74000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	0	0	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	0	0	.	.	.

---------- AX=500 ----------

Level of	----------DATA----------		
VA	N	Mean	SD
0	3	164.740000	21.2456019

Appendic 11 (continued)
Astaxanthin contents in shrimp

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	219134.51697778	43826.90339556	65.29	0.0001
Error	12	8055.06273333	671.25522778		
Corrected Total	17	227189.57971111			

R-Square	C.V.	Root MSE	DATA Mean
0.964545	10.50452	25.90859371	246.64222222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	35749.23315556	17874.61657778	26.63	0.0001
AX	2	170535.48528889	85267.74264444	127.03	0.0001
VA*AX	1	12849.79853333	12849.79853333	19.14	0.0009

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	25544.53388889	12772.26694444	19.03	0.0002
AX	2	170535.48528889	85267.74264444	127.03	0.0001
VA*AX	1	12849.79853333	12849.79853333	19.14	0.0009

Duncan's Multiple Range Test for variable: Astaxanthin content

Alpha= 0.05 df= 12 MSE= 671.2552

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 4.909091

Number of Means 2 3

Critical Range 36.03 37.71

Duncan Grouping	Mean	N	VA
A	286.37	9	0
B	247.31	3	40000
C	186.72	6	20000

Duncan's Multiple Range Test for variable: Astaxanthin content

Alpha= 0.05 df= 12 MSE= 671.2552

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 4.909091

Number of Means 2 3

Critical Range 36.03 37.71

Duncan Grouping	Mean	N	AX
A	456.12	3	500
B	252.46	6	200
C	172.94	9	0

---------- VA=0 ----------

Appendic 11 (continued)

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	179442.87715556	89721.43857778	115.39	0.0001
Error	6	4665.24073333	777.54012222		
Corrected Total	8	184108.11788889			

R-Square	C.V.	Root MSE	DATA Mean
0.974660	9.737233	27.88440643	286.36888889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	2	179442.87715556	89721.43857778	115.39	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	2	179442.87715556	89721.43857778	115.39	0.0001

---------- VA=0 ----------

Duncan's Multiple Range Test for variable: Astaxanthin content

Alpha= 0.05 df= 6 MSE= 777.5401

Number of Means 2 3

Critical Range 55.71 57.74

Duncan Grouping	Mean	N	AX
A	456.12	3	500
B	292.57	3	200
C	110.41	3	0

---------- VA=20000 ----------

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3942.40666667	3942.40666667	6.50	0.0633
Error	4	2424.96973333	606.24243333		
Corrected Total	5	6367.37640000			

R-Square	C.V.	Root MSE	DATA Mean
0.619157	13.18658	24.62199085	186.72000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	1	3942.40666667	3942.40666667	6.50	0.0633

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	1	3942.40666667	3942.40666667	6.50	0.0633

---------- VA=20000 ----------

Duncan's Multiple Range Test for variable: Astaxanthin content

Alpha= 0.05 df= 4 MSE= 606.2424

Number of Means 2

Critical Range 55.82

Appendic 11 (continued)

Duncan Grouping	Mean	N	Astaxanthin
A	212.35	3	200
A			
A	161.09	3	0

---------- VA=40000 ----------

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	0	0.00000000		.	.
Error	2	964.85226667	482.42613333		
Corrected Total	2	964.85226667			

R-Square	C.V.	Root MSE	DATA Mean
-.000000	8.881362	21.96420118	247.30666667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AX	0	0	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AX	0	0	.	.	.

---------- VA=40000 ----------

Level of	DATA	
AX	N	Mean	SD
0	3	247.306667	21.9642012

---------- AX=0 ----------

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	28741.45982222	14370.72991111	38.84	0.0004
Error	6	2220.05200000	370.00866667		
Corrected Total	8	30961.51182222			

R-Square	C.V.	Root MSE	DATA Mean
0.928296	11.12299	19.23560934	172.93555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	2	28741.45982222	14370.72991111	38.84	0.0004

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	2	28741.45982222	14370.72991111	38.84	0.0004

---------- AX=0 ----------

Duncan's Multiple Range Test for variable: Astaxanthin content

Alpha= 0.05 df= 6 MSE= 370.0087

Duncan Grouping	Mean	N	Vitamin A
A	247.31	3	40000
B	161.09	3	20000
C	110.41	3	0

---------- AX=200 ----------

Appendic 11 (continued)

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	9652.87260000	9652.87260000	20.67	0.0104
Error	4	1867.84353333	466.96088333		
Corrected Total	5	11520.71613333			

R-Square	C.V.	Root MSE	DATA Mean
0.837871	8.559373	21.60927771	252.46333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	1	9652.87260000	9652.87260000	20.67	0.0104

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	1	9652.87260000	9652.87260000	20.67	0.0104

---------- AX=200 ----------

Duncan's Multiple Range Test for variable: Astaxanthin content

Alpha= 0.05 df= 4 MSE= 466.9609

Number of Means 2

Critical Range 48.99

Duncan Grouping	Mean	N	Vitamin A
A	292.57	3	0
B	212.35	3	20000

---------- AX=500 ----------

Dependent Variable: Astaxanthin content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	0	0.00000000	.	.	.
Error	2	3967.16720000	1983.58360000		
Corrected Total	2	3967.16720000			

R-Square	C.V.	Root MSE	DATA Mean
-.000000	9.764413	44.53744043	456.12000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
VA	0	0	.	.	.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
VA	0	0	.	.	.

---------- AX=500 ----------

Level of	----------DATA----------		
VA	N	Mean	SD
0	3	456.120000	44.5374404

BIOGRAPHY

Miss Rungjit Yoddee was born on August 15, 1980 in Radburee Province. She graduated with a Bachelor degree from the Department of Biotechnology, Faculty of Science, King Mongkut's Institute Technology of Ladkrabang in 2002. She began to study Master's degree in Biotechnology program, Faculty of Science, Chulalongkorn University in 2002. During studying, She had an oral presentation titled ' Effects of kelp *Ascophyllum nodosum* as feed supplement on growth, survival, and salinity stress of *Penaeus monodon* larvae' at the 4th National Symposium on Graduate Research on August 11, 2004 at Lotus Hotel Pang Suan Kaew, Chiang Mai.