ผลของแอสทาแซนธินและน้ำมันปลาในอาหารต่อความสมบูรณ์เพศของกุ้งกุลาดำ
Penaeus monodon จากบ่อเลี้ยง

นายชลี ไพบูลย์กิจกุล

EFFECTS OF DIETARY ASTAXANTHIN AND FISH OIL ON MATURATION
OF POND-REARED BLACK TIGER PRAWN *Penaeus monodon*

Mr. Chalee Paibulkichakul

Thesis Title   Effect of dietary astaxanthin and fish oil on maturation of
                      pond-reared black tiger prawn *Penaeus monodon*
By   Mr. Chalee Paibulkichakul
Field of Study   Marine Science
Thesis Advisor   Associate Professor Somkiat Piyatiratitivorakul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctor's Degree

....Dean of Faculty of Science

(Associate Professor Wanchai Phothiphichitr, Ph.D.)

THESIS COMMITTEE

....Chairman

(Supichai Tangjaitrong, Ph.D.)

....Thesis Advisor

(Associate Professor Somkiat Piyatiratitivorakul, Ph.D.)

....Member

(Professor Piamsak Menasveta, Ph.D.)

....Member

(Voranop Viyakarn, Ph.D.)

....Member

(Sorawit Powtongsook, Ph.D.)

....Member

(Mali Boonyaratpalin, Ph.D.)

ชลี ไพบูลย์กิจกุล : ผลของแอสทาแซนธินและน้ำมันปลาในอาหารต่อความสมบูรณ์เพศ
ของกุ้งกุลาดำ *Penaeus monodon* จากบ่อเลี้ยง (EFFECT OF DIETARY
ASTAXANTHIN AND FISH OIL ON MATURATION OF POND-REARED
BLACK TIGER PRAWN *Penaeus monodon*) อ. ที่ปรึกษา : รศ.ดร.สมเกียรติ   ปิ
ยะธีรธิติวรกุล, 163 หน้า. ISBN 974-17-3037-3.

        การศึกษาผลของน้ำมันปลา และแอสทาแซนธิน ต่อความความสมบูรณ์เพศของกุ้งกุลาดำระยะวัยรุ่นและระยะ
โตเต็มวัยจากการเพาะเลี้ยง การศึกษาแบ่งเป็น 2 การทดลอง การทดลองแรกศึกษาผลของการเสริม แอสทาแซนธินใน
อาหารต่อการเจริญเติบโต, การรอด และความสมบูรณ์เพศในกุ้งกุลาดำระยะวัยรุ่นโดยใช้อาหารอัดเม็ดที่มีแอสทาแซนธิน
3 ระดับ (0, 300 และ 500 mg kg^{-1}) ทดลองในกุ้งอายุ 4 เดือน ระยะเวลาในการทดลอง 120 วัน ผลการศึกษาไม่พบความ
สัมพันธ์ร่วมระหว่างแอสทาแซนธินกับเพศต่อการเจริญเติบโต, การรอด และปริมาณแอสทาแซนธินในเนื้อเยื่อกุ้ง กุ้งที่ได้
รับแอสทาแซนธิน 300 mg kg^{-1} มีการเจริญเติบโตมากกว่ากุ้งที่ไม่ได้รับแอสทาแซนธินอย่างมีนัยสำคัญ (*P*<0.05) แต่กุ้ง
เพศเมียมีการเจริญเติบโตมากกว่ากุ้งเพศผู้อย่างมีนัยสำคัญ (*P*<0.05) กุ้งเพศเมียไม่มีการพัฒนาของรังไข่ในระหว่างการ
ทดลอง กุ้งเพศผู้ที่ได้รับอาหารที่มีแอสทาแซนธินมีปริมาณเซลล์อสุจิมากกว่ากลุ่มที่ไม่ได้รับแอสทาแซนธินอย่างมีนัย
สำคัญ (*P*<0.05) กุ้งที่เลี้ยงด้วยอาหารที่มีแอสทาแซนธิน 500 mg kg^{-1} จะมีการสะสมของแอสทาแซนธินในกล้ามเนื้อและ
ตับอ่อนมากกว่ากลุ่มที่ได้รับแอสทาแซนธิน 300 mg kg^{-1} และกลุ่มที่ไม่ได้รับแอสทาแซนธินอย่างมีนัยสำคัญ เพศไม่มีผล
ต่อการสะสมแอสทาแซนธินในเนื้อเยื่อกุ้ง
        การทดลองที่สองศึกษาผลของน้ำมันปลา และแอสทาแซนธิน ต่อความสมบูรณ์เพศในกุ้งกุลาดำระยะโตเต็มวัย
โดยใช้อาหารอัดเม็ดที่มีระดับน้ำมันปลา 2 ระดับ (3 และ 8%) และแอสทาแซนธิน 2 ระดับ (100 และ 500 mg kg^{-1}) ผลการ
ศึกษาไม่พบความสัมพันธ์ร่วมระหว่างน้ำมันปลาและแอสทาแซนธิน, และระหว่างน้ำมันปลาและเพศ ต่อการเจริญเติบโต,
ปริมาณไข่, ปริมาณเซลล์อสุจิ และการสะสมของแอสทาแซนธิน และกรดไขมันในเนื้อเยื่อกุ้ง น้ำมันปลาไม่มีผลการเจริญ
เติบโตของกุ้ง แต่ในกุ้งเพศผู้ที่ได้รับแอสทาแซนธินในปริมาณสูงจะมีการเจริญเติบโตสูงกว่ากุ้งที่ได้รับแอสทาแซนธินใน
ระดับต่ำอย่างมีนัยสำคัญ กุ้งกลุ่มที่ได้รับน้ำมันปลา และแอสทาแซนธินในระดับสูงจะมีปริมาณไข่ และเซลล์อสุจิมากกว่า
กลุ่มที่ได้รับน้ำมันปลา และแอสทาแซนธินในระดับต่ำอย่างมีนัยสำคัญ (*P*<0.05) น้ำมันปลาไม่มีผลต่อการสะสมของ
แอสทาแซนธินในเนื้อเยื่อกุ้ง กุ้งที่ได้รับแอสทาแซนธินในระดับสูงจะมีการสะสมของแอสทาแซนธินในกล้ามเนื้อและรัง
ไข่มากกว่ากุ้งที่ได้รับแอสทาแซนธินในระดับต่ำ ในกุ้งกลุ่มที่ได้รับแอสทาแซนธินระดับสูงพบว่ากุ้งเพศเมียมีการสะสม
แอสทาแซนธินในตับอ่อนมากกว่ากุ้งเพศผู้อย่างมีนัยสำคัญ กุ้งที่ได้อาหารที่มีน้ำมันปลาและแอสทาแซนธินระดับสูงจะพบ
กรดไขมัน 22:6(n-3), n-3 PUFA และ n-3 HUFA ในกล้ามเนื้อและรังไข่มากกว่ากุ้งที่ได้รับน้ำมันปลาในระดับต่ำอย่างมีนัย
สำคัญ ส่วนกรดไขมัน 20:4(n-6), 20:5(n-3), 22:6(n-3), n-6 PUFA, n-3 PUFA และ n-3 HUFA พบว่าจะสะสมมากในตับ
อ่อนของกุ้งที่ได้รับน้ำมันปลาในระดับสูงอย่างมีนัยสำคัญ จากการศึกษาแสดงให้เห็นว่าการเพิ่มน้ำมันปลา และ/หรือ
แอสทาแซนธินในอาหารจะช่วยเพิ่มความสมบูรณ์เพศของกุ้งกุลาดำจากการเพาะเลี้ยงในระยะวัยรุ่น และระยะโตเต็มวัย

ภาควิชา วิทยาศาสตร์ทางทะเล   ลายมือชื่อนิสิต

สาขาวิชา วิทยาศาสตร์ทางทะเล   ลายมือชื่ออาจารย์ที่ปรึกษา

ปีการศึกษา 2545

This study was determine designed to the effect of dietary astaxanthin and/or fish oil on reproductive performance of pond-reared adolescent and broodstock black tiger shrimp *Penaeus monodon*. This study was divided into two experiments. The first experiment studied effects of dietary astaxanthin on growth, survival and reproductive performance of pond reared immature black tiger shrimp *P. monodon* by using pelleted diets containing 3 levels of astaxanthin (0, 300 and 500 mg kg^{-1}). Four months old, shrimp used in this experiment had no appearance of spermatopore or ovary development before the trial. The experiment was operated 120 days. No interaction between dietary astaxanthin and sex was found on growth, survival and astaxanthin accumulation. Weight gain of shrimp fed diet supplemented with astaxanthin 300 mg kg^{-1} had significantly higher ($P<0.05$) than those fed diet without astaxanthin. Female shrimp had significantly greater growth rate ($P<0.05$) than that of male. Survival rate of all groups was not significantly different ($P>0.05$) among levels of dietary astaxanthin and sex. Ovarian development did not appear in all treatments. Male shrimp fed diet supplemented with astaxanthin 300 and 500 mg kg^{-1} had significantly higher amount of spermatozoa ($P<0.05$) than those of the control. Shrimp fed diet supplemented with 500 mg kg^{-1} had significantly higher astaxanthin content in muscle and hepatopancreas ($P<0.05$) than those of other groups. In shell, shrimp fed diet supplemented with astaxanthin had significantly greater astaxanthin content ($P<0.05$) than those of the control. Sexes had not effect on astaxanthin content in all tissues of adolescent shrimp. The present study suggested that supplementation of astaxanthin to adolescent *P. monodon* diet could enhance reproductive performance in male shrimp.

The second experiment studied the improvements for reproductive performance of pond-reared black tiger shrimp broodstock *P. monodon* by using diets containing 2 levels of fish oil, 3 and 8%, and 2 levels of astaxanthin 100 and 500 mg kg^{-1}. Female shrimp weighting 48-50 g and male shrimp weighting 35-38 g were randomly separated to fed the experimental diets for 4 months. The result indicated no interaction between fish oil and astaxanthin, and fish oil and sex on average weight gain was found but astaxanthin and sex had significant ($P<0.05$) interaction. Fish oil was no effect on average weight gain. In male, shrimp fed high level of astaxanthin had significantly higher average weight gain ($P<0.05$) than those fed low level of astaxanthin. There was no interaction between fish oil and astaxanthin on amount of egg and spermatozoa. Shrimp fed diet containing high levels of fish oil and astaxanthin had significantly higher amount of eggs and spermatozoa than those fed diet containing low levels of them. No interaction was found on fish oil and astaxanthin, and fish oil and sex on astaxanthin content in all tissues. Astaxanthin content in all tissues was not significantly different among level of fish oil. Astaxanthin content in muscle, hepatopancreas, ovary and shell, only interaction between astaxanthin and sex was found. In muscle and ovary, shrimp fed diet containing high level of astaxanthin had significantly high astaxanthin content. In hepatopancreas, female shrimp fed diet containing high level of astaxanthin had significantly higher astaxanthin content than male shrimp. In shell, no significant difference among levels of fish oil, astaxanthin and sex was found on astaxanthin content. The interaction between fish oil, astaxanthin and sex was not found on fatty acid content in muscle, hepatopancreas and ovary. Shrimp fed diets containing high level of fish oil and astaxanthin had significantly high 22:6(n-3), total n-3 PUFA and total n-3 HUFA contents in muscle and ovary, whereas 20:4(n-6), 20:5(n-3), 22:6(n-3), total n-6 PUFA, total n-3 PUFA and total n-3 HUFA contents were significantly high accumulated in hepatopancreas of shrimp fed diet containing high level of fish oil. The supplementation of dietary fish oil and/or astaxanthin in practical broodstock shrimp diet could enhance reproductive performance of pond-reared broodstock shrimp.

Program Marine Science   Student's signature

Field of study Marine Science   Advisor's signature

Academic year 2002

ACKNOWLEDGMENTS

CONTENTS

CONTENTS (Cont.)

LIST OF TABLES

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

LIST OF FIGURES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Shrimp farmings in coastal and low salinity areas, are generally known as one of the major aquacultural industries of Thailand. In the first-half of 2002, shrimp industry provided substantial income for more than 30,000 million baht and the amount of exported shrimp products has been recorded to the levels of 90,000 metric ton. Shrimp farming need billions of high quality postlarvae to maintain the continued successful shrimp culture industry. However, high quality shrimp larval productions for grow out are depend on wild-caught broodstocks in present. In order to ensure enough shrimp larval supplies, we improve reproductive performance of broodstock shrimp. Although wild-caught broodstocks can produce high quality shrimp larvae, they may be carriers of viral diseases (Flegel and Alday-Sanz, 1998; Otta *et al*., 1999) that are the cause of mass mortality of pond-reared shrimp during first months of rearing. The quantity of mature wild female rapidly decrease from time to time and price of the female shrimp are extremely high. To solve the problem, substitution of wild-caught broodstocks with pond-reared broodstocks need more research work for providing the knowhow to produce high quality domesticated pond-reared broodstock. Moreover, pond-reared broodstocks are not popular due to poor reproductive performance. One of the main causes of poor reproductive performance in pond-reared broodstocks is the lack of knowledge in dietary requirement. Therefore, the study of pond-reared broodstocks maturation diet to improved reproductive performance and larval production will be necessary to maintain continued success of shrimp culture industry.

Shrimp maturation diet is similar to other shrimp diets as it compose of the basal ingredients e.g. carbohydrate, protein, lipid, vitamin, mineral but it has additional these fundamental aspects. First, adequate nutrient and energy statuses appear necessary for the onset of gonadal maturation. Secondly, the maturation technique of eyestalk ablation induces rapid precocious gonadal development even without the requisite accumulation of nutrients to support reproduction. Under these circumstances it appears that maternal nutrient intake during ovarian development and right up to spawning may be especially critical and can influence the composition of the ovaries and the nutritional status of the eggs. Thirdly, crustacean embryos and prefeeding larvae are lecithotrophic; they rely exclusively on the nutrients and energy supplied by the yolk (Harrison, 1997).

Lipids and astaxanthin are interesting nutritional factor for improving reproductive performance in shrimp broodstock. Lipids play major roles in sexual development and breeding, for instant steroids are used as sex hormones and some essential fatty acids. Lipids are precursors of prostaglandins (Gurr and Harwood, 1991). Lipids in particular are suspected to have a large significant effect on the reproductive process and egg quality (Bray *et al.*, 1990) and are transferred from hepatopancreas to ovaries during maturation (Teshima *et al.*, 1988a).

Although the major pigment found in shrimp is astaxanthin, astaxanthin can not be synthesized by most of animals (Goodwin, 1984). Despite of *de novo* synthesis lack, shrimp can transform β-carotene, lutein, echinenone, zeaxanthin and canthaxanthin and other intermediate carotenoids of the astaxanthin syntheses pathway into astaxanthin (Katayama *et al.*, 1972b). However, several studies showed that the deposition rates or pigmentation of those intermediate carotenoids were less

efficient than those of pure astaxanthin (Yamada *et al*., 1990; Chien and Jeng, 1992; Negre-Sadargues *et al*., 1993). The proposed functions for astaxanthin in aquaculture have been those of: provitamin A activity, antioxidant properties, positive effect on embryonic or larval development, cellular protection from photodynamic damage, enhancement of growth, maturation and reproduction, and fecundity, formation of in-chain epoxides that act as oxygen reserves under anoxic condition (Goodwin, 1960). The concentration and distribution of lipids and pigments storages in penaeids varies with species, life history stages, tissues or organs of the animals and rearing conditions, mainly the food sources.

The purposes of this study were to improve reproductive performance of the shrimp by using astaxanthin and fish oil in adolescent and broodstock black tiger shrimp *Penaeus monodon* in order to determine effect of dietary levels of essential fatty acid and astaxanthin on tissues composition as well as the levels required for the growth, survival, eggs and spermatozoa production were studied.

The thesis is organized in the following parts

1. Literature reviews describe the reproductive biology of black tiger shrimp *P. monodon*, classification and role of astaxanthin and lipid in aquatic diets (Chapter II).

2. Enhancement of reproductive performance of adolescent pond-reared black tiger shrimp *P. monodon* by dietary astaxanthin (Chapter III).

3. Dietary enrichment of astaxanthin and fish oil for maturation improvement of pond-reared *P. monodon* broodstock (Chapter IV).

4. Summary of the overall results (Chapter V).

CHAPTER II

LITERATURE REVIEW

Life history of giant black tiger shrimp *Penaeus monodon*

Penaeus monodon broodstocks spawn offshore at depths of 20-70 m at salinities between 30 to 35 ppt. The semi-buoyant fertilized eggs hatch within 24 hours and the larvae drift with water currents. The larvae successively change their shape with every molting and go through various stages of development: 6 nauplial, 2 protozoal and 3 mysis stages. The nauplii are nourished by their own yolk reserves, while the protozoea and mysis feed on microscopic plants and animals. After mysis stage, the postlarvae acquire the familiar shrimp-like appearance and start to crawl and live on the bottom or on substrates.

The postlarvae move shoreward and start appearing in coastal waters around two weeks into their postlarvae life. They continue migration towards mangrove and other brackishwater areas which serve as their nurseries or feeding grounds, growing to larger juvenile, postjuveniles and subadults. These nursery areas are sheltered, protected from waves and wind, and rich in food, but have wide temperature (20-30 ºC) and salinity fluctuation (5-32 ppt). At subadults stage, *P. monodon* is mainly carnivorous, feeding on slow-moving small animals, crabs, shrimps, mollusks, marine worms and detritus.

After about 2-4 months in brackishwater, the adolescent and sub-adult shrimps start leaving the nursery grounds back to the sea. The gonad of the shrimp mature as they reach full saline water (>32 ppt) outside of estuaries. *P. monodon* reaches sexual

maturity and spawns when it is about 47 mm carapace length (CL) or nearly one year old.

When shrimps mate, a sperm capsule or spermatophore from the male is inserted into receptacles within the thelycum of the female. This happens when the female is in the soft-shelled condition (newly molted) and the male is hard-shelled. The female carries the sperm as its own shell hardens again. A female *P. monodon* may develop from 100 000 to one million eggs. The eggs are released through the the female genital pores at the base of the third pair of walking legs while the sperm is released from her thylycum between the fourth and fifth pairs of walking legs. Fertilization takes place in water column. First spawning may occur near the mouths of estuaries after the shrimps leave the nursery grounds. Subsequent spawnings occur offshore where the adults live (Bagarinao *et al.*, 1986).

Biology of reproductive-penaeid shrimp

P. monodon is heterosexual. At mature, the female attains a relatively larger size than the male. The sexually mature prawn can be distinguished by the presence of the external genital organs; "thelycum" in female and "petesma" in male (Solis, 1988).

Age and size of first breeding adults

The best age for first breeding broodstock of *P. monodon* is about one year old (Bagarinao *et al.*, 1986), but it is difficult to detect the actual age of them. In a general rule the size of animals capable of breeding corresponds to the size at which onset of maturation occurs in the wild. Because size of a poikilotherm at a given age extremely depends on culture temperature, as well as adequate nutrition, age alone is not a good

index of breeding readiness. In nature, as well as in ponds, attainment of potential for sexual maturity in tropical marine shrimp usually occurs in 8 to 10 months. In *P. monodon*, size of males about 60 g and females of about 90 g can be recommended to use for breeding (Bray and Lawrence, 1992). A simple indicator of breeding readiness is appearance of ovarian development in some females the population. The youngest reported spawning in captive *P. monodon* is five month-old (Primavera, 1978).

Motoh (1981) reported that subadult stage began at the onset of maturation, i.e. males possessing spermatozoa in terminal ampules, and females possessing first spermatozoa inside the thelycum through copulation. A sex size disparity occurred at almost 30 mm in CL, and hereafter, the growth of females become greater than males. The size at onset of maturation in both male and female is independent on fishing areas and season. Males are sexually matured at a smaller size than females in *P. monodon*.

Male reproductive system

The male reproductive system is composed of paired testes and paired vasa deferentia terminating in ampules containing spermatophores, or sperm packets, at gonopores in the base of the fifth pair of pereiopods. The multi-lobed, transparent testes are located above the heart, which is situated dorsally over the digestive gland. The milky-colored vasa deferentia extend posteriorly from the testes, then ventrally to connect with the gonopores. In mature males, spermatophores are clearly visible through the exoskeleton from ventral or lateral views at the fifth pair of pereiopods. The two spermatophores, one from each terminal ampule, become fused longitudinally at the time of extrusion (mating with a female) and then are referred to

as the compound spermatophore or simply the spermatophore in open thelycum species (Bray and Lawrence, 1992).

Males have a specialized structure called the petasma which is presumed to be used in spermatophore transfer. The petasma is a roughly triangular, membranous flap which connects the first pair of pleopods. The petasma in young animals is unjoined, but becomes zippered in subadulthood (Bray and Lawrence, 1992).

Motoh (1981) reported that specimens at average size of 11 mm CL possess a small rudimentary petasma in the form of a knife-shaped projection situates on the subapical portion of the protopod. Until at a size of 31 mm CL, the modified endopod closely resembles the petasma of the adult. When the prawn reaches about 34 mm CL, the petasma is complete to the adult form. The two halves are now large enough so that their inner margins meet at the median line and are thus united or fused together with the aid of numerous minute hooks. However, the two components could be easily separated by physical force.

Spermatozoa are contained within the spermatophore in a viscous, slightly grayish or milky medium. These sperms are non-motile with characteristic spherical portion and cap, with spike extending outward from the spherical portion (King, 1948). Trujillo (1990) has shown that number of sperm is positively correlated with male total weight in *P. setiferus*. An adult male *P. setiferus* of 35 g may carry some 70 million sperms per spermatophore.

Female reproductive system

The female reproductive system consists of two bilaterally symmetrical, partly fused ovaries which extend almost the entire length of the female. Later stages are

visible dorsally in those species with light-colored exoskeletons. In the carapace, the anterior lobes extend to the anterior portion of the gastric mill and laterally in the cephalothorax area, while posterior lobes extend the length of the abdomen dorsally. Oviducts terminate to gonopores at the base of the third pair of pereiopods. The maturation of the ovary has been categorized into five stages, the classification of which is based on ovum size, gonad expansion, and coloration (Motoh, 1981) that can be described as follows:

Stage I and V (undeveloped and spent stages). Ovaries are thin, transparent, and not visible through the dorsal exoskeleton.

Stage II (developing stage). Referred to as early maturing stage, the ovaries are flaccid and white to olive green in color, and discernible as a linear band through the exoskeleton.

Stage III (nearly rip stage). Ovaries have pale yellow-green color with the anterior portion thick and expanded. They are very visible through the exoskeleton, particularly at the first abdominal segment, when viewed against the light.

Stage IV (ripe stage). The ovary classified as ripe (matured) stage is diamond-shaped, expansion through the exoskeleton of the first abdominal segment. The isolated ovary appears dark olive green, filling up all the available space in the body cavity.

Tan-Fermin and Pudadera (1989) recommended a simplified ovarian stage system for *P. monodon* which includes previtellogenic (P stage), vitellogenic (V), cortical rod (C), and spent (S) stages. The final flush of color change during ovarian maturation seems to occur within hours of spawning, and may be associated with

breakdown of follicle cells surrounding the ova in preparation for spawning. Ovary evaluation is implemented with a powerful light source such as a underwater dive flashlight or bright overhead illumination.

Under captive conditions, particularly over time, ovary colors vary somewhat from wild-matured ovaries, possibly due to dietary differences such as carotenoid content, hormonal changes related to eyestalk ablation, or the tank color in which the females are cultured. AQUACOP (1977) noted that ovary color, although varying from the wild, can be fairly dependable as a staging determinant in *P. vannamei* and *P. stylirostris*. The size of the gonad in the first thoracic segment, compression of the posterior cephalic lobes observed at the thoracic abdominal junction, and a granular texture, must be used as a determinants of immediate spawning in *P. monodon*, *P. merguiensis* and *P. japonicus*.

External examination is used to monitor the internal process of oocyte development, or ovarian development, includes oocyte proliferation in a germinal zone of the ovary, development of previtellogenic oocytes surrounded by round follicular cells outside of the germinal zone, and primary and secondary vitellogenesis, in which egg yolk is produced and accumulated. The final stage is retraction of follicle cells from around mature oocytes (Bray and Lawrence, 1992).

Mating characteristics

Penaeid species are grouped into two broad categories based on differences in morphology of the female thelycum, which occupies the area from the third to the fifth pereiopods. The open thelycum species receive a spermatophore or sperm packet from the male, and then retain it externally for a few hours prior to spawning. In contrast, the closed thelycum species are those which mate each time the female

molts. The closed thelycum female receives the spermatophore into her thelycum, her new exoskeleton hardens over it, and she retains the spermatophore until she utilizes the sperm in one to several spawnings, or until she molts again. In both groups, males with hardened exoskeletons transfer spermatophores to females. Multiple spawns may occur within one intermolt period for both open and closed thelycum species (Bray and Lawrence, 1992).

In closed thelycum species, when the spermatophores are inserted into the thelycum, a portion remains visible protruding from the thelycal opening (seminal receptacle) for about 24 hour until the exoskeleton hardens. Mating seems to very closely follow molting in time sequence. Primavera (1979) observed a female *P. monodon* in the laboratory which molted, and then related within an hour. Mating in closed thelycum species, in conjunction with molting, occurs at night. Primavera (1979) found that 88% of *P. monodon* molting occurred between 1800 and 0600 hours over a five-month period.

Fecundity

Penaeids are extremely fecund, and may produce from 100,000 to over 1,000,000 eggs per spawning in the wild (Martosubroto, 1974). There is a positive correlation between female size and number of eggs produced, and larger species, such as *P. monodon* produce higher numbers of eggs per spawn than smaller species such as *P. indicus*. In captivity, spawns usually range from 50 000 to 300 000 eggs. Some species such as *P. monodon* consistently produce larger spawns than others, such as *P. indicus* and *P. merguiensis*.

Precise data concerning the number of spawns produced by a female in nature are unavailable. However, evidence of repeated spawning in nature has been

presented for a number of species. Several to more than five times have been predicted for *P. indicus* (Emmerson, 1980). Beard and Wickens (1980) have also observed ablated *P. monodon* to spawn up to 6 times in one molt cycle.

Recent technique for maturation stimulating

Unilateral eyestalk ablation

The process of unilateral eyestalk ablation used to stimulate maturation in marine shrimp has been employed for both research and commercial. This method usually uses to induce ovarian development in female shrimp in captivity. The benefits of the technique are induce ovary development and ensure reproductive success, increase fecundity and percentage of fertility (Bray and Lawrence, 1992).

Unilateral eyestalk ablation can be accomplished in the following ways as described by Makinouchi and Honculada-Primavera (1987). The techniques are:

1. Simple pinching of the eyestalk, usually performed half to two-thirds down the eyestalk. This method may leave an open wound.

2. Slitting one eye with a razor blade, the crushing eyestalk, with thumb and index fingernail, beginning one-half to two-thirds down the eyestalk and moving distally until the contents of eyes have been removed. This method, sometimes called enucleation, leaves behind the transparent exoskeleton so that clotting of hemolymph, and closure of the wound, may occur more rapidly.

3. Cauterizing through the eyestalk with either an electrocautery device or and instrument such as a red-hot wire or forceps. If correctly performed, this method closes the wound completely and allows scar tissue to form more readily. A variation

of this technique is to use scissors or sharp blade to sever the eyestalk, and then to cauterize the wound.

4. Ligation by tying off the eyestalk tightly with surgical or other thread. This method also has the advantage of immediate wound closure.

Once females have been subjected to eyestalk ablation, complete ovarian development often ensues within a little as 3 to 10 days, assuming the animals were removed from a breeding or ready-to-breed population, of adequate size for reproduction, and not subjected to too much transfer stress. If the animals have been removed from non-conducive environmental conditions, a longer than normal latency period between eyestalk ablation and ovarian development can be anticipated, probably due to seasonal hormonal cycling. Duration of the latency period between eyestalk ablation and maturation of ovaries is determined by the readiness of the population at the time of eyestalk ablation (Bray and Lawrence, 1992).

Artificial insemination

Artificial insemination is routinely used to accomplish fertilization when environmental conditions are not suitable or can not stimulate mating process. While artificial insemination can be useful in increasing production in a laboratory, or in the captive and wild spawners, it also has long-term significance in its potential for interspecific and intraspecific pairings of individual males and females for genetic selection of desirable traits in domestic stocks. In the closed thelycum species, spermatophores can be implanted into the thelycum while the female exoskeleton is soft (at molt, prior to ovarian maturation) or during the intermolt period (Lin and Ting, 1986).

Nutrition

Nutrition is profoundly important to reproduction of *Penaeus*, and the success of reproduction is closely related to nutrient ingestion accompanying ovarian development. Diets used for reproduction consist of one or more fresh marine organism ingredients, with the most common being squid, mussels, clams, shrimp, brine shrimp and polychaete worms either alone or in combination with pelleted diets (Bray and Lawrence, 1992). Nutritional value of fresh diets can vary in nutritional quality with species, lifestage, season of collection, nutritional condition and storage method. Therefore, pelleted diets are additional choice for stimulation of broodstock.

In the wild, 85% of ingested food of broodstock *P. monodon* consisted of small crabs, shrimps and mollusks (Marte, 1980). The more frequent occurrence of mollusks and other non-crustaceans during months when *P. monodon* showed a higher feeding index may reflect changes in dietary requirements related to gonad development during the spawning season (Marte, 1982).

Wild immature *P. monodon* females showed an increase in ovarian lipid levels upon reaching full maturity from 5.8 to 17.0% and from 7.5 to 21.9% in unablated and ablated females, respectively (Millamena *et al*., 1985). The fatty acid profile showed 12.14-24.87% and 11.81-21.50% total fatty acids in unablated and ablated females consisting of 20:4(n-6), 20:5(n-3) and 22:6(n-3). Similar proportions of the same polyunsaturated fatty acids were found in the spawned eggs, indicating their importance in the reproductive process.

Even under ideal conditions, eyestalk ablation causes some degree of stress to the female shrimp. It should therefore be considered a stop-gap measure until less stressful methods, along environmental and dietary manipulation, are developed.

Many parameters including light intensity, light quality, photoperiod, salinity, substrate and tank color are used to stimulate maturation. Among various environmental parameters, the control of light appears to be the most promising (Primavera, 1988).

Carotenoids

Nature and chemical structure of carotenoids

Carotenoids are natural, lipid soluble pigments known as lipochromes. In nature, they are produced via an isoprenoid pathway shared with such diverse chemical compounds as essential fatty acids, steroids, and vitamins A, D, E and K. Within the various classes of natural pigments, the carotenoids are among the most widespread and structurally diverse pigmenting agents. They are almost universally distributed, occurring in the most primitive bacteria and algae up to the highly developed flowering plants and mammals. At least 600 different natural carotenoids have been identified. They are responsible for many of the brilliant yellow to red colors in plants and animals, as well as the variety of bluish, greenish, purplish, brownish and blackish colors seen in many fish and crustaceans (Latscha, 1990; 1991).

Carotenoids are structurally related to vitamin A and β-carotene, the main source of vitamin A for animals. Both in nature and through chemical synthesis, β-carotene can be considered as the basic compound for many chemical reactions. It consisted of 40 carbon atoms arranged in two β-ionone rings connected by a chain of conjugated double bonds representing the chromophore which is responsible for the typical color of carotenoids (**Figure 2-1**).

Beta-carotene

Zeaxanthin

Canthaxanthin

Astaxanthin
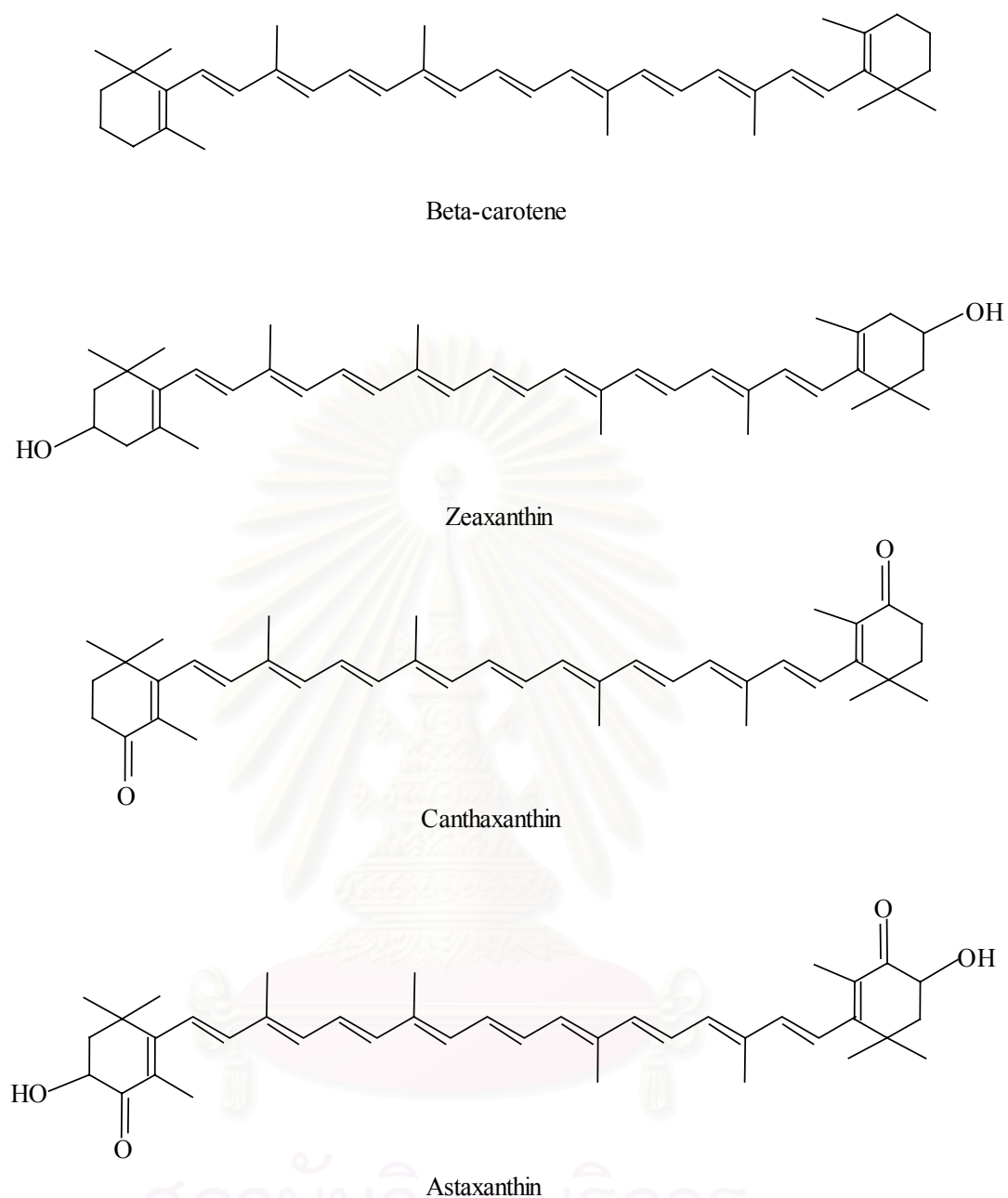
Figure 2-1. The chemical structure of some selected carotenoids.

Despite their distribution in almost all living matter and their widespread abundance in a variety of aquatic animals, carotenoids are synthesized *de novo* only by plants and some microorganisms. Thus, animals are dependent on an exogenous dietary supply of carotenoids to meet metabolic nutritional requirements. Provision of a natural source of these pigments is impractical and limited. Efficiency is primarily

related to the proximity of the dietary carotenoids to astaxanthin in the metabolic

pathway of synthesis (**Figure 2-2**).
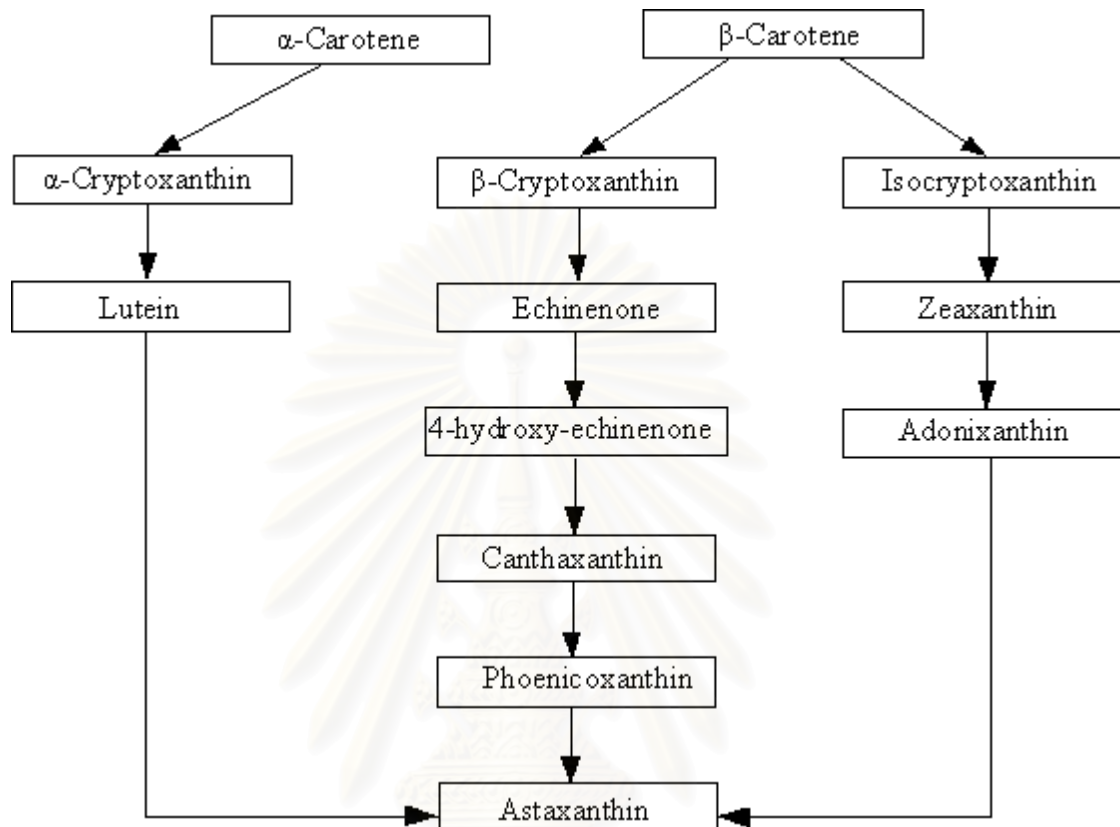
Figure 2-2. Metabolic pathways of carotenoid synthesis in crustaceans (Latscha,
1990).

Bicyclic carotenoids, β-carotene or xanthophylls (zeaxanthin) are ingested

from plants and may be converted to astaxanthin(Simpson *et al*., 1981; Torrissen *et
al*., 1989; Latscha, 1990). Organisms feeding on lower forms may be able to absorb

pigments that are intermediate between β-carotene and astaxanthin. These carotenoids

may be deposited unaltered or metabolically transformed. Animals often demonstrate

a marked degree of selectivity in absorbing specific carotenoids or metabolically

transforming them. The type of pigments absorbed and the specific rate of absorption

can vary considerably between families or species. Thus, while some carotenoids may be used as astaxanthin precursors by certain species, they may not be used by others.

The ability of marine organisms to convert dietary carotenoids into astaxanthin (**Figure 2-1**) falls into three general categories (Meyers and Chen, 1982).

1. Those which cannot oxidize the β-ionone ring and can only utilize the specific oxygenated derivatives like astaxanthin for deposition are called the salmonid or sea bream type.

2. Those which can oxidize the 4 and 4' positions of the β-ionone rings and can convert dietary zeaxanthin to astaxanthin depositing the majority of dietary carotenoids unchanged are called the carp type.

3. Those which can oxidize the 3 and 3' positions and 4 and 4' positions of the β-ionone and may convert β-carotene, zeaxanthin or intermediates to astaxanthin are called the crustacean type.

Carotenoids in crustaceans

Most crustaceans contain carotenoid pigments in some combination in carapace as well as in eyes, blood, eggs, midgut gland, and ovary. The usual pigments isolated from various classes of crustacean are astaxanthin, β-carotene, echinenone and canthaxanthin. Such carotenoids can be of dietary origin directly or derived from metabolic transformation of another dietary carotenoid.

Astaxanthin has been described as the most frequent end-product of carotenoid metabolism in crustaceans (Katayama *et al*., 1972a) and identified as the main pigment in the adult prawn, *Penaeus japonicus* (Katayama *et al*., 1971; 1972a;

Katagiri *et al*., 1987). Tanaka *et al*. (1976) indicated that astaxanthin was the most prominent pigment in both carapaces and internal organs of seven crustacean species and other carotenoids that isolated from them included β-carotene, echinenone, canthaxanthin, lutein, zeaxanthin, 3-hydroxy-canthaxanthin and 3, 3-dihydroxy-carotene. A variety of factors has affected amounts and distribution of carotenoids in crustaceans, including embryogenesis, sexual cycle, molting, background colors and hormonal control (Goodwin, 1960).

Yamada *et al*. (1990) examined the effect of dietary carotenoids, i.e., β-carotene, astaxanthin and canthaxanthin on pigmentation of *P. japonicus*. All three carotenoid sources were deposited in the tissue of the prawn as astaxanthin esters. After 8 weeks, total carotenoid and astaxanthin ester concentrations in prawns fed astaxanthin-supplemented feed were significantly higher than those of prawns supplied β-carotene or canthaxanthin-supplemented feed. Total carotenoid and astaxanthin concentrations increased as dietary levels of pigment increased up to 200 ppm, deposition also increases to a maximum of 29.1 mg kg^{-1} body weight.

Negre-Sadargues *et al*. (1993) examined utilization of synthetic carotenoids in *P. japonicus* and found accumulation of dietary astaxanthin in the integument (carapace and epidermis) and hepatopancreas. Investigations of the carotenoid composition in the exoskeleton of *P. monodon* (Okada *et al*., 1994) demonstrated astaxanthin to be the major carotenoid, accounting for 86-98% of total carotenoids. Cultured prawn accumulated mainly astaxanthin monoester in their exoskeleton, converting all precursors to astaxanthin until a certain level (8 mg 100 g^{-1}) of carotenoid was reached. It was postulated that *P. monodon* may need to store carotenoids, mainly free astaxanthin, in order to build carotenoprotein. When the

prawn receives sufficient free astaxanthin, excess dietary carotenoids are accumulated as astaxanthin esters or other carotenoids like β-carotene.

Astaxanthin and astaxanthin esters are the primary pigments of the fresh water prawn, *Macrobrachium rosenbergii* (Maugle *et al*., 1980). Evidence suggested that *M. rosenbergii* can convert β-carotene into astaxanthin via isocryptaxanthin, echinenone and canthaxanthin. Eye stalk ablation affected deposition and metabolism of carotenoids, in all likelihood through acceleration of the molting cycle (Maugle *et al*., 1980; Castillo and Negre-Sadargues, 1991). D'Abramo *et al*. (1983) established the effectiveness of dietary β-carotene, echinenone and canthaxanthin in the production of the primary tissue carotenoid astaxanthin of by these astaxanthin precursors was related to its proximity to the astaxanthin end product. Free astaxanthin represented the bulk of carotenoids in the unhatched embryo whereas larval, postlarval and juvenile stages exhibited the typical carotenoid pattern in which esterified forms of astaxanthin predominated.

Carotenoid astaxanthin, is the main pigment of crustaceans. Due to the presence of two identical asymmetric C-atoms at position 3, astaxanthin exists in the form of four different optical isomers (3S, 3'S), (3S, 3'R), (3R, 3'S) and (3R, 3'R) of which two (3S, 3S'R), (3R, 3'S) are identical (**Figure 2-3**). Various studies have focused on these isomers relative to pigmentation (Foss *et al*., 1984; Katsuyama *et al*., 1987). Of the geometric isomers known, the *trans*-form is the most stable and abundant.

[3S, 3'S]

[3R, 3'S meso]

[3R, 3'R]
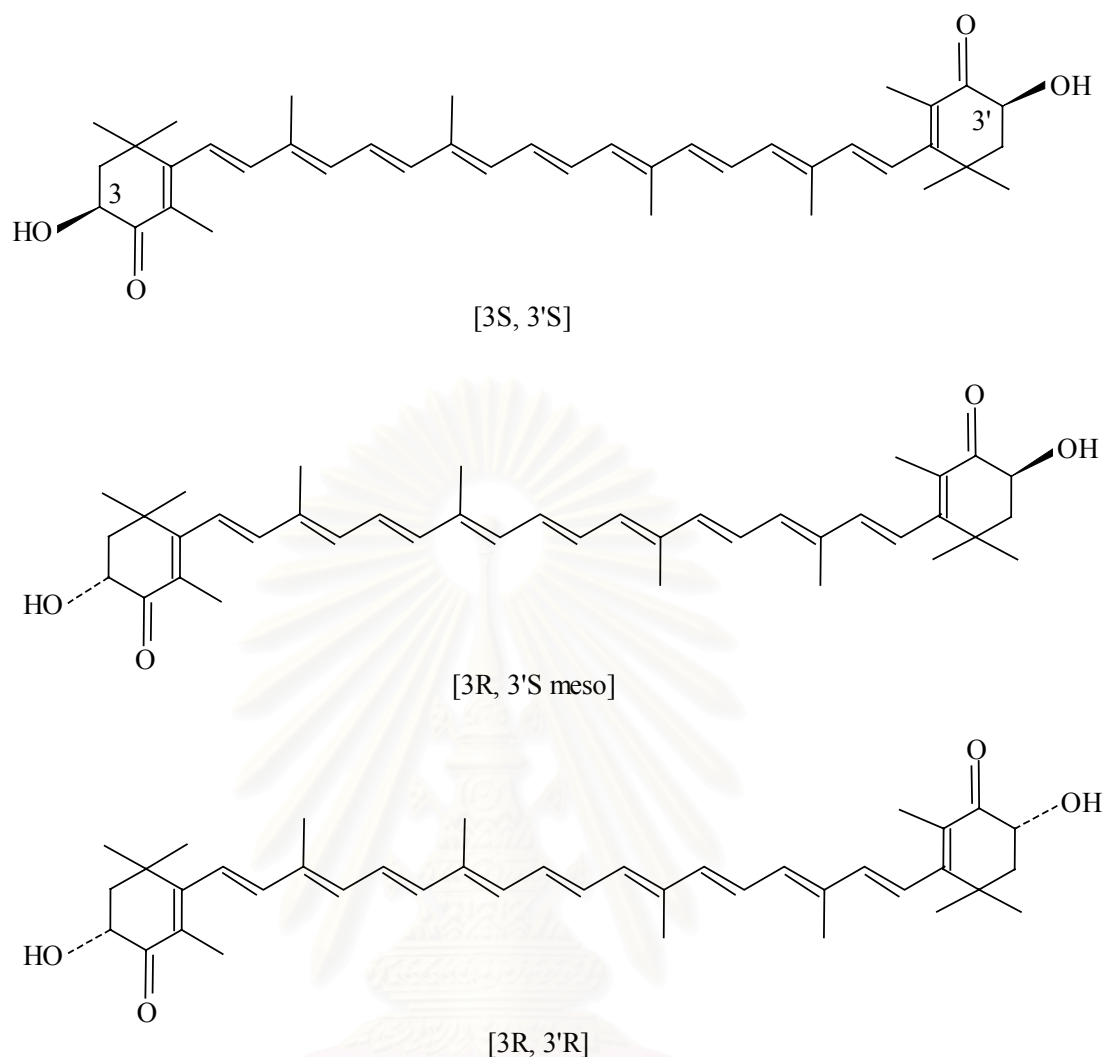
Figure 2-3. Optical isomers of astaxanthin.

Astaxanthin is present in nature either in the free form, esterified to long chain fatty acids, or associated with proteins forming carotenoproteins. All three forms are found in crustaceans. The bulk of pigments in the crustacean epidermal tissue is in the ester form with dominates monoesters, while complex carotenoproteins and carotenolipoproteins dominate in the exoskeleton. A keto group is generally essential for formation of carotenoid protein complexes and two keto groups in astaxanthin, are necessary for optimal bonding.

Total carotenoid concentrations found in caridae and penaeidae range from about 60 mg kg^{-1} to as much as 499 mg kg^{-1}, the latter having been observed in some members of the penaeidae. Interspecific differences may be as large as 300%. Most wild specimens of penaeid species involved in shrimp farming have levels of total body carotenoids ranging between 80 to 200 mg kg^{-1}. Qualitative composition varies in group- and species-specific. The vast majority of decapod crustaceans are characterized by a predominant accumulation of astaxanthin which accounts for 65-98% of the total tissue carotenoid content. In the case of *P. monodon*, the remaining 2% may be attributed to various yellow to red carotenoids which may represent transient products of carotenoid metabolism (Latscha, 1991).

The carotenoid content of a crustacean is essentially based on body weight. However, content is more precisely related to the animal's body surface because the vast majority of carotenoid pigments are concentrated in the thin exoskeleton. Given a specific carotenoid content, pigment concentration will decrease with growth suggesting an apparent pigment loss over time. This apparent loss in a decline in the average concentration of carotenoid and loss of pigments contained within the exoskeleton during molting. While the latter loss may be as high as 85% of the total carotenoid content in some lobster species, only a relatively small proportion attributed to the impact of body mass or surface increase (Meyers and Latscha, 1997). The larger proportion (approximately 70%) is attributed to the impact of body mass or surface increase. Total average losses in *P. monodon* during various pigmentation trials resulted in a mean loss of 33.6% after 30 days of culture(Meyers and Latscha, 1997).

Function of dietary carotenoids

Astaxanthin is the most common carotenoid of the marine invertebrate zooplankton (Fisher *et al.*, 1964). Astaxanthin in selected species of crustaceans found to be free and esterified forms (Foss *et al.*, 1987). Studies of copepods, genus *Calanus* and euphausid, have repeatedly demonstrated the presence of astaxanthin (in the free and esterified 3R, 3'R form) as the predominant. In 11 species of Euphausiacea, including *Euphausia superba*, astaxanthin or its esters were the only carotenoid found (Fisher *et al.*, 1955). Such crustaceans form a vital link in the transfer of nutrients from phytoplankton to zooplankton to higher marine invertebrates, with conversion of astaxanthin by euphausids to vitamin A.

The tissue carotenoid, astaxanthin, of marine zooplankton cannot be synthesized *de novo*, but requires assimilation of a precursor which serves as a template for production (Kleppel *et al.*, 1985). In crustaceans, the chief precursor is β-carotene. Diet changes in body carotenoid levels of natural marine zooplankton correlated with in situ feeding activity have been reported (Kleppel *et al.*, 1985).

Biological function of astaxanthin

Torrissen (1990) indicated that among the proposed functions for astaxanthin in aquaculture have been those of: provitamin A activity, antioxidant properties, positive effect on embryonic or larval development, cellular protection from photodynamic damage, enhancement of growth, maturation and reproduction rate and fecundity, formation of in-chain epoxides that act as oxygen reserves under anoxic conditions.

Carotenoids may be divided into two groups according to physiological function. Phenological functions are intraspecific and interspecific interactions and behavioral patterns influenced by color, such as luring prey, warning, camouflaging or reproduction. In confined aquaculture, animals in all likelihood are deprived of their natural food sources. Thus, if the respective carotenoids normally present in the natural diet are not included in the feed, the color of integuments and eggs of fish and crustaceans will fade or disappear completely. In the natural environment, functions related to predation and defense provided by carotenoids, along with sex attraction based on color, are of ecological and biological significance.

Metabolic function

Torrissen (1990) reported that stresses the vital role of carotenoids in the physiology and overall health of plants and animals and concluded that carotenoids are essential and should be included in all aquatic animal diets. Similarity in function of astaxanthin and canthaxanthin to alpha-tocopherol (vitamin E) and retinol (vitamin A) has led to suggestions that these two carotenoids be listed among the fat soluble vitamins.

Apart from its obvious role in pigmentation, increasing attention is being directed toward defining the biological activities of astaxanthin in aquatic animals, with the majority of the research focusing on the Atlantic salmon and rainbow trout. The review articles by Torrissen *et al*. (1989) and Torrissen (1990) concluded that carotenoids were essential nutrients for salmonids and noted similarity in action of astaxanthin comparable to that of vitamins A and E. Other studies using ^{14}C-labeled compounds in salmonid feeding trials have demonstrated the function of astaxanthin in various metabolic processes as well as its association with vitamin A metabolism.

Among the diversity of physiological functions, provitamin A activity of carotenoids containing β-ionone rings like astaxanthin is centrally important. Vitamin A is not available from plants and is derived from the conversion of provitamin A active carotenoids.

Antioxidant properties

Carotenoids can be characterized by their capacity to interact with a chemically reactive species of oxygen called "singlet oxygen" (Ranby and Rabek, 1978). The latter participates in oxidative reactions which can impair or destroy important cellular parts, including membranes, enzymes, and DNA nucleic acids. Singlet oxygen reactions can lead to formation of "free radicals" another species of reactive molecules capable of causing damage to cellular components. Certain carotenoids, notably β-carotene, due to their particular chemical structure, can neutralize the reactivity of singlet oxygen, thus serving as an effective antioxidant protector. β-carotene is recognized as a lipid antioxidant which causes lipid peroxidation and photosensitivity. β-corotene's lipid protecting effect complements that of vitamin E, depending on the oxygen content of the tissue. Increasing investigations are demonstrating the beneficial metabolic role of β-carotene as an antioxidant vitamin in protection against chemically induced toxicity (Kornhauser *et al.*, 1994).

Antioxidant activity of astaxanthin is higher than β-carotene 10 times, and vitamin E approximately 100 times (Miki, 1991). Palozza and Krinsky (1992) studies further document the role of astaxanthin as an extremely effective antioxidant. Carotenoids also are active in cross membrane calcium transfer, serving as oxygen reservoirs in the neuronal respiratory chain and protecting sensitive tissues and

reactive compounds from damage due to oxidation. Thus, carotenoids are now thought to function as antioxidants during hemolymph transport and within eggs, protecting nutrients and embryonic tissues from oxidative damage. They also confer structural stability to lipoproteins by forming nonstochiometric complexes, thus protecting the nutrients until required and used. Canthaxanthin and astaxanthin are more effective antioxidants than β-carotene in stabilization of free radicals (Terao, 1989).

Membrane stabilization

Carotenoids in conjunction with protein moieties, i.e., carotenoproteins of crustaceans, appear to improve the stabilization of proteins and their tertiary structure (Chessman *et al.*, 1967). These interactions are believed to be involved in changes in membrane permeability and composition and consequently may contribute indirectly to maintenance of water balance.

In nauplius to postlarval stages, shrimp initially obtains precursors of astaxanthin in vitellin reserves, then in algae and *Artemia* (Petit *et al.*, 1991). These investigators also studied the ontogeny of carotenoid metabolism in *P. japonicus* and hypothesized a storage of astaxanthin in the prawn eggs. This condition suggested a possible role of carotenoids in reproduction and during embryonic and post-embryonic development. While astaxanthin is deposited as the free form in larvae, it occurs mainly as esterified forms in postlarvae with the percentages of these forms increasing until stage P20. The ability to metabolize precursor pigments appeared in the postlarval stages and was linked to the development of oxidation and esterification pathways of carotenoids.

Desy *et al*. (1995) studied carotenoid metabolism in early developmental stages of the European lobster *Homarus gamarus* and observed a rapid decrease of pigment concentration immediately after hatching. Free astaxanthin represented the massive of carotenoids in the unhatched embryo whereas larval, postlarval, and juvenile stages exhibited the typical carotenoid pattern in which esterified forms of astaxanthin predominated. During certain physiological processes, such as molting or maturation, significant quantities of pigments may be relocated to other tissues like hemolymph, midgut gland, gonads, and eggs. Menasveta *et al*. (1994a) showed that prawns fed astaxanthin added diet has significantly greater egg diameter but no increase in maturation or spawning frequency of non-ablated prawns fed the astaxanthin added diet.

Fatty acids

The differential responses to lack or provision of dietary oils is partially due to the constituent fatty acids. Fatty acids may be divided into three different groups as follows (D'Abromo, 1997):

1. Fatty acids that can be synthesized de novo from acetate. This group includes all even number carbon, straight chain, saturated fatty acids, including those composed of 20 or 22 carbons. The most abundant is 16:0 (palmitic acid). Crustaceans, like other animals, apparently possess a delta-9-desaturase enzyme system which can convert these saturated fatty acids to monoenoic (monounsaturted) forms that contain one double bond.

2. Unusual fatty acid groups, this group includes a) fatty acids composed of an odd number of straight chain carbon atoms such as 13:0, 15:0, 17:0 and 19:0; b) Non-

methylene interrupted fatty acids which have two or more double bonds that are separated by more than three carbons; and cyclic forms such as cyclopropanoic and cyclopropenoic fatty acids.

3. Essential fatty acids (EFA) composed of the linoleic (n-6) and linolenic (n-3) families of polyunsaturated fatty acids (PUFA). These fatty acids have 2 or more unsaturated bonds. The first double bond of a fatty acid of the linoleic family occurs at the sixth carbon from the methyl end of the molecule. These fatty acids have the greatest EFA value for homothermic animals. Fatty acids of the linolenic (n-3) family have their first double bond located between the third and fourth carbon from the methyl end. Marine animals derive the greatest EFA value from this family of fatty acids.

Neither the linolenic nor linoleic families of fatty acids are synthesized *de novo* by crustaceans (Kayama *et al*., 1980). Within these two families, fatty acids that consist of a chain of 20 or more carbon atoms and more than 3 unsaturated bonds are generally termed highly unsaturated fatty acids.

The body tissue of marine crustaceans generally tends to contain proportionately higher levels of HUFA and PUFA of the linolenic family than that of freshwater crustaceans (Chanmugam *et al*., 1983). Accordingly, freshwater species of crustaceans characteristically have higher levels of fatty acids of the linoleic family in their body tissue. The linolenic family have the greatest EFA value for marine animals (Castell and Boghen, 1979).

General function of lipids

Lipid are important sources of metabolic energy (ATP). In fact, the lipids are the most energy rich of all classes of nutrients. Gross energy value of lipid is 9.5 kcal g^{-1}. In this respect, dietary lipids may be used to spare the more valuable protein for growth. In particular, free fatty acids derived from triglycerides are the major aerobic fuel source for energy metabolism of aquatic animal muscle.

Lipids are essential components of all cellular and subcellular membranes (lipids classes that are involved include the polyunsaturated fatty acid containing phospholipids, and sterol esters). Lipids serve as biological carriers for the absorption of the fat soluble vitamins A, D, E and K. Lipids are a source of essential fatty acids, which turn to be essential for maintenance and integrity of cellular membranes, are required for optimal lipid transport, and are precursors of the prostaglandin hormones (Tacon, 1990).

The major functions of essential fatty acids are related to their roles as components of phospholipids and as precursors of prostaglandins. Essential fatty acids are found in the highest concentration in phospholipids and, as such, are important in maintaining the flexibility and permeability of biological membranes, in lipid transport, and in activation of certain enzymes. As precursors of prostaglandins they are probably involved in many diverse physiological and metabolic functions.

Essential fatty acid, a role in maturation, fecundity and egg hatchability

Provisions of dietary polyunsaturated fatty acids 20:4(n-6), 20:5(n-3) and 22:6 (n-3) also appear to be necessary for successful ovarian maturation and spawning of *P. setiferus* (Midleditch *et al.*, 1979; 1980). During induced ovarian maturation,

through bilateral eyestalk ablation, the neutral lipids of the ovary of *P. setiferus*
contained higher proportions of monoenes and 22:6(n-3) and lower proportions of
20:4(n-6) and 20:5(n-3) than those of non-destalked prawns (Teshima *et al*., 1988b).
The ovarian development of *P. japonicus* was retarded in the absence of n-3 HUFA
(Alava, 1993). The results of investigations by Xu *et al*. (1992; 1994) suggest that
dietary 20:5(n-3) and 22:6(n-3) positively influence fecundity and egg hatchability,
respectively, in *P. chinensis*.

Lipids in particular are suspected to have a large effect on the reproductive
process and egg quality (Bray *et al*., 1990). Teshima *et al*. (1988a) has shown a high
transfer of lipids from hepatopancreas to ovaries during maturation, with triglyceride
and phosphatidylcholine as the major lipid classes. Alava *et al*. (1993) demonstrated
that diets deficient in either phospholipids or n-3 HUFA retarded ovarian
development in *P. japonicus*. Kanazawa *et al*. (1979) demonstrated the essentialbility
of linolenic and linoleic acids in the diet of *Penaeus japonicus* and also showed that
DHA was more effective as an essential fatty acid than either linoleic or linolenic.
Three dietary long-chain polyunsaturated fatty acid, 20:4(n-6), 20:5(n-3) and 22:6(n-3), were suggested to be involved in the shrimp reproductive process (Middleditch *et
al*., 1979). Cahu *et al*. (1994) demonstrated that fatty acids composition of eggs is
readily influenced by dietary fatty acids, as the consequence of a very fast secondary
vitellogenesis in penaeids, and a low ability for bioconversion of C18 fatty acids into
HUFA. Lytle *et al*. (1990) examined PUFA and HUFA profiles of natural foods used
in the successful maturation of *P. vannamei* and suggested that arachidonic acid, 20:4
(n-6), as well as EPA, 20:5(n-3) and DHA, 22:6(n-3) should be included and balance
of the n-3 and n-6 acids in maturation diets.

CHAPTER III
EFFECT OF ASTAXANTHIN ON REPRODUCTIVE PERFORMANCE OF POND-REARED ADOLESCENT BLACK TIGER SHRIMP *Penaeus monodon*

Introduction

Marine shrimp industries in Thailand have been used high quality shrimp larvae produced from wild-caught broodstocks for more than 3 decades. Due to rapid decline of spawners and viral disease carries from the wild shrimp. Some shrimp hatcheries begin to develop domesticated broodstocks using specific pathogen free pond-reared stocks. However, pond reared broodstocks are smaller in size and have low reproductive performances such as low fecundity and low larval quality. Development of matured broodstocks needs an understanding of adolescent or juvenile shrimp nutrition. Improvement of growth and reproductive potential in adolescent shrimp can be succeeded by micronutrient enrichment.

Shrimp and other crustaceans are unable to produce astaxanthin *de novo*. The proposed functions for astaxanthin in aquaculture have been those of: provitamin A activity, antioxidant properties, positive effect on embryonic or larval development, cellular protection from photodynamic damage, enhancement of growth, maturation and reproduction, formation of in-chain epoxides that act as oxygen reserves under anoxic condition (Torrissen, 1990). Astaxanthin has been reported as the most frequent end product of carotenoid metabolism in crustaceans and identified as the main pigment in the adult penaeids (Katayama *et al*., 1971, 1972; Okada *et al*., 1994).

Many previous reports of astaxanthin supplement in crustacean nutrition have focused on postlarval and young juvenile shrimp rather than adolescent stage (Chien and Jeng, 1992; Negre-Sadargues *et al*., 1993; Thongrod *et al*., 1995; Pan *et al*., 2001). Moreover, some researchers reported that astaxanthin had an effect on reproductive performance and larval development in marine shrimp (Menasveta *et al*., 1994; Dall, 1995; Wyban *et al*., 1995; Pangantihon-Kuhlmann *et al*., 1998).

The objective of present study is to determine effects of dietary astaxanthin on the reproductive performance in male and female of pond-reared the adolescent black tiger shrimp, *Penaeus monodon.*

Materials and methods

Experimental design

The experiment was operated for 120 days. A completely randomized design involved 3x2 factorials was used for then study. Three dietary treatments (0, 300, and 500 mg astaxanthin kg feed^{-1} and both sexes) were used. Four replications were designed for each treatment combination.

Diet and its preparation

Astaxanthin at concentration of 0, 300 and 500 mg was added into a kilogram of basal diet to formulate the experimental diets. The basal diet ingredients are shown in **Table 3-1**. All ingredients were grounded into 200 mesh-size powder and mixed by a twin blade rolling mixer for 30 min. Then, astaxanthin was gently added as concentration designed. Carophyll Pink, a synthetic astaxanthin provided by Hoffman-La Roche, Switzerland was used as astaxanthin source in the experiment. Vitamin A, C and E were added in experimental diets at levels of 20,000 IU kg^{-1}, 200 and 100 mg kg^{-1}, respectively. After homogenized mixtures, each mash was pelleted using a California Pelleting Machine (CPM), streamed at 95 ºC for 5 min and dried by hot air oven at 60 ºC for 2 hrs. The size of the finishing pellet was 2 mm in diameter and 4 mm in length. The pelleted diets were kept in dark container, flushed with nitrogen gas and storaged at -20 ºC. The experimental diets were analyzed for crude protein, lipid, ash, fiber and moisture contents with methods described by AOAC (1995).

Table 3-1. Feed ingredients of adolescent shrimp diets.

Ingredients	Dry weight (g 100^{-1} g of diet)		
	Astaxanthin 0 mg kg^{-1}	Astaxanthin 300 mg kg^{-1}	Astaxanthin 500 mg kg^{-1}
Fish meal	56	56	56
Shrimp head meal	10	10	10
Wheat flour	16	16	16
Refined tuna fish oil	6	6	6
Cellulose	6.883	6.508	6.258
Mineral mixture[a]	1	1	1
Vitamin mixture[b]	1	1	1
Cholesterol[c]	1	1	1
Lecithin[d]	1	1	1
Binder[e]	1	1	1
Astaxanthin[f]	0	0.375	0.625
Vitamin A[g]	0.04	0.04	0.04
Vitamin C[h]	0.057	0.057	0.057
Vitamin E[i]	0.02	0.02	0.02
Total	100	100	100

[a]Mineral mixture 100 g contains: K_2HPO_4 2.0 g, $Ca_3(PO_4)_2$ 2.720 g, $MgSO_4$ $7H_2O$ 3.041 g, NaH_2PO_4 $2H_2O$ 0.790 g.

[b]Vitamin mixture 100 g contains: ρ-aminobenzoic acid 10.0 mg; biotin 0.40 mg, inositol 400.0 mg; nicotinic acid, 40.0 mg; Ca-pantothenate, 60.0 mg; pyridoxine-HCl, 12.0 mg; riboflavin, 8.0 mg; thiamin-HCl, 4.0 mg; menadione, 4.0 mg; cyanocobalamine, 0.08 mg; calciferol, 1.20 mg; folic acid, 0.80 mg; choline chloride, 120.0 mg.

[c]Ninety five percent cholesterol, laboratory grade, Sigma.

[d]Soy lecithin, feed grade.

[e]Aquabind, Du Pont.

[f]Chlorophyll pink 8%, Roche.

[g]Five hundred thousand IU g^{-1}, feed grade, Roche.

[h]Stay C 35%, Roche.

[i]Fifty percent, feed grade, Roche.

Experimental animals

Shrimp used in the experiment were adolescent *P. monodon*, with average weight of 22.3 g for female and 21.3 g for male, collected from commercial earthen pond reared at the age of 4 months. The adolescent shrimp were acclimated under laboratory condition for at least 15 days before the beginning of experiment. Shrimp were doubly tagged; a plastic numbering glued at the carapace and a rubber ring with the same number tagged around the eyestalk for individual biological data monitoring of growth, molting, and reproductive stages. At initial, all unique size of healthy adolescent shrimp were randomly selected, weighted, tagged and transferred into each experiment unit. The 5 animals (3 females and 2 males) were acclimated for a few days with life food. If no sign of unhealthy shrimp was observed, the feeding regime of the designed experiment then began.

Experimental pond and reared condition

The rearing system in this experiment was an outdoor closed recirculating water system covered with shading to reduce light intensity of about 70%. The system was a circular shape, consisted a rearing tank with 30 tons of water and a biological treatment unit of 8 tons of water at the center. Water depth was maintained at 1.0 m. The detail of the experimental pond has been described by Menasveta (1982). Twelve trapezoid net cages with 1.5 m^2 of rearing area each were established in the rearing unit.

Feeding strategy

The experimental diets regime; pelleted diet and fresh diet were fed 3 times per day, 0600 1200 and 1800. The fresh diet was chopped squid (*Loligo sp.*) given 10% of shrimp body weight at 0600. The pelleted diets fed at 1200 and 1800 at about 2% of shrimp weight per day. Uneaten diets, fecal matters and particular detritus were removed before the first feed each day.

Data collection

In female shrimp, ovarian development was observed by flashing a light through dorsal part of the abdomen every 2 day (Motoh, 1981). The gravid shrimp with ripe stage was sacrificed for detecting number of eggs and tissues collecting of muscle, hepatopancreas, ovary and shell for analyzing astaxanthin contents. In male, spermatophores were obtained by electrical stimulation at 2-4 volts, and 0.3-0.5 amperes using a method similar to that described by Sandifer *et al*. (1984). Amount of spermatozoa was determined at the end of the experiment in term of total sperm count followed by Leung-Trujillo and Lawrence (1987). The adolescent growth rate and survival of both sexed were determined monthly. At the end of experiment, the remainder shrimp was sacrificed and then muscle, hepatopancreas, ovary and shell were collected for astaxanthin analysis using high performance liquid chromatography as a method described by Weber (1988). The detail of astaxanthin analysis is shown in Appendix C.

Water quality

Ammonia, nitrite and nitrate (NH_4-N, NO_2-N and NO_3-N) in water determined weekly by using test kits (Sera, Germany). Temperature, dissolved oxygen, pH and alkalinity were monitored every day during the whole experiment period.

Statistical analysis

Effects of astaxanthin and sex on weight gain were analyzed using analysis of covariance. Effects of astaxanthin and sex on survival rate, amount of spermatozoa and tissue astaxanthin contents were analyzed using analysis of variance and Duncan's New Multiple Range Test (Cody and Smith, 1997).

Results

The proximate analysis of experimental feeds and astaxanthin content is shown in **Table 3-2**. Average protein in diets was $45.64 \pm 0.57\%$. Astaxanthin concentrations in diet supplemented with astaxanthin 0, 300 and 500 mg kg^{-1} were 9.85 ± 0.37, 283.38 ± 2.06 and 394.91 ± 10.39 mg kg^{-1}, respectively. Water quality of the experiment was described in **Table 3-3**.

Table 3-2. Proximate analysis of adolescent shrimp diet as fed basis (means $\pm$ s.d.).

Proximate	Treatments		
	Astaxanthin 0 mg kg^{-1}	Astaxanthin 300 mg kg^{-1}	Astaxanthin 500 mg kg^{-1}
Protein (%)	46.16 ± 0.16	44.95 ± 0.11	45.81 ± 0.13
Lipid (%)	13.52 ± 0.28	13.18 ± 0.24	13.75 ± 0.17
Fiber (%)	2.48 ± 0.18	2.52 ± 0.20	2.05 ± 0.23
Ash (%)	13.39 ± 0.06	13.38 ± 0.04	14.03 ± 0.13
Moisture (%)	9.51 ± 0.25	11.66 ± 0.59	9.79 ± 0.23
NFE[a] (%)	14.94 ± 0.45	14.31 ± 0.57	14.57 ± 0.28
Astaxanthin (mg kg^{-1})	9.86 ± 0.37	283.39 ± 2.06	394.91 ± 10.39

[a]NFE, Nitrogen free extract.

The interaction between dietary astaxanthin and sex on total growth, survival, amount of spermatozoa and astaxanthin content in muscle, hepatopancreas and shell had not significant difference ($P>0.05$). Therefore, the effects of astaxanthin and sex on total growth, survival, amount of spermatozoa and astaxanthin content in muscle, hepatopancreas and shell were discussed independently.

Table 3-3. Water quality of the adolescent shrimp experiment.

Parameters	Range
Salinity (ppt)	29-30
Temperature (°C)	26-29
Dissolved oxygen (mg l^{-1})	6.5-7.7
Alkalinity (mg l^{-1})	120-200
pH	7.5-8.5
Ammonia (mg l^{-1})	0-0.5
Nitrite (mg l^{-1})	0-0.3
Nitrate (mg l^{-1})	0-10

The effects of dietary astaxanthin and sex on weight gain are shown in **Figure 3-1**. The weight gain of shrimp was presented by linear regression models. Linear slope of weight gain of the shrimp is shown in **Table 3-4**. There was significantly different ($P<0.05$) of the weight gain among levels of astaxanthin during the experimental period. Female shrimp had significantly greater weight gain than that of the male shrimp ($P<0.05$).

The effects of dietary astaxanthin and sex on survival are shown in **Figure 3-2**. The highest survival rates of 58.35 ± 16.70 and 75.00 ± 50.00% were observed in female and male shrimp fed diet supplemented with astaxanthin 300 and 500 mg kg^{-1}, respectively, at 4 months of experiment. There was not significant difference of survival rate between levels of astaxanthin treatment diets and sexs after 4 months of experiment.
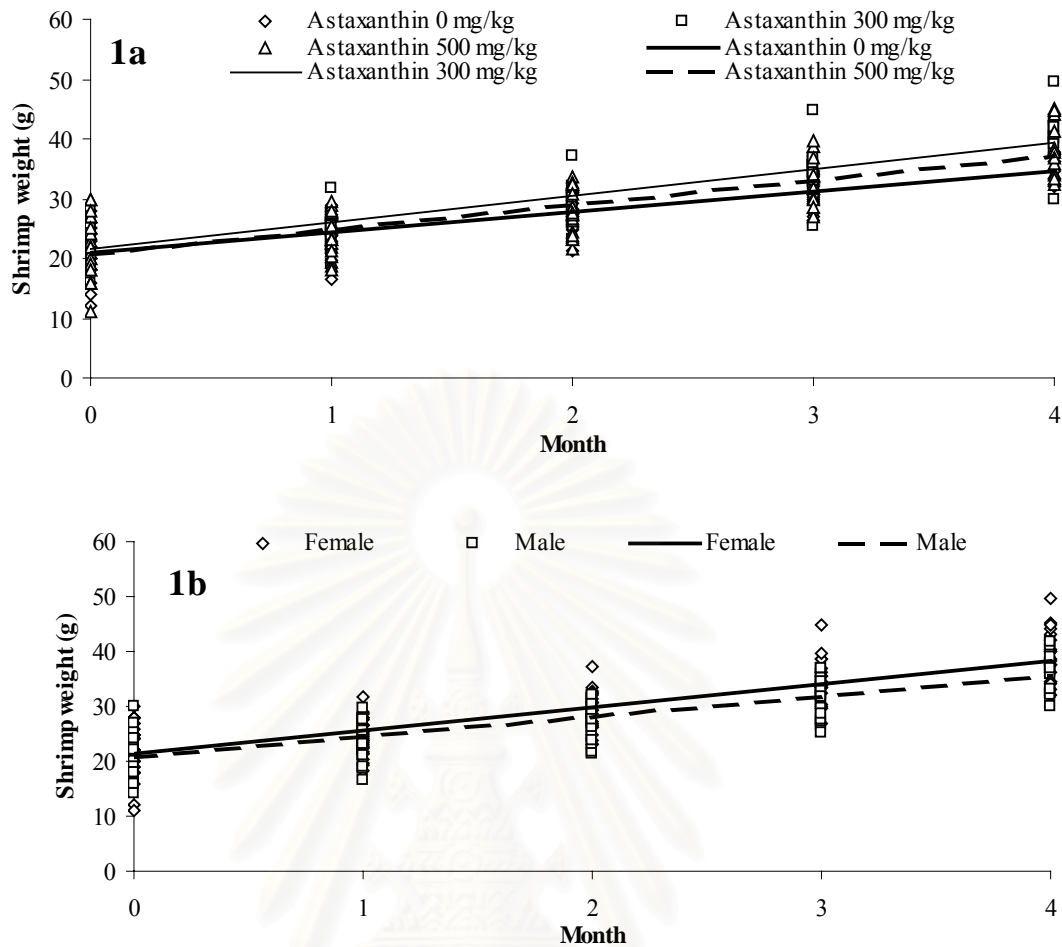
Figure 3-1. Effect of astaxanthin (1a) and sex (1b) on total growth of adolescent

shrimp *P. monodon*.

In female shrimp, no any ovarian development was observed in all treatments

during 4 months of experiment, but in the male, dietary astaxanthin had an effect on

the amount of spermatozoa (**Figure 3-3**). Male shrimp fed diets supplemented with

astaxanthin 300 and 500 mg kg^{-1}, the amount of spermatozoa ($1.24 \times 10^6 \pm 5.18 \times 10^5$

and $1.47 \times 10^6 \pm 4.96 \times 10^5$ individuals shrimp^{-1}), respectively were significantly higher

($P < 0.05$) than those of the control ($8.40 \times 10^5 \pm 2.60 \times 10^5$ individuals shrimp^{-1}).

Table 3-4. Effect of astaxanthin and sex on linear slope of total growth of adolescent

shrimp *P. monodon* (means). Means within each factor with the different

superscripts are significantly different (*P*<0.05).

Factors	Growth rate (g month^{-1})
Astaxanthin 0 mg kg^{-1}	3.36^{b}
Astaxanthin 300 mg kg^{-1}	4.57^{a}
Astaxanthin 500 mg kg^{-1}	3.94^{ab}
Female	4.35^{a}
Male	3.72^{b}

The effects of dietary astaxanthin and sex on astaxanthin content in muscle,

hepatopancreas and shell are shown in **Figure 3-4**. Shrimp fed diet supplemented with

astaxanthin 500 mg kg^{-1} had significantly higher astaxanthin content in muscle and

hepatopancreas (*P*<0.05) than those of the control and those fed diet supplemented

with astaxanthin 300 mg kg^{-1}. In the shell, shrimp fed diet supplemented with

astaxanthin had significantly greater astaxanthin content (*P*<0.05) than those fed

without astaxanthin supplementation. No significant difference on astaxanthin content

in muscle, hepatopancreas and shell among sex were found
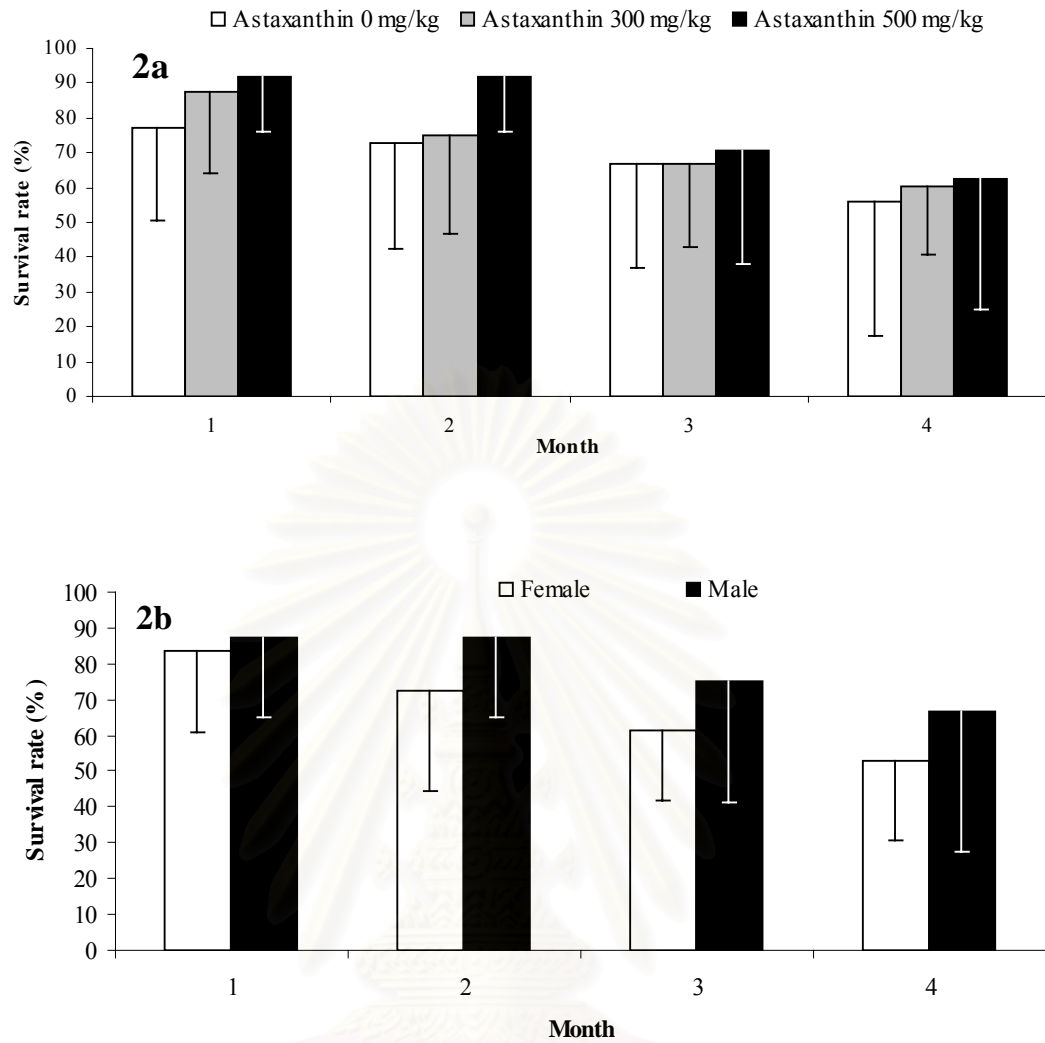
Figure 3-2. Effect of astaxanthin (2a) and sex (2b) on survival rate of adolescent

shrimp *P. monodon*. The different superscripts within each month at the top of

each bar are significantly different (*P*<0.05).
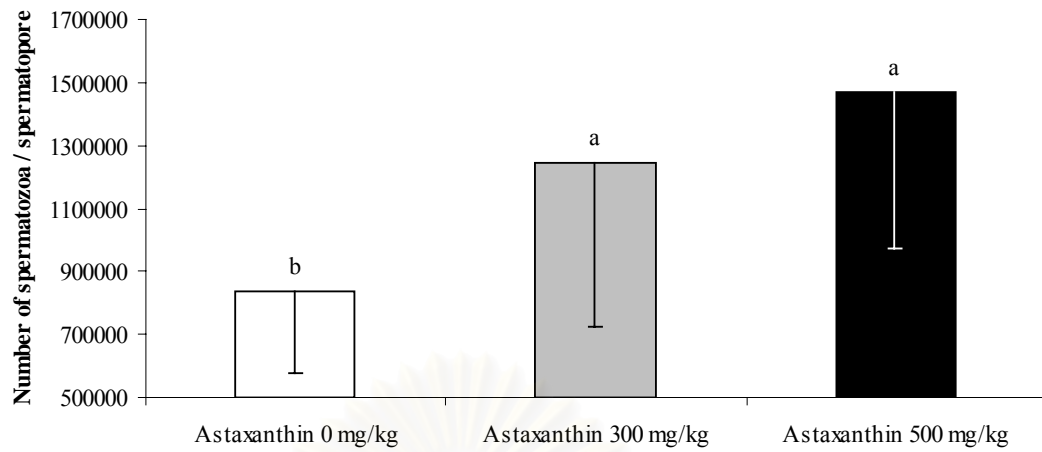
Figure 3-3. Effect of astaxanthin on amount of spermatozoa of adolescent male

shrimp *P. monodon*. The different superscripts at the top of each bar are

significantly different (*P*<0.05).
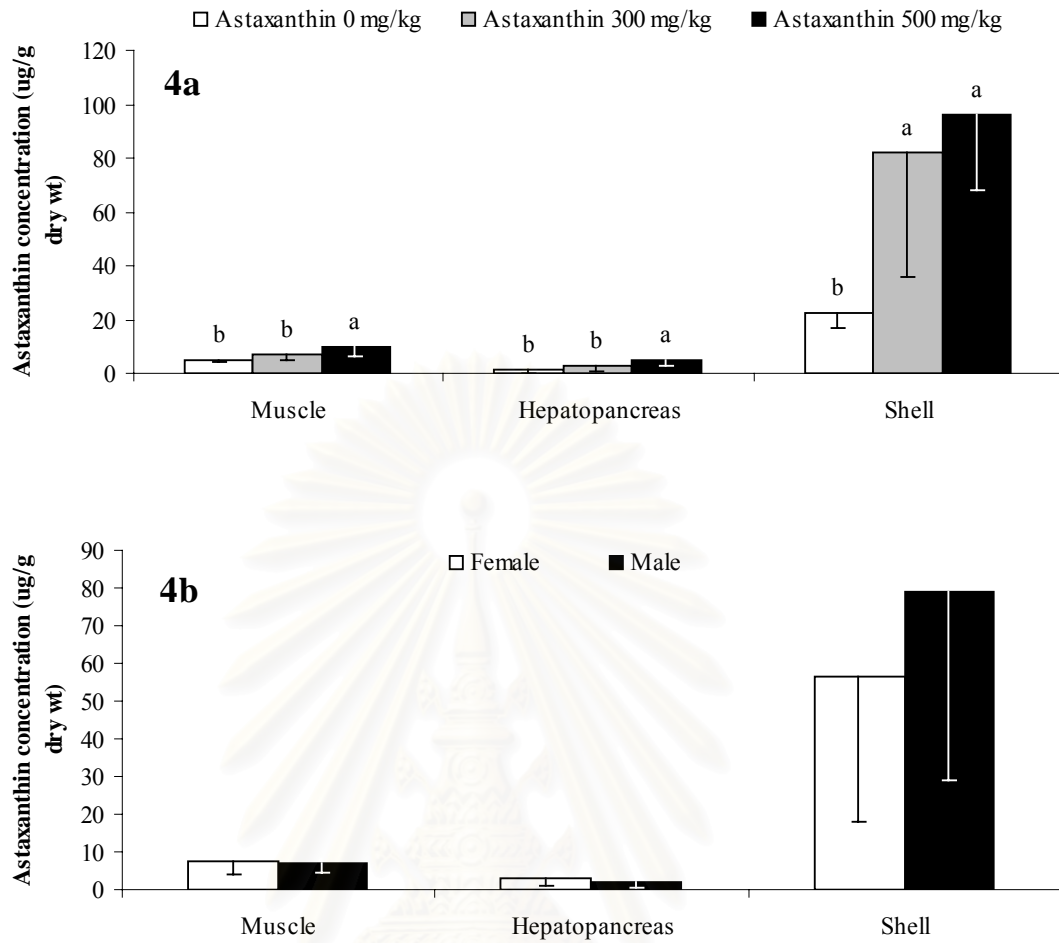
Figure 3-4. Effect of astaxanthin (4a) and sex (4b) on astaxanthin content in muscle, hepatopancreas and shell of adolescent shrimp *P. monodon*. The different superscripts within each type of tissues at the top of each bar are significantly different (*P*<0.05).

Discussion

The results in this study illustrated that dietary astaxanthin could improve growth of pre-matured shirmp in both sexes (**Table 3-4**). There was no interactive effect between dietary astaxanthin and sexes on weight gain, survival and astaxanthin content in muscle, hepatopancreas and shell. In general, astaxanthin which is a main carotenoid in crustacean has a potential effect on shrimp growth and/or survival (Chien and Jeng, 1992; Thongrod *et al*., 1995; Petit *et al*., 1997; Pan *et al*., 2001). Petit *et al*. (1997) found that the moulting cycle of *P. japonicus* postlarvae fed with diet supplementing astaxanthin was significantly shorten. Thongrod *et al*. (1995) indicated that survival rate of *P. monodon* postlarvae could be improved by supplement of dietary astaxanthin. Pan *et al*. (2001) reported that astaxanthin concentrations in shrimp decreased significantly due to rapid growth, but there was no significant difference of astaxanthin on growth was observed in their study. Chien and Jeng (1992) reported that the different pigment sources and their various levels had not effect on growth rate but a positive correlation between pigment concentration in shrimp tissue and survival rates was observed in *P. japonicus*. In the present study, astaxanthin supplemented diets did no promote survival rate of the shrimp during the experimental period. The similar results also reported by Negre-Sadargues *et al*. (1993) and Boonyaratpalin *et al*. (2001).

The adolescent female shrimp grew faster than the male shrimp. It is quite common for the penaeid species when they reach to a pre-adult stage of life cycle the female growth is faster than the male. Bray and Lawrence (1998) reported the growth rate of female shrimp was 3.9 g week^{-1} and the male was 2.5 g week^{-1}.

The adolescent ovary did not develop during the whole experimental period in all treatments. It can be explained by the reasons that; the shrimp at the end of the experiment is still pre-matured (an average age of 8 months and size of less than 50 g), there is no any maturation induction technique applied to the shrimp in the experiment, and the experiment was outdoor with natural light cycle, no light intensity control as in most of the maturation hatcheries. High light intensity is a negative effect on ovarian development and spawning of broodstock shrimp (Hoang *et al*., 2002a). Recommended size for matured female *P. monodon* is 90 g (Bray and Lawrence, 1992). Pangantihon-Kuhlmann *et al*. (1998) reported that eight-month-old female could reach a size over 110 g in earthen pond. The female at this size can be matured if fed them with diet supplemented astaxanthin 100 mg kg^{-1}.

In the present study, shrimp fed diet-supplemented astaxanthin could enhance maturation performance in males. Higher number of spermatozoa was found in males fed with diets containing astaxanthin. Male shrimp can mature earlier than that of the females, they can produce sperm at smaller size and younger ages (Hoang *et al*., 2002b). Primavera (1978) reported that both pond and wild male *P. monodon* of at size more than 40 g body weight can be matured and have sperm production, but the one can produce high number and high of sperm.

Astaxanthin may promote spermtozoa in male shrimp via the provitamin A activity. Ratinoid act in testicular development, especially on germ cells, via retinoic acid receptor and/or retinoid X receptors (Boulogne *et al*., 1999). Akmal *et al*.(1997) reported in the mutation study of spermatid and found that retinoic acid receptor alpha played an essential role in spermatogenesis. Characterization of retinoic acid receptor

alpha expression revealed time and location of the vitamin A requirement during spermatogenesis.

Astaxanthin components in muscle, hepatopancreas and shell of adolescent shrimp mainly were all-trans-astaxanthin, 9-cis and 13-cis isomers. Similar with a report by Muriana *et al*. (1993) as the main component of astaxanthin in hepatopancreas and muscle of shrimp *P. japanicus* was all-trans-astaxanthin, which was accompanied by an epimer and its cis-isomer. There no effect of differenct sex on astaxanthin content in muscle, hepatopancreas and shell of adolescent shrimp (**Figure 3-4b**). Female shrimp usually have high accumulation of carotenoid pigment, especially astaxanthin in vitellogenesis process during ovarian development (Nelson *et al*. 1988; Quinito *et al*., 1989; 1990; Harrison, 1990; Dall, 1995). Sagi *et al*. (1996) found the strong correlation between the increasing diameter of the oocyte and concentration of astaxanthin in the ovary and hemolymph in female crayfish *Cherax quadricarinatus*. In the present study the females are still pre matured with no ovary development, so that no astaxanthin accumulation can be seen at this stage.

The main tissues of astaxanthin deposit in adolescent shrimp were found in the shell while muscle and hepatopancreas had minor astaxanthin deposit. Similar was reported by Dall *et al*. (1995) as female *P. esculentus* contained 82 to 94% of the total carotenoid in the integument, but at maturity the digestive gland contained $10.7 \pm 3.4\%$ and the ovary $5.6 \pm 0.9\%$ of the total carotenoid. During maturity, digestive gland concentrations of astaxanthin increased (total 20 to 120 $\mu g\ g^{-1}$), but levels in the muscle and integument varied little throughout maturation (total similar to 0.4 and 100 $\mu g\ g^{-1}$, respectively). Astaxanthin accumulation in the shrimp tissues in this study had significantly higher in the group fed diet containing higher level of astaxanthin.

This was also reported by Menasveta *et al*. (1994) that shrimp fed diet with astaxanthin had significant greater total carotenoid and astaxanthin content in hepatopancreas than those fed diet without astaxanthin.

In conclusion, dietary astaxanthin can enhance reproductive performance in adolescent male and promote growth in both sexes of pond-reared *P. monodon.* This finding may benefit for the farmers in domestication of marine shrimp broodstock. Better preparation of pre-adult will get the best quality of broodstock.
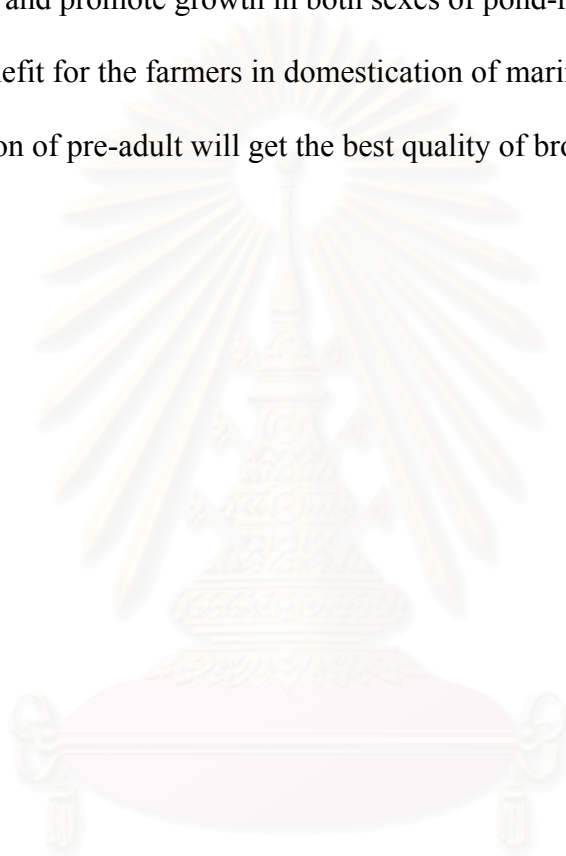
CHAPTER IV

NUTRITIONAL ENRICHMENT OF FISH OIL AND ASTAXANTHIN FOR MATURATION IMPROVEMENT OF POND-REARED BROODSTOCK BLACK TIGER SHRIMP
(*Penaeus monodon* Fabricius)

Introduction

In Thailand, high quality shrimp larvae have been mostly produced by wild-caught broodstocks. Although the wild-caught broodstocks produce high quality shrimp larvae, they may be carriers of viral diseases (Flegel and Alday-Sanz, 1998; Otta *et al*., 1999) and cause mass mortality of shrimp after 1 or 2 months of rearing in the earthen pond. The amounts of matured wild female rapidly decrease on time to time. Then, the substitution of wild-caught broodstocks with pond-reared broodstocks will be sustainable developed. However, pond-reared broodstocks are not favorite use for broodstock because they have lower maturation performance than wild-caught broodstocks. One of the main causes of low maturation performance is due to less nutritional information. Therefore, the study of pond-reared broodstocks nutrition to improve maturation and larvae production is necessary.

Astaxanthin and lipid are important nutrient to promote reproductive performance in shrimp (Middleditch *et al*., 1979; Millamena, 1989; Bray *et al*., 1990; Menasveta *et al*., 1994a; Pangantihon-Kuhlmann *et al*., 1998). The proposed functions for astaxanthin in aquaculture have been those of provitamin A activity, antioxidant properties, positive effect on embryonic and larval development, cellular protection from photodynamic damage, enhancement of growth, maturation and

formation of in-chain epoxides that act as oxygen reserves under anoxic condition (Torrissen, 1990). Astaxanthin has been reported as the most frequent end product of carotenoid metabolism in crustaceans (Katayama *et al.*, 1972) and identified as the main pigment in the adult prawn, *Penaeus japonicus* (Katayama *et al.*, 1971; 1972). A variety of factors affects amounts and distribution of carotenoids in crustaceans, including embryogenesis, reproductive cycle, molting, background colors, and hormonal control (Goodwin, 1960).

Lipid plays major roles in reproductive processes of crustaceans. Levels and composition of dietary lipids profoundly affect ovarian maturation and reproductive success (Harrison, 1990). Middleditch *et al.* (1979; 1980) investigated that penaeid shrimp needs polyunsaturated fatty acid (PUFA) in order to develop shrimp gonad tissue. The purpose of this study is to improve reproductive performance of pond-reared black tiger shrimp broodstock *Penaeus monodon* by dietary fish oil and astaxanthin.

Materials and methods

Experimental design

The experiment was operated 120 days. The experimental design was 2×2×2 factorials in completely randomized design. Two levels of fish oil 3 and 8%, 2 concentrations of astaxanthin 100 and 500 mg kg^{-1} and 2 sexes (male and female) were used. The composition of the tested diet was described in **Table 4-1**. Eight replication was done in each treatment.

Diet and dietary preparation

The experimental diets contained 48% crude protein. The four experimental diets were: LFLA, supplemented with 3% fish oil and 100 mg kg^{-1} astaxanthin; LFHA, supplemented with 3% fish oil and 500 mg kg^{-1} astaxanthin; HFLA, supplemented with 8% fish oil and 100 mg kg^{-1} astaxanthin; and HFHA, supplemented with 8% fish oil and 500 mg kg^{-1} astaxanthin. Dietary ingredients were grounded into powder and mixed by a twin blade rolling mixer for 30 min. Astaxanthin were added to contain levels according to formula. Astaxanthin source was a synthetic one and bought from Hoffman-La Roche, Switzerland. Vitamin A, C and E were added in experimental diets at levels of 20 000 IU kg^{-1}, 200 and 100 mg kg^{-1}, respectively. The homogenized mixture of each diet's mesh was pelletized by a pelleting machine (CPM, California Pelleting Machine), streamed for 5 min and then dried by hot air oven at 60 ºC for 2 hr. The pelleted dietary size was 3 mm of diameter and 5 mm of length. The pelleted diets were kept in dark container and flushed with nitrogen gas before storage at -20 ºC.

Table 4-1. Feed ingredients of broodstock shrimp diets.

Ingredients	Dry weight (g 100 g^{-1} of diet)			
	LFLA	LFHA	HFLA	HFHA
Fish meal	56	56	56	56
Shrimp head meal	10	10	10	10
Wheat flour	16	16	16	16
Refined tuna fish oil	3	3	8	8
Chlorophyll pink[a]	0.125	0.625	0.125	0.625
Cellulose	9.758	9.258	4.758	4.258
Mineral mixture[b]	1	1	1	1
Vitamin mixture[c]	1	1	1	1
Cholesterol[d]	1	1	1	1
Lecithin[e]	1	1	1	1
Binder[f]	1	1	1	1
Vitamin A[g]	0.04	0.04	0.04	0.04
Vitamin C[h]	0.057	0.057	0.057	0.057
Vitamin E[i]	0.02	0.02	0.02	0.02
Total	100	100	100	100

[a]Chlorophyll pink contains 8% active form of astaxanthin, Roche.

[b]Mineral mixture 100 g contains: K_2HPO_4 2.0 g, $Ca_3(PO_4)_2$ 2.720 g, $MgSO_4$ $7H_2O$ 3.041 g, NaH_2PO_4 $2H_2O$ 0.790 g.

[c]Vitamin mixture 10 g contains: ρ-aminobenzoic acid 10.0 mg; biotin 0.40 mg, inositol 400.0 mg; nicotinic acid, 40.0 mg; Ca-pantothenate, 60.0 mg; pyridoxine-HCl, 12.0 mg; riboflavin, 8.0 mg; thiamin-HCl, 4.0 mg; menadione, 4.0 mg; cyanocobalamine, 0.08 mg; calciferol, 1.20 mg; folic acid, 0.80 mg; choline chloride, 120.0 mg.

[d]Ninety five percent cholesterol, laboratory grade, Sigma.

[e]Soy lecithin, feed grade.

[f]Aquabind, Du Pont.

[g]Five hundred thousand IU g^{-1}, feed grade, Roche.

[h]Stay C 35%, Roche.

[i]Fifty percent, feed grade, Roche.

Experimental animals

Shrimp used in the experiment were subadult *P. monodon* at 48-50 g body weight in female and 35-38 g body weight in male. All of the shrimp were collected from a commercial earthen pond at the age of 6 months. The broodstocks were acclimated under experimental condition (temperature 28 ± 1 ºC and salinity 35 ppt) for at least 15 days before beginning of the experiment. Shrimp were doubly tagged, a plastic numbering glued at the carapace and a rubber tube with the same number around the eyestalk for biological data monitoring on an individual basis such as molting, and/or ovary maturation. At initial, all broodstock shrimp were randomly selected into each experiment unit.

Experimental pond and reared condition

The rearing system in this experiment was an indoor closed recirculating water system covered with shading to reduce light intensity of about 100%. The system was a circular shape, consisted a rearing tank with 30 tons of water and a biological treatment unit of 8 tons of water at the center. Water depth was maintained at 1.0 m. The detail of the experimental pond has been described by Menasveta (1982). Thirty two rectangular net cages with 0.36 m^2 of rearing area each were established in the rearing unit. At initial, 2 broodstock shrimp, 1 female and 1 male were stocked in each cage.

Feeding strategy

The experimental feeding regime, pelleted diet and fresh diet were fed 3 times a day at 0600, 1200 and 1800. The fresh diet was chopped squid (*Loligo sp.*) given 10% of shrimp body weight at 0600. The pelleted diets fed at 1200 and 1800 for about

2% of shrimp weight a day. Uneaten diets, fecal matters and particular detritus were removed before the first feed.

Data collection

The newly molted female broodstocks were induced to spawn by unilateral eyestalk ablation. In female shrimp, ovarian development was observed by flashing a light through dorsal part of the abdomen every 2 days (Motoh, 1981).

The gravid broodstock in ripe stage was sacrificed for detecting number of eggs and tissues sampling of muscle, hepatopancreas, ovary and shell for analyzing astaxanthin contents and fatty acids concentration. In male, spermatophores were obtained by electrical stimulation at 2-4 volts, and 0.3-0.5 amperes using a method similar to that described by Sandifer et al. (1984). Amount of spermatozoa was determined at the end of experiment in term of total sperm count followed by Leung-Trujillo and Lawrence (1987). The broodstocks growth rates were measured by weighting every 30 day. Survival of shrimp was not determined because dead shrimp were replaced with the new same sex shrimp in the first two months of experiment. At the end of experiment, the remainder shrimp were sacrificed and then muscle, hepatopancreas, ovary and shell were collected for astaxanthin and fatty acid analysis using high performance liquid chromatography and gas chromatography as method described by Weber (1988) and Christie (1989), respectively. The details of astaxanthin and fatty acid analytical methods are shown in Appendix C and D, respectively.

Proximate analysis of the experimental diets

The experimental diets were analyzed for crude protein, lipid, ash, fiber and moisture with methods as described by AOAC (1995).

Water quality

Ammonia, nitrite and nitrate (NH_3-N, NO_2-N and NO_3-N) in water were determined weekly by using test kits (Sera, Germany). Temperature, dissolved oxygen, alkalinity, and pH were monitored every day during the experiment period.

Statistical analysis

Effects of astaxanthin, fish oil and sex on weight gain, amount of eggs and spermatozoa, astaxanthin contents and fatty acid concentration were analyzed using Analysis of Variance and Duncan's New Multiple Range Test (Cody and Smith, 1997).

Results

Water quality at the experiment was maintains as described in **Table 4-2**. All water quality parameters were in the range that was suitable for aquatic animals. The proximate analysis of experimental diets and astaxanthin content are show in **Table 4-3**. Average protein content in LFLA, LFHA, HFLA and HFHA diets was 48.64 ± 1.71, 48.96 ± 0.28, 47.52 ± 0.20 and $48.83 \pm 0.83\%$, respectively. Astaxanthin concentrations in LFLA, LFHA, HFLA and HFHA diets were 45.58 ± 1.71, 296.41 ± 2.86, 46.16 ± 1.30 and 264.55 ± 6.00 mg kg^{-1}, respectively. The fatty acid contents in diets and fresh diet, squid was showed in **Table 4-4**.

Table 4-2. Water quality of the broodstock shrimp experiment.

Parameters	Range
Salinity (ppt)	35 ± 1
Temperature (ºC)	28 ± 1
Dissolved oxygen (mg l^{-1})	6.5-7.7
Alkalinity (mg l^{-1})	148-220
pH	7.5-8.5
Ammonia (mg l^{-1})	0-0.5
Nitrite (mg l^{-1})	0-0.3
Nitrate (mg l^{-1})	0-20

Table 4-3. Proximate analysis of experimental broodstock diets as fed basis (means ± s.d.).

Proximate	Diets			
	LFLA	LFHA	HFLA	HFHA
Protein (%)	48.64 ± 1.71	48.96 ± 0.28	47.52 ± 0.20	48.83 ± 0.83
Lipid (%)	7.70 ± 0.03	7.78 ± 0.16	11.51 ± 0.13	12.04 ± 0.30
Moisture (%)	13.56 ± 0.11	12.18 ± 0.08	12.95 ± 0.16	12.87 ± 0.23
Ash (%)	12.51 ± 0.10	12.52 ± 0.37	12.20 ± 0.51	12.40 ± 0.06
Fiber (%)	2.83 ± 0.04	2.92 ± 0.31	2.96 ± 0.17	2.61 ± 0.11
NFE[a] (%)	14.76 ± 1.85	15.64 ± 0.16	12.86 ± 0.85	11.25 ± 0.82
Astaxanthin(mg kg^{-1})	45.58 ± 1.71	296.41 ± 2.86	46.16 ± 1.30	264.55 ± 6.00

[a]NFE = Nitrogen free extract.

The effect of dietary fish oil, astaxanthin and sex on average weight gain of shrimp is shown in **Figure 4-1**. There was no significant ($P>0.05$) interaction between fish oil and astaxanthin, and fish oil and sex on average weight gain but astaxanthin and sex had significant ($P<0.05$) interaction. The effect of fish oil on average weight gain was discussed separately, while the effect of astaxanthin and sex on average weight gain was reported together. Shrimp fed diet containing fish oil 3 and 8% had not significantly different ($P>0.05$) on average weight gain after 4 months of experiment. In shrimp fed diet supplemented with astaxanthin 100 mg kg^{-1}, female shrimp had significantly higher average weight gain ($P<0.05$) than those of male. In shrimp fed diet supplemented with astaxanthin 500 mg kg^{-1}, no significant difference ($P>0.05$) of sex was found on average weight gain. In female, there was not significant difference ($P>0.05$) among levels of astaxanthin on average weight gain, while male shrimp fed supplemented with astaxanthin 500 mg kg^{-1} had significantly greater average weight gain ($P<0.05$) than those of obtained astaxanthin 100 mg kg^{-1}.

Table 4-4. Fatty acid composition of the experimental diet and squid (means ± s.d., % of total fatty acids).

Fatty acid	Pelleted diets				Squid
	LFLA	LFHA	HFLA	HFHA	
14:0	4.1 ± 0.2	4.8 ± 0.1	3.0 ± 0.3	4.3 ± 0.2	2.2 ± 0.4
16:0	22.8 ± 1.0	23.9 ± 3	17.3 ± 2.1	22.0 ± 0.1	26.3 ± 0.6
16:1(n-7)	4.3 ± 0.3	5.2 ± 0.1	3.2 ± 0.3	4.7 ± 0.3	0.6 ± 0.1
18:0	6.0 ± 0.0	5.5 ± 1.0	6.8 ± 0.5	6.3 ± 0.2	7.7 ± 0.5
18:1(n-9)	15.7 ± 0.4	12.9 ± 1.0	14.1 ± 1.4	13.6 ± 0.1	2.8 ± 0.1
18:1(n-7)	2.7 ± 0.1	2.6 ± 0.2	3.7 ± 0.6	2.7 ± 0.1	1.3 ± 0.1
18:2(n-6)	11.9 ± 0.2	9.0 ± 0.7	9.4 ± 1.2	7.6 ± 0.1	0.2 ± 0.1
19:0	0.3 ± 0.0	0.4 ± 0.1	0.9 ± 0.2	0.4 ± 0.0	0.2 ± 0.0
18:3(n-3)	1.1 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.0	0.0 ± 0.1
18:4(n-3)	1.3 ± 0.0	1.2 ± 0.2	1.5 ± 0.1	1.4 ± 0.0	0.3 ± 0.1
20:0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	nd[a]
20:1(n-9)	0.6 ± 0.1	0.7 ± 0.0	0.9 ± 0.0	0.8 ± 0.1	1.5 ± 0.6
20:3(n-6)	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	nd
20:4(n-6)	1.4 ± 0.1	1.4 ± 0.2	1.8 ± 0.1	1.7 ± 0.3	5.3 ± 0.5
20:4(n-3)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.0 ± 0.0
20:5(n-3)	6.7 ± 0.1	6.6 ± 0.4	7.7 ± 0.2	7.1 ± 0.1	8.7 ± 0.3
21:5(n-3)	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.2	0.2 ± 0.0	0.1 ± 0.1
22:5(n-6)	0.9 ± 0.0	1.1 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	2.2 ± 0.2
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0 .0 ± 0.0	nd
22:5(n-3)	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.0	0.7 ± 0.2
22:6(n-3)	14.9 ± 0.3	16.6 ± 1.5	20.6 ± 0.3	19.2 ± 0.4	36.2 ± 0.6
Σ Saturated[b]	35.5 ± 1.3	37.8 ± 2.4	30 ± 2.1	35.4 ± 0.3	38.5 ± 0.7
Σ Monoenes	24.3 ± 0.4	23.1 ± 0.7	23.8 ± 1.3	23.0 ± 0.2	7.0 ± 0.4
Σ n-6 PUFA	14.5 ± 0.4	12.0 ± 0.5	13.4 ± 1.1	11.0 ± 0.1	8.3 ± 0.4
Σ n-3 PUFA	25.7 ± 0.5	27.1 ± 2.3	32.8 ± 0.3	30.5 ± 0.6	46.2 ± 0.7
Σ n-3 HUFA[c]	23.2 ± 0.5	25.0 ± 2.0	30.2 ± 0.3	28.1 ± 0.6	45.9 ± 0.6

[a]nd: not detected.

[b]Sums include minor fatty acids not shown in table.

[c]$\geq$20:3(n-3).
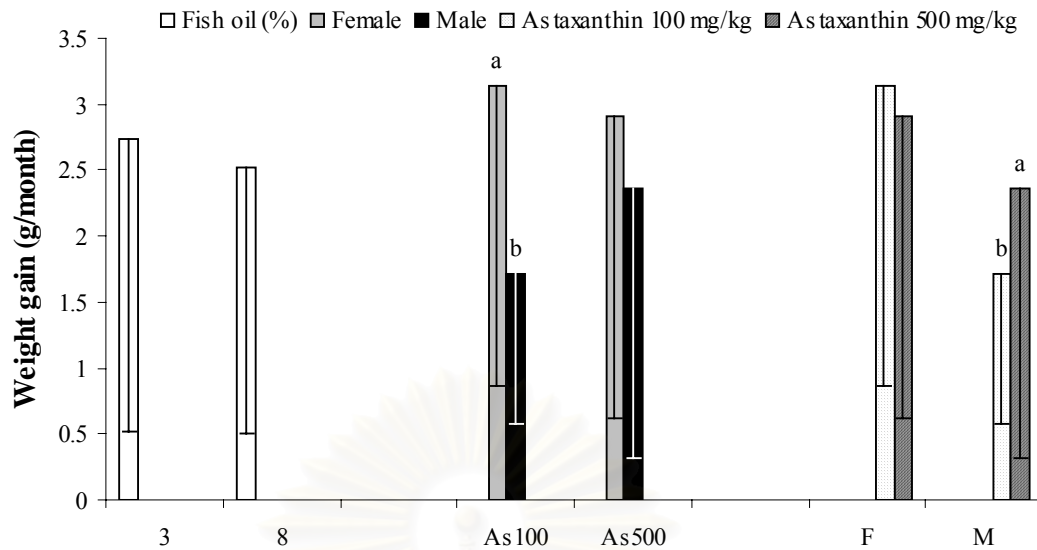
Figure 4-1. Effect of fish oil and sex on weight gain of broodstock *P. monodon*

(As100, Astaxanthin 100 mg kg^{-1}; As500, Astaxanthin 500 mg kg^{-1}; F,

Female; M, Male). The different superscripts within each group at the top of

each bar are significantly different ($P<0.05$).

Effect of fish oil and astaxanthin on amount of egg in female shrimp is shown

in **Figure 4-2**. The interaction between fish oil and astaxanthin on amount of egg had

not significant difference ($P>0.05$). Therefore, the effects of fish oil and astaxanthin

on amount of egg were discussed independently. Female shrimp fed diet containing

8% fish oil had significantly greater amount of eggs ($P<0.05$) than those fed diet

containing 3% fish oil. Female shrimp fed diet supplemented with astaxanthin 500 mg

kg^{-1} had higher amount of eggs ($P<0.05$) than those fed diet supplemented with

astaxanthin 100 mg kg^{-1}.
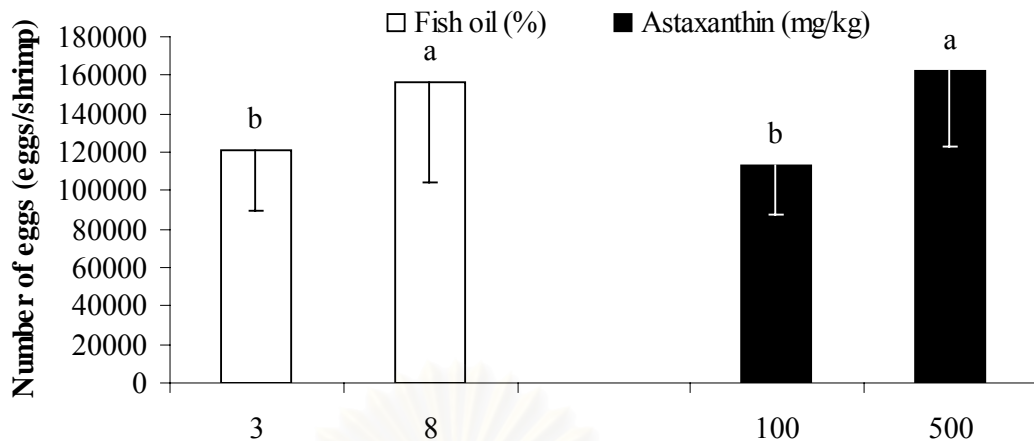
Figure 4-2. Effect of fish oil and astaxanthin on amount of egg of female broodstock *P. monodon*. The different superscripts within each group at the top of each bar are significantly different (*P*<0.05).

The effect of dietary fish oil and astaxanthin on amount of spermatozoa is shown in **Figure 4-3**. The effects of fish oil and astaxanthin on amount of spermatozoa were discussed independently because there was no significant (*P*>0.05) interaction between dietary fish oil and astaxanthin. Male shrimp fed diet containing 8% fish oil had significantly greater amount of spermatozoa (*P*<0.05) than those fed diet containing 3% fish oil. Male shrimp fed diet supplement with astaxanthin 500 mg kg^{-1} had higher amount of spermatozoa (*P*<0.05) than those fed diet supplemented with astaxanthin 100 mg kg^{-1}.
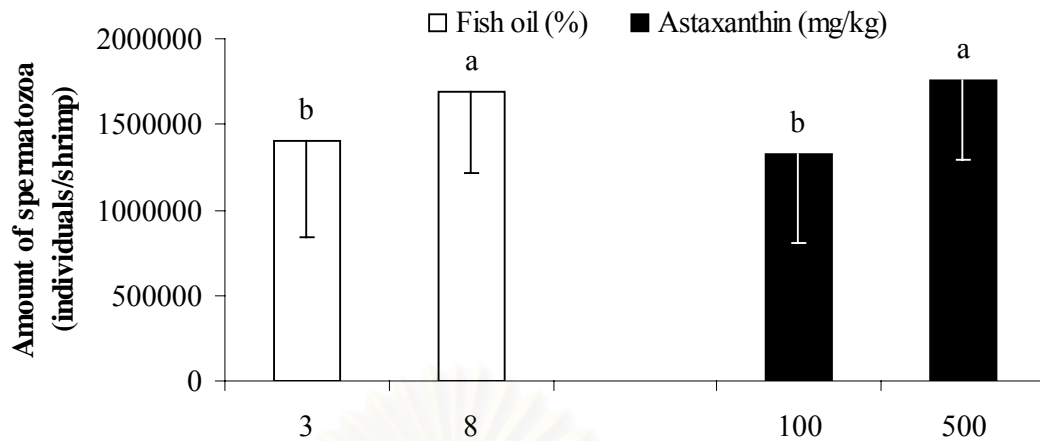
Figure 4-3. Effect of fish oil and astaxanthin on amount of spermatozoa of male broodstock *P. monodon*. The different superscripts within each group at the top of each bar are significantly different (*P*<0.05).

The effect of dietary fish oil, astaxanthin and sex on astaxanthin accumulation in muscle is presented in Figure **4-4a**. There was no significant (*P*>0.05) interaction between fish oil and astaxanthin, fish oil and sex, and astaxanthin and sex on astaxanthin accumulation in muscle. Therefore, the effects of fish oil, astaxanthin and sex on astaxanthin accumulation in muscle were discussed independently. No significant differences (*P*>0.05) among levels of astaxanthin and sex were found on astaxanthin content in muscle. Shrimp fed diet supplemented with astaxanthin 500 mg kg^{-1} had significantly higher astaxanthin content in muscle (*P*<0.05) than those fed diet supplemented with astaxanthin 100 mg kg^{-1}.

The effect of dietary fish oil, astaxanthin and sex on astaxanthin accumulation in hepatopancreas of shrimp is shown in **Figure 4-4b**. There was no significant (*P*> 0.05) interaction between fish oil and astaxanthin, and fish oil and sex on astaxanthin accumulation in hepatopancreas but interaction between astaxanthin and sex was

found significant difference ($P<0.05$). Thus, the effect of fish oil on astaxanthin accumulation in hepatopancreas was discussed individually, while the effect of astaxanthin and sex on astaxanthin accumulation in hepatopancreas was discussed together. There was not significantly different ($P>0.05$) between 2 levels of fish oil on astaxanthin content in hepatopancreas during experimental period. In shrimp fed diet supplemented with astaxanthin 100 mg kg^{-1}, no significant difference ($P>0.05$) of sex was found on astaxanthin accumulation in hepatopancreas. In shrimp fed diet supplemented with astaxanthin 500 mg kg^{-1}, female shrimp had significantly higher astaxanthin accumulation in hepatopancreas ($P<0.05$) than those of male. Female shrimp fed supplemented with astaxanthin 500 mg kg^{-1} had significantly greater astaxanthin accumulation in hepatopancreas ($P<0.05$) than those of obtained astaxanthin 100 mg kg^{-1}, while in male shrimp, there was not significant difference ($P>0.05$) among levels of astaxanthin on astaxanthin accumulation in hepatopancreas.

The effect of dietary fish oil and astaxanthin on astaxanthin accumulation in ovary is shown in **Figure 4-4c**. The effects of fish oil and astaxanthin on astaxanthin accumulation in ovary were discussed independently because there was no significant ($P>0.05$) interaction between them. No significant difference ($P>0.05$) of fish oil was found on different astaxanthin content in ovary, while female shrimp fed diet supplemented with astaxanthin 500 mg kg^{-1} had significant higher astaxanthin content in ovary ($P<0.05$) than those fed diet supplemented with 100 mg kg^{-1}.

The effect of dietary fish oil, astaxanthin and sex on astaxanthin accumulation in shell is presented in **Figure 4-4d**. There was no significant ($P>0.05$) interaction between fish oil, astaxanthin and sex on astaxanthin accumulation in the shell. Therefore, the effects of fish oil, astaxanthin and sex on astaxanthin accumulation in

shell were discussed independently. There was no significantly different (P>0.05) in levels of fish oil, astaxanthin, and sex on astaxanthin content in shell.

Fatty acid contents in muscle, hepatopancreas and ovary of female and male broodstock are shown in **Table 4-5**. The effects of fish oil, astaxanthin and sex on on fatty acid content in muscle, hepatopancreas and ovary were discussed independently because there were no significant (P>0.05) interaction between fish oil and astaxanthin, fish oil and sex, and astaxanthin and sex.

The effects of fish oil is shown in **Table 4-6**. No significant difference (P> 0.05) of dietary fish oil was found on 18:2(n-6), 18:3(n-3), and total saturated contents in muscle, hepatopancreas and ovary. Shrimp fed diet containing 8% fish oil had significant higher 20:4(n-6), 20:5(n-3), total monoenes and total n-6 PUFA contents in hepatopancreas (P<0.05) than those fed diet containing 3% fish oil. Shrimp fed diet containing 8% fish oil had significant higher 22:6(n-3), total n-3 PUFA and total n-3 HUFA contents in muscle, hepatopancreas and ovary (P<0.05) than those fed diet containing 3% fish oil.

The effect astaxanthin is shown in **Table 4-7**. There was no significant (P> 0.05) of 18:2(n-6), 18:3(n-3), 20:4(n-6), 20:5(n-3), total saturated, total monoenes and total n-6 PUFA contents in muscle, hepatopancreas and ovary on shrimp fed diet containing differed levels of astaxanthin. While, shrimp fed diet supplemented with astaxanthin 500 mg kg^{-1} had significant greater 22:6(n-3), total n-3 PUFA and total n-3 HUFA contents in muscle and ovary than those fed diet supplemented with astaxanthin 100 mg kg^{-1}.

The effect of sex on fatty acid content in muscle, hepatopancreas and ovary is shown in **Table 4-8**. There was no any significant ($P>0.05$) of all fatty acid contents in muscle, hepatopancreas and ovary between male and female shrimp.
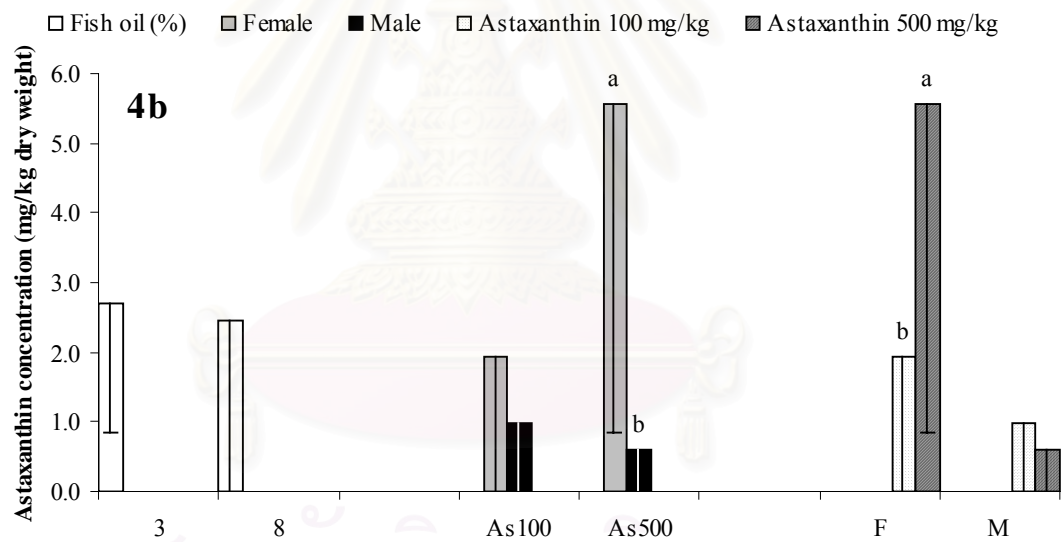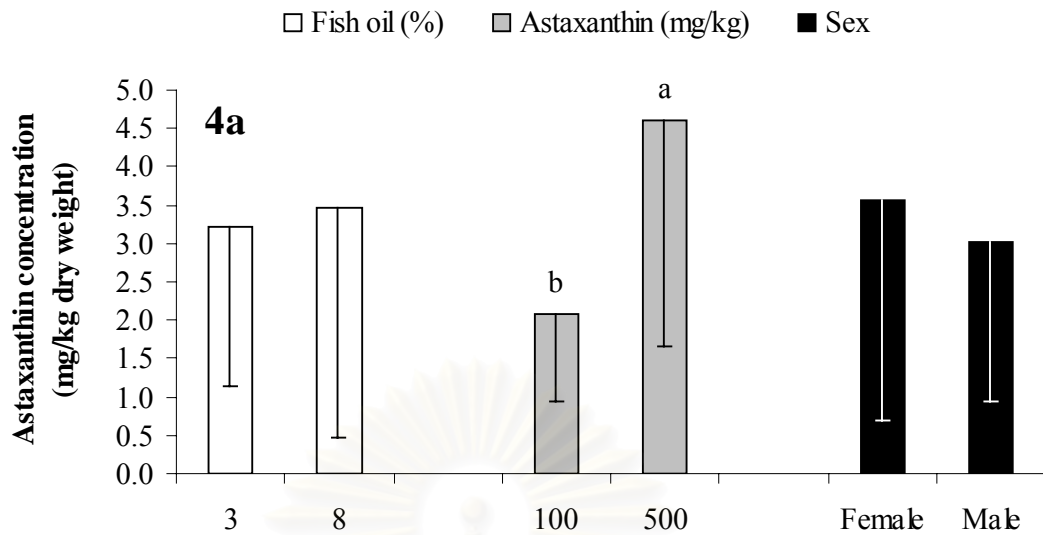
Figure 4-4. Effects of fish oil, astaxanthin and sex on astaxanthin content in muscle (4a), hepatopancreas (4b), ovary (4c) and shell (4d) of broodstock *P. monodon* (As100, Astaxanthin 100 mg kg^{-1}; As500, Astaxanthin 500 mg kg^{-1}; F, Female; M, Male). The different superscripts within each group at the top of each bar are significantly different ($P<0.05$).
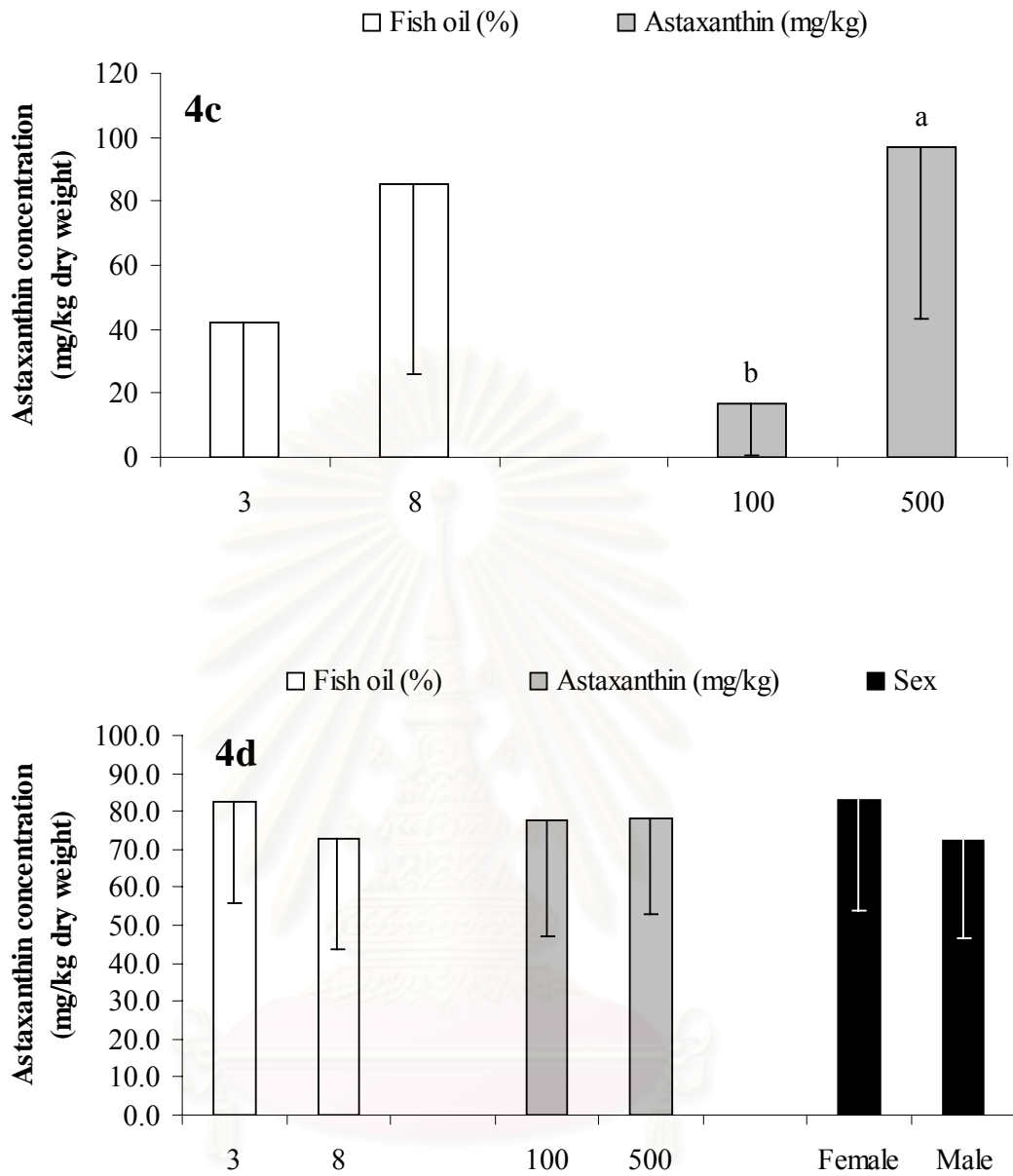
Figure 4-4. (Continued)

Table 4-5. Fatty acid composition (means ± s.d., % of total fatty acids) in muscle, hepatopancreas and ovary of female, and muscle and hepatopancreas of male broodstock *P. monodon*.

Fatty acids	Female			Male	
	Muscle	Hepatopancreas	Ovary	Muscle	Hepatopancreas
14:0	2.6 ± 2.3	1.1 ± 1.0	1.3 ± 1.6	3.6 ± 3.4	1.9 ± 2.1
16:0	36.0 ± 21.8	14.4 ± 13.9	15.1 ± 14.8	39.1 ± 23.1	24.4 ± 20.4
16:1(n-7)	4.4 ± 3.4	1.7 ± 1.8	1.6 ± 1.7	4.8 ± 3.6	3.4 ± 3.1
18:0	9.2 ± 5.1	10.5 ± 4.7	9.6 ± 3.2	8.0 ± 5.1	7.1 ± 1.7
18:1(n-9)	8.6 ± 4.8	19.0 ± 7.2	19.7 ± 5.9	7.0 ± 4.6	15 ± 7.8
18:1(n-7)	1.4 ± 0.8	4.2 ± 1.4	3.4 ± 1.1	1.1 ± 0.8	3.2 ± 1.5
18:2(n-6)	5.0 ± 3.1	7.2 ± 4.2	3.7 ± 2	4.6 ± 3.1	7.0 ± 4.3
19:0	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1
18:3(n-3)	0.3 ± 0.2	0.5 ± 0.3	0.2 ± 0.1	0.2 ± 0.2	0.6 ± 0.3
18:4(n-3)	0.5 ± 0.4	0.8 ± 0.3	0.7 ± 0.2	0.5 ± 0.3	0.8 ± 0.4
20:0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:1(n-9)	0.4 ± 0.2	2.2 ± 1.1	1.2 ± 0.4	0.3 ± 0.2	1.3 ± 0.4
20:3(n-6)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
20:4(n-6)	5.5 ± 3.0	4.9 ± 2.5	6.5 ± 2.3	4.9 ± 3.4	3.1 ± 0.8
20:4(n-3)	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.2
20:5(n-3)	9.8 ± 5.4	8.1 ± 2.5	11.3 ± 4.2	8.5 ± 5.5	5.7 ± 2
21:5(n-3)	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1
22:5(n-6)	0.5 ± 0.3	1.4 ± 0.6	1.1 ± 0.3	0.5 ± 0.4	1.2 ± 0.6
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
22:5(n-3)	0.2 ± 0.2	0.7 ± 0.6	0.5 ± 0.4	0.2 ± 0.2	1.1 ± 0.6
22:6(n-3)	10.6 ± 6.3	17.3 ± 10.1	18.2 ± 10.9	11.1 ± 7.4	18.1 ± 8.3
Σ Saturated[a]	52.1 ± 19.9	28.4 ± 14.7	28.8 ± 16	55.5 ± 22	36.6 ± 22.6
Σ Monoenes	15.5 ± 3.2	29.4 ± 8.4	28.2 ± 5.2	14.1 ± 2.6	24.7 ± 6.5
Σ n-6 PUFA	11.0 ± 6.0	14.1 ± 4.4	11.7 ± 4.0	10.0 ± 6.6	11.8 ± 5.2
Σ n-3 PUFA	21.5 ± 11.5	28.1 ± 9.7	31.4 ± 11.9	20.4 ± 13.2	26.8 ± 11.7
Σ n-3 HUFA[b]	20.7 ± 11.1	26.7 ± 9.8	30.5 ± 11.9	19.7 ± 12.8	25.4 ± 11.1

[a]Sums include minor fatty acids not shown in table.

[b]$\geq$20:3(n-3).

Table 4-6. Effect of fish oil on fatty acid content (means ± s.d., mg g^{-1} dry weight) in muscle, hepatopancreas and ovary. Mean with the different superscripts within each row indicate a significant difference ($P<0.05$).

Fatty acid	Tissue	Fish oil (%)	
		3	8
18:2(n-6)	Muscle	1.42 ± 0.28	1.33 ± 0.50
	Hepatopancreas	8.93 ± 6.49	11.42 ± 8.82
	Ovary	5.10 ± 0.8	3.10 ± 2.45
18:3(n-3)	Muscle	0.06 ± 0.05	0.07 ± 0.05
	Hepatopancreas	0.64 ± 0.52	0.97 ± 0.65
	Ovary	0.20 ± 0.08	0.26 ± 0.13
20:4(n-6)	Muscle	1.53 ± 0.32	1.55 ± 0.25
	Hepatopancreas	3.91^b ± 1.72	7.82^a ± 5.89
	Ovary	6.65 ± 1.01	6.84 ± 1.26
20:5(n-3)	Muscle	2.68 ± 0.19	2.69 ± 0.36
	Hepatopancreas	7.40^b ± 3.89	12.85^a ± 6.74
	Ovary	11.23 ± 1.45	12.34 ± 1.27
22:6(n-3)	Muscle	2.62^b ± 0.99	3.65^a ± 0.45
	Hepatopancreas	13.11^b ± 9.59	37.76^a ± 15.24
	Ovary	11.08^b ± 11.72	29.68^a ± 7.19
Σ Saturated	Muscle	27.10 ± 30.37	23.18 ± 22.48
	Hepatopancreas	39.69 ± 32.33	51.62 ± 30.94
	Ovary	43.10 ± 35.2	66.28 ± 56.24
Σ Monoenes	Muscle	5.59 ± 3.88	5.30 ± 2.39
	Hepatopancreas	30.57^b ± 20.65	49.43^a ± 19.81
	Ovary	27.98 ± 4.71	36.54 ± 12.02
Σ n-6 PUFA	Muscle	3.08 ± 0.27	3.03 ± 0.56
	Hepatopancreas	14.78^b ± 8.86	22.90^a ± 11.94
	Ovary	13.25 ± 1.75	11.7 ± 2.58
Σ n-3 PUFA	Muscle	5.58^b ± 0.95	6.65^a ± 0.64
	Hepatopancreas	23.49^b ± 12.62	55.79^a ± 21.3
	Ovary	23.98^b ± 12.57	44.24^a ± 8.57
Σ n-3 HUFA	Muscle	5.37^b ± 0.96	6.43^a ± 0.64
	Hepatopancreas	22.00^b ± 11.86	53.37^a ± 20.58
	Ovary	23.08^b ± 12.52	43.26^a ± 8.47

Table 4-7. Effect of astaxanthin on fatty acid content (means ± s.d., mg g^{-1} dry weight) in muscle, hepatopancreas and ovary. Mean with the different superscripts within each row indicate a significant difference ($P<0.05$).

Fatty acid	Tissue	Astaxanthin (mg kg^{-1})	
		100	500
18:2(n-6)	Muscle	1.43 ± 0.24	1.32 ± 0.52
	Hepatopancreas	9.63 ± 4.87	10.8 ± 9.82
	Ovary	4.68 ± 1.95	3.44 ± 2.24
18:3(n-3)	Muscle	0.08 ± 0.04	0.05 ± 0.05
	Hepatopancreas	0.77 ± 0.51	0.85 ± 0.70
	Ovary	0.25 ± 0.13	0.22 ± 0.11
20:4(n-6)	Muscle	1.59 ± 0.32	1.49 ± 0.24
	Hepatopancreas	6.99 ± 6.00	5.06 ± 3.32
	Ovary	6.52 ± 1.07	6.94 ± 1.19
20:5(n-3)	Muscle	2.65 ± 0.23	2.72 ± 0.34
	Hepatopancreas	11.01 ± 7.00	9.62 ± 5.39
	Ovary	11.52 ± 0.81	12.1 ± 1.79
22:6(n-3)	Muscle	2.86[b] ± 1.09	3.43[a] ± 0.62
	Hepatopancreas	22.53 ± 18.39	29.34 ± 17.12
	Ovary	13.67[b] ± 16.05	27.60[a] ± 6.79
Σ Saturated	Muscle	27.8 ± 28.25	22.55 ± 24.7
	Hepatopancreas	47.84 ± 34.56	44.33 ± 29.82
	Ovary	39.78 ± 32.73	68.94 ± 55.81
Σ Monoenes	Muscle	5.89 ± 3.13	5.03 ± 3.17
	Hepatopancreas	39.83 ± 18.71	41.15 ± 25.24
	Ovary	27.45 ± 5.26	36.96 ± 11.44
Σ n-6 PUFA	Muscle	3.17 ± 0.33	2.95 ± 0.51
	Hepatopancreas	19.31 ± 8.65	18.85 ± 13.37
	Ovary	12.85 ± 2.71	12.02 ± 2.1
Σ n-3 PUFA	Muscle	5.86[b] ± 1.02	6.39[a] ± 0.85
	Hepatopancreas	37.67 ± 24.16	43.11 ± 24.11
	Ovary	26.92[b] ± 17.66	41.88[a] ± 7.88
Σ n-3 HUFA	Muscle	5.61[b] ± 1.01	6.22[a] ± 0.83
	Hepatopancreas	35.71 ± 23.69	41.10 ± 22.93
	Ovary	26.00[b] ± 17.51	40.92[a] ± 7.86

Table 4-8. Effect of sex on fatty acid content (means ± s.d., mg g^{-1} dry weight) in muscle, hepatopancreas and ovary. Mean with the different superscripts within each row indicate a significant difference ($P<0.05$).

Fatty acid	Tissue	Sex	
		Female	Male
18:2(n-6)	Muscle	1.34 ± 0.46	1.43 ± 0.31
	Hepatopancreas	10.65 ± 8.92	9.54 ± 5.53
	Ovary	3.99 ± 2.09	
18:3(n-3)	Muscle	0.07 ± 0.05	0.07 ± 0.05
	Hepatopancreas	0.79 ± 0.65	0.86 ± 0.55
	Ovary	0.23 ± 0.11	
20:4(n-6)	Muscle	1.55 ± 0.26	1.52 ± 0.32
	Hepatopancreas	6.90 ± 5.74	4.33 ± 1.56
	Ovary	6.76 ± 1.09	
20:5(n-3)	Muscle	2.72 ± 0.24	2.62 ± 0.36
	Hepatopancreas	11.48 ± 6.86	8.16 ± 4.05
	Ovary	11.84 ± 1.39	
22:6(n-3)	Muscle	3.03 ± 1.05	3.40 ± 0.51
	Hepatopancreas	25.57 ± 17.92	27.09 ± 18.3
	Ovary	21.41 ± 13.17	
Σ Saturated	Muscle	28.49 ± 30.95	18.91 ± 13.42
	Hepatopancreas	46.80 ± 30.28	44.55 ± 35.38
	Ovary	55.98 ± 46.85	
Σ Monoenes	Muscle	5.72 ± 3.69	4.94 ± 1.83
	Hepatopancreas	44.05 ± 23.67	34.30 ± 18.18
	Ovary	32.73 ± 10.05	
Σ n-6 PUFA	Muscle	3.03 ± 0.48	3.08 ± 0.39
	Hepatopancreas	20.53 ± 12.56	16.47 ± 8.18
	Ovary	12.39 ± 2.27	
Σ n-3 PUFA	Muscle	6.04 ± 1.07	6.32 ± 0.74
	Hepatopancreas	41.04 ± 23.88	39.66 ± 25.02
	Ovary	35.23 ± 14.49	
Σ n-3 HUFA	Muscle	5.83 ± 1.07	6.10 ± 0.74
	Hepatopancreas	39.02 ± 23.12	37.74 ± 24.04
	Ovary	34.29 ± 14.41	

Discussion

Many reports studied potential of pond-reared shrimp broodstock for larvae production by comparing with wild-caught broodstock (Menasveta *et al*., 1993, 1994b; Sangpradub *et al*., 1994; Ramos *et al*., 1995; Cavalli *et al*., 1997; Palacios *et al*., 1999). Menasveta *et al*. (1994b) reported total eggs produced by large females was significantly greater than for small shrimp; nevertheless, shrimp source (pond-reared and wild-caught) and size had no significant influence on amount of eggs spawned per spawning event. Palacios *et al*. (1999) reported that wild-caught shrimp had higher mating and spawning frequencies compared to pond-reared broodstock. However, the number of nauplii per spawn was higher for wild shrimp, but fertilization and hatching rates were higher for pond-reared spawners.

The result of this study illustrated that fish oil could enhance reproductive performance in pond-reared broodstock shrimp. Shrimp fed diet containing high level of fish oil can produce higher amount of eggs and spermatozoa than those fed diet containing low level (**Figure 4-2** and **4-3**) and they accumulated high contents of 20:4 (n-6), 20:5(n-3) and total n-6 PUFA in hepatopancreas (**Table 4-6**). Many reports noted that n-6 PUFA, especially 20:4(n-6), is precursor in the synthesis of prostaglandins in vertebrates and insects which serve many functions in reproduction, spanwing and larval production (Middleditch *et al*., 1979; Johnson *et al*., 1983; Spaziani *et al*., 1991; Alava *et al*., 1993; Harrison, 1997).

This study found that 22:6(n-3) had the most effect to improve reproductive performance in pond-reared broodstock shrimp. The positive relationship between 22:6(n-3), total n-3 PUFA and n-3 HUFA contents in muscle, hepatopancreas and ovary, and amount of egg and spermatozoa in pond-reared broodstock shrimp was

found on this study. Shrimp fed diet supplemented with high level of fish oil had significantly higher amount of egg and spermatozoa, and 22:6(n-3), total n-3 PUFA and n-3 HUFA contents in muscle, hepatopancreas and ovary than those fed diet supplement with low level of fish oil. This was also reported by Kanazawa *et al.* (1979) and Xu *et al.* (1994) as 22:6(n-3) had more effective as an essential fatty acid than other essential fatty acids. Cerolini *et al.* (1997) and Conner *et al.* (1997) noted that n-3 and n-6 PUFA may have important roles for sperm production. The results of this study indicate that fish oil contained high contents of total n-3 PUFA and n-3 HUFA that could improve reproductive performance of pond-reared shrimp.

The current study indicated that astaxanthin could improve reproductive performance of pond-reared shrimp. Shrimp fed diet supplemented with high level of astaxanthin had significantly higher amount of egg and spermatozoa than those fed diet supplemented with low level of astaxanthin. Moreover, high astaxanthin accumulation in muscle and ovary were detected in shrimp fed diet supplemented with high level of astaxanthin (**Figure 4-4a** and **4-4c**). A similar several previous studies showed the important effects of astaxanthin on ovarian maturation (Menasveta *et al.*, 1994a; Sagi *et al.*, 1996; Pangantihon-Kuhlmann *et al.*, 1998; Ribeiro *et al.*, 2001). During ovarian maturation, crustaceans accumulate and mobilize astaxanthin from hepatopancreas to ovary via the haemolymph with lipovitellin in the oocytes (Nelson *et al.*, 1988; Quinito *et al.*, 1989; 1990; Harrison, 1990).

Astaxanthin may be promoting amount of spermatozoa in male broodstock via the provitamin A activity. Ratinoid act on the testicular development, especially on germ cell, via retinoic acid receptor and/or retinoid X receptors (Boulogne *et al.*, 1999). Akmal *et al.*(1997) reported that mutational studies of spermatid have

identified retinoic acid receptor alpha as having an essential role in spermatogenesis. Characterization of retinoic acid receptor alpha expression revealed the time and location of the vitamin A requirement during spermatogenesis.

In this study, the amounts of astaxanthin in diet supplemented with astaxanthin 100 and 500 mg kg^{-1} were 45.87 ± 1.51 and 280.48 ± 4.43 mg kg^{-1}, repectively. The analytical concentration of astaxanthin in diet supplemented with astaxanthin 500 mg kg^{-1} was less than the expected concentration due to loss of astaxanthin during feed pelleting process and feed storage.

This study found that shell was the main tissue for astaxanthin deposit and muscle and hepatopancreas were the subsequent tissues for astaxanthin deposit. Similar with reported by Negre-Sandargues et al. (1993) as dietary astaxanthin was found to be stored in the integument (carapace and epidermis) and hepatopancreas. Moreover, the all-trans-astaxanthin was found as the main form of astaxanthin in this study. The 9-cis and 13-cis astaxanthin was found minor content. This was also reported by Muriana et al. (1993) as study of astaxanthin identify using HPLC suggested all-trans-astaxanthin to be the main component, which was accompanied by an epimer and its cis-isomer.

This study was not detected the interaction between dietary fish oil and astaxanthin, and fish oil and sex, while dietary astaxanthin had interaction with sex on astaxanthin content in hepatopancreas and growth of shrimp (**Figure 4-1** and **4-4b**). Female shrimp fed diet containing high level of astaxanthin had significantly higher accumulate astaxanthin content in hepatopancreas than those fed diet containing low level of astaxanthin but male shrimp had not significantly different astaxanthin content in hepatopancreas among levels of astaxanthin supplemented in shrimp diet.

Astaxanthin had high accumulated in muscle, hepatopancreas and ovary of female

shrimp fed diet containing high astaxanthin supplementation, while it had only high

accumulated in muscle of male shrimp. This indicated that both sexes of shrimp had

different rates of astaxanthin accumulation and transfer among tissues during

reproductive maturation. Dall *et al*. (1995) reported that the maturing ovary of *P.*

esculentus contained high levels of carotenoids.

The interaction between astaxanthin and sex on weight gain indicated that

both sexes of shrimp required astaxanthin for growth at different levels. Effect of

astaxanthin on weight gain had not found in female broodstock shrimp but detected

only in male broodstock shrimp. Male shrimp fed diet containing high level of

astaxanthin had significantly greater weight gain than those fed diet containing low

level of astaxanthin. It may be that female shrimp accumulated high content of

astaxanthin during ovarian maturation. The astaxanthin content had higher than the

requirement of astaxanthin for growth. So, the study found no effect of astaxanthin on

growth of female shrimp. However, Negre-Sadargues *et al*. (1993) reported that

shrimp fed diet supplemented with astaxanthin were not significantly different on

growth rate.

The result of this study found that fish oil had not effect on growth of pond-

reared broodstock shrimp after 4 month of experiment. The average total lipids of

experimental diet supplemented with 3 and 8% fish oil were 7.74 ± 0.10 and $11.78 \pm$

0.22%, respectively. The total lipids in diets of this study found in range of lipid

requirement levels for juvenile to broodstock shrimp. In crustacean diet, the best

survival and growth responses are achieved when the dietary level of one oil or a

mixture of oils is between 5 to 8% (D'Abramo, 1997). Bray *et al*. (1990) reported that

the broodstock diet supplemented with 11.1% lipid has high nauplii production.

The result of present study found that shrimp fed diet containing high level of

astaxanthin had higher astaxanthin content in muscle and ovary than those fed diet

containing low level of astaxanthin ovary (**Figure 4-4a** and **4-4c**). The accumulated

astaxanthin in muscle and ovary had effect on accumulation of 22:6(n-3), total n-3

PUFA and total n-3 HUFA contents in muscle and ovary (**Table 4-7**). It may be that

the accumulated astaxanthin esterified to long chain fatty acids, especially 22:6(n-3).

So, the increasing of fatty acid content in muscle and ovary was found, when shrimp

had increased astaxanthin content in their tissues. Meyers and Latscha (1997) reported

that astaxanthin is present in nature either in the free form, esterified to long chain

fatty acids, or associated with proteins forming carotenoproteins. All three forms are

found in crustaceans.

In summary, dietary fish oil (HUFA) and astaxanthin can promote

reproductive performance in pond-reared broodstock *P. monodon* of both sexes. In the

current result, 20:4(n-6) and 22:6(n-3) were prominent fatty acids to promote amount

of egg and spermatozoa in pond-reared broodstock. The practical pond-reared

broodstock shrimp diet supplemented with astaxanthin 300 mg kg^{-1} and/or 12.0%

total lipid could enhance reproductive performance of broodstock shrimp *P. monodon*.

CHAPTER V

SUMMARY

1. Dietary astaxanthin could improve reproductive performance in male adolescent shrimp and improve growth in both sexes of adolescent shrimp but it had not effect on survival. Dietary astaxanthin had not induced ovarian development but could improve amount of spermatozoa in pond-reared adolescent shrimp via the provitamin A activity. This study found no interaction between dietary astaxanthin and sex found on growth, survival and astaxanthin accumulation in pond-reared adolescent shrimp *P. monodon*. Dietary astaxanthin had effect on astaxanthin content in muscle and hepatopancreas of adolescent shrimp. The first study suggested that supplementation of astaxanthin to adolescent *P. monodon* diet could enhance reproductive performance in pond-reared adolescent male shrimp.

2. Dietary fish oil and astaxanthin could enhance reproductive performance in both sexes of pond-reared broodstock shrimp. Fish oil had not effect on growth rate of pond-reared broodstock shrimp, while astaxanthin had only effect on growth rate of male. Dietary astaxanthin had effect on astaxanthin content in muscle of male shrimp and had effect on astaxanthin content in muscle, hepatopancreas and ovary of female shrimp but fish oil had not effect on astaxanthin content in all tissues of pond-reared broodstock shrimp. The interaction between fish oil, astaxanthin and sex was not found on fatty acid content in muscle, hepatopancreas and ovary of broodstock shrimp. Dietary fish oil had effect on 20:4(n-6), 20:5(n-3), total monoenes and total n-6 PUFA contents in hepatopancreas and had effect on 22:6(n-3), total n-3 PUFA and total n-3 HUFA contents in muscle, hepatopancreas and ovary of broodstock shrimp,

while dietary astaxanthin had effect on 22:6(n-3), total n-3 PUFA and total n-3 HUFA contents in muscle and ovary of broodstock shrimp. The second study indicated that the supplementation of 12.0% total lipid and/or 300 mg kg^{-1} astaxanthin in practical broodstock shrimp diet could promote reproductive performance of pond-reared broodstock shrimp *P. monodon*.

This study demonstrated the potential dietary fish oil and astaxanthin to improve reproductive performance of pond-reared shrimp *P. monodon* and can be directly applied to aquacultural activity and to further studies. However, to increase the effectiveness, the feed pelleting process could be improved for reduction of astaxanthin loss during preparing pelleted diet.

REFERENCES

Akmal, K.M., Dufour, J.M. and Kim K.H. 1997. Retinoic acid receptor alpha gene expression in the rat testis: Potential role during the prophase of moiosis and in the transition from round to elongation spermatids. Biology of Reproduction 56: 549-556.

Alava, V.R., Kanazawa, A., Teshima, S.I. and Koshio, S. 1993. Effect of dietary phospholipids and n-3 highly unsaturated fatty acids on ovarian development of Kuruma prawns. Bulletin of the Japanese Society of Scientific Fisheries 59: 345-351.

AQUACOP. 1977. Reproduction in captivity and growth of *Penaeus monodon* Fabricius in Polynesia. Proc. World Maricul. Soc. 8: 927-945.

Association of Official Analytical Chemists. 1995. Official methods analysis. Washington: Association of Official Analytical Chemists.

Bagarinao, T.U., Solis, N.B., Villaver, W.R., and Villaluz, A.C. 1986. Important fish and shrimp fry in Philippine coastal waters: identification, collection and handling. Aquaculture Extension Manual No. 10. SEAFDEC Aquaculture Department. 52 p.

Beard, T.W. and Wickens, J.F. 1980. Breeding of *Penaeus monodon* Fabricius in laboratory recirculation systems. Aquaculture 20: 79-89.

Boonyaratpalin, M., Thongrod, S., Supamattaya, K, Britton, G. and Schlipalius, L.E. 2001. Effect of β-carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. Aquaculture Research 32: 182-190.

Boulogne, B., Levacher, C., Durand, P. and Habert, R. 1999. Retinoic acid receptors and retinoid X receptors in the rat testis during fetal and postnatal

development: Immonolocalization and implication in the control of the number of gonocytes. Biology of Reproduction 61: 1548-1557.

Bray, W.A., Lawrence, A.L. and Lester, L.J. 1990. Reproduction of eyestalk-ablated *Penaeus stylirostris* fed various levels of total dietary lipid. J. World Aquacul. Soc. 21: 41-52.

Bray, W.A. and Lawrence, A.L. 1992. Reproduction of Penaeus species in captivity. In Fast, A.W. and Lester, J.L. (eds.), Marine shrimp culture: principles and practices. Amsterdam: Elsevier Science Publishers. pp. 93-170.

Bray, W.A. and Lawrence, A.L. 1998. Successful reproduction of *Penaeus monodon* following hypersaline culture. Aquaculture 159: 275-282.

Cahu, C., Guillaume, J.C., Stephan, G. and Chim, L. 1994. Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg and tissue composition in *Penaeus vannamei* fed semi-purified diets. Aquaculture 126: 159-170.

Castell, J.D. and Boghen, A.D. 1979. Fatty acid metabolism in juvenile lobsters (*Homarus americanus*) fed a diet low in methionine and histidine. Proceedings of the World Mariculture Society 10: 720-727.

Castillo, R. and Negre-Sadargues, G. 1991. Effect of eye stalk ablation on the metabolism of carotenoid pigments of the hermit crab *Clibanarius erythropus* Lalreille (1818) (Crustacea Decapoda Anoroura). Comparative Biochemistry and Physiology 99B: 187-192.

Cavalli, R.O., Scardua, M.P. and Wasielesky, W.Jr. 1997. Reproductive performance of different sized wild and pond-reared *Penaeus paulensis* females. Journal of the World Aquaculture Society 28: 260-267.

Cerolini, S., Surai, P., Maldjian, A., Gliozzi, T. and Noble, R. 1997. Lipid composition of semen in different fowl breeders. Poultry and Avian Biology Reviews 8: 141-148.

Chanmugam, P., Donovan, J., Wheeler, C.J. and Hwang, D.H. 1983. Differences in the lipid composition of freshwater prawn (*Macrobrachium rosenbergii*) and marine shrimp. J. Food Science 48: 1440-1443.

Chessman, D.F., Lee, W.L. and Zogalsly, P.F. 1967. Carotenoproteins in invertebrates. Biological Review 42: 131-158.

Chien, Y.H. and Jeng, S.C. 1992. Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levels and feeding regimes. Aquaculture 102: 333-346.

Christie, W.W. 1989. Gas chromatography and lipids: a practical guide. The Oily Press, UK: Ayr.

Conner, W.E., Lin, D.S. and Neuringer, M. 1997. Biochemical markers for puberty in the monkey testis: Desmosterol and docosahexaenoic acid. Journal of Clinical Endocrinology and Metabolism 82: 1911-1916.

Cody, R.P. and Smith, J.K. 1997. Applied statistics and the SAS programming language. New Jersey: Simon & Schuster / A Viacom Company. 445 p.

D'Abramo, L.R., Baum, N.A., Bordner, C.E. and Conklin, D.E. 1983. Carotenoids as a source of pigmentation in juvenile lobsters fed a purified diet. Canadian Journal of Fisheries and Aquatic Sciences 40: 699-704.

D'Abramo, L.R. 1997. Triacylglycerols and fatty acids. In D'Abramo, L.R., Conklin, D.E. and Akiyama, D.M. (eds.), Crustacean Nutrition. Louisiana: The World Aquaculture Society. pp. 71-84.

Dall, W., Smith, D.M. and Moore, L.E. 1995. Carotenoids in the tiger prawn *Peanaeus esculentus* during ovarian maturation. Marine Biology 123: 435-441.

Dall, W. 1995. Carotenoids versus retenoids (vitamin A) as essential growth factors in penaeid prawns (*Penaeus semisulcatus*). Marine Biology 124: 209-213.

Desy, M., Mantiri, H., Negre-Sandargues, G., Castillo, R. and Trilles J.P. 1995. Evolution of carotenoid metabolic capabilities during the early development of the european lobster *Homarus gammarus* (Linne 1758). Comp. Biochem. Physiol. IIIB: 553-558.

Emmerson, W.D. 1980. Induced maturation of prawn *Penaeus indicus*. Mar. Ecol. Prog. Ser. 2: 121-131.

Fisher, L.R., Kon, S.K. and Thompson, S.Y. 1955. Vitamin A and carotenoids in certain invertebrates. III Euphausiacea. Journal of the Marine Biological Association 34: 81-100.

Fisher, L.R., Kon, S.K. and Thompson, S.Y. 1964. Vitamin A and carotenoids in certain invertebrates. VII Crustacean: Copepoda. Journal of the Marine Biological Association 44: 685-692.

Flegel, T.W. and Alday-Sanz, V. 1998. The crisis in Asian shrimp aquaculture: current status and future needs. Journal of Applied Ichthyology 14: 269-273.

Folch, J., Lees, M. and Sloane-Stanley, G.M. 1957. A simple method for the isolation and purification of total lipid from animal tissue. J. Biol. Chem. 226: 497-509.

Foss, P., Renstrom, B. and Liaasen-Jensen, S. 1987. Natural occurrence of enantiometric and meso astaxanthin 7-crustaceans including zooplankton. Comparative Biochemistry and Physiology 86B: 313-314.

Goodwin, T.W. 1960. Biochemistry of pigments. In Waterman, T.H. (ed.), The physiology of crustacea, Vol. 1. Metabolism and growth. London: Academic Press. pp. 101-140.

Goodwin, T.W. 1984. The biochemistry of the carotenoids. Vol II. Animals. London: Chapman and Hall.

Gurr, M.I. and Harwood, J.L., 1991. Lipid biochemistry. London: Chapmand and Hall. 406 p.

Harrison, K.E. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. Journal of Shellfish Research 9: 1-28.

Harrison, K.E. 1997. Broodstock nutrition and maturation diets. In D'Abramo, L.R., Conklin, D.E. and Akiyama, D.M. (eds.), Crustacean Nutrition. Louisiana: The World Aquaculture Society. pp. 390-408.

Hoang, T., Lee, S.Y., Keenan, C.P. and Marsden, G.E. 2002a. Effect of light intensity on maturation and spawning of ablated female *Penaeus merguiensis*. Aquaculture 209: 347-358.

Hoang, T., Lee, S.Y., Keenan, C.P. and Marsden, G.E. 2002b. Observations on growth, sexual maturity and spawning performance of pond-reared *Penaeus merguiensis*. Aquaculture Research 33: 863-873.

Johnson, M., Carey, F. and McMillan, R.M. 1983. Alternate pathways of arachidonate metabolism: prostaglandins, thromboxane, and leukotrienes. In Cambell, P.N. and Marshall, R.D. (eds.), Essays in biochemistry, Vol. 19. New York: Academic Press. pp. 40-141.

Kanazawa, A., Teshima, S., Tokiwa, S., Kayama, M. and Hirata, M. 1979. Essential fatty acids in the diet of prawn. II. Effects of docosahexaenoic acid on growth. Bull. Jpn. Soc. Sci. Fish. 45: 1151-1153.

Katagiri, K., Koshimo, Y., Maoki, T. and Matsuno, T. 1987. Occurrence of pirardixanthin derivatives in the prawn *Penaeus japonicus*. Comparative Biochemistry and Physiology 87B: 161-163.

Katayama, T., Hirata, K. and Chichester, C.O. 1971. The biosynthesis of astaxanthin-IV. The carotenoids in the prawn *Penaeus japonicus* Bate (Part I). Bull. Japan. Soc. Sci. Fish. 37: 614-620.

Katayama, T., Katama, T. and Chichester, C.O. 1972a. The biosynthesis of astaxanthin in the prawn *Penaeus japonicus* Bate (Part II). International Journal of Biochemistry 3: 363-368.

Katayama, T., Katama, T., Shimaya, M., Deshimaru, O. and Chichester, C.O. 1972b. The biosynthesis of astaxanthin-VIII. The conversion of labelled β-carotene-15,15'-3H2 into astaxanthin in prawn, *Penaeus japonicus* Bate. Bull. Japan. Soc. Sci. Fish. 37: 614-620.

Katsuyama, M., Komari, T. and Matsuno, T. 1987. Metabolism of three stereoisomers of astaxanthin in the fish, rainbow trout and tilapia. Comparative Biochemistry and Physiology 86B: 1-5.

Kayama, M., Hirata, M., Kanazawa, A., Tokiwa, S. and Saito, M. 1980. Essential fatty acids in the diet of prawn III. Lipid metabolism and fatty acid composition. Bull. Japan. Soc. Sci. Fish. 46: 483-488.

King, J.E. 1948. A study of the reproductive organs of the common marine shrimp, *Penaeus setiferus* (Linnaeus). Biol. Bull. 94: 244-262.

Kleppel, G.S., Willbanks, L. and Pieper, R.E. 1985. Diet variations in body carotenoid content and feeding activity in marine zooplankton assemblages. Journal of Plankton Research 7: 569-580.

Kornhauser, A., Wamer, W.G., Lambert, L.A. and Wei, R.R. 1994. β-carotene inhibition of chemically induced toxicity in vivo and in vitro. Food and Chemical Toxicology 32: 149-154.

Latscha, T. 1990. Carotenoids in animal nutrition: their nature and significance in animal feeds. Roche Publication No. 2175, F. Hoffmann-La Roche, Animal Nutrition and Health, Basel, Switzerland.

Latscha, T. 1991. Carotenoids in aquatic animal nutrition. In Akiyama, D.M. and Tan, R.K.H. (eds.), Proceedings of the aquaculture feed processing and nutrition workshop, Thailand and Indonesia. pp. 68-79.

Lepage, G. and Roy, C.C. 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. J. Lip. Res. 25: 1391-1396.

Leung-Trujillo, J.R. and Lawrence, A.L. 1987. Observations on the decline in sperm quality of Penaeus setiferus under laboratory conditions. Aquaculture, 65: 363-370.

Lin, M.-N. and Ting, Y.-Y. 1986. Spermatophore transplantation and artificial fertilization in grass shrimp. Bull. Japan. Soc. Sci. Fish. 52: 585-589.

Lytle, J.S., Lytle, T.F. and Ogle, J.T. 1990. Polyunsaturated fatty acid profiles as a comparative tool in assessing maturation diets of Penaeus vannamei. Aquaculture 89: 287-299.

Makinouchi, S. and Honculada-Primavera, J. 1987. Maturation and spawning of Penaeus indicus using different ablation methods. Aquaculture 62: 73-86.

Marte, C.L. 1980. The food and feeding habit of Penaeus monodon Fabricius (Decapoda, Natantia) collected from Makato River, Aklan, Philippines. Crustaceana 38: 225-236.

Marte, C.L. 1982. Seasonal variation in food and feeding of *Penaues monodon* Fabricius (Decapoda, Natantia). <u>Crustaceana</u> 42: 250-255.

Martosubroto, P. 1974. Fecundity of pink shrimp *Penaeus duorarum* Burkenroad. <u>Bull. Mar. Sci</u>. 24: 606-627.

Maugle, P., Kamata, T., McLeon, S., Simpson, K.L. and Katayama, T. 1980. The influence of eyestalk ablation on the carotenoid composition of juvenile *Macrobrachium rosenbergii*. <u>Bulletin of the Japanese Society of Scientific Fisheries</u> 46: 901-904.

Menasveta, P., Choosuwan, J., Piyatiratitivorakul, S., Fast, A.W. and Latscha, T. 1994a. Effect of dietary astaxanthin on gonadal maturation and spawning of giant tiger prawn, (*Penaeus monodon* Fabricius). In Chou, L.M., Munro, A.D., Chen, T.W., Cheong, L.K.K., Ding, J.K., Hooi, K.K., Khoo, H.W., Phang, V.P.E., Shim, K.F. and Tan, C.H. (eds.). <u>The third asian fisheries forum</u>. Asian Fisheries Society, Manila, Philippines. pp. 713-716.

Menasveta, P., Piyatiratitivorakul, S., Rungsupa, S., Moree, N. and Fast, A.W. 1993. Gonadal maturation and reproductive performance of giant tiger prawn (*Penaeus monodon* Fabricius) from the Andaman Sea and pond-reared sources in Thailand. <u>Aquaculture</u> 116: 191-198.

Menasveta, P., Sangpradub, S., Piyatiratitivorakul, S. and Fast, A.W. 1994b. Effects of broodstock size and source on ovarian maturation and spawning of *Penaeus monodon* Fabricius from the Gulf of Thailand. <u>Journal of the World Aquaculture Society</u> 25: 41-49.

Menasveta, P. 1982. Improvement of the closed recirculating water system for giant prawn (*Macrobrachium rosenbergii*) larviculture. In <u>Technical report of the working party on small-scale shrimp/prawn hatcheries in southeast Asia</u>. FAO/UNDP South China Sea Development and Coordinating programme, SCS/GEN/82/40. pp. 95-99.

Meyers, S.P. and Chen, H.M. 1982. Astaxanthin and its role in fish culture. In Stickney, R.R. and Meyers, S.P. (eds.), <u>Warmwater fish culture workshop</u>. World Aquaculture Society Special Publication No. 3, World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 153-165.

Meyers, S.P. and Latscha, T. 1997. Carotenoids. In D'Abramo, L.R., Conklin, D.E. and Akiyama, D.M. (eds.), <u>Crustacean Nutrition</u>. Louisiana: The World Aquaculture Society. pp. 164-193.

Middleditch, B.S., Missler, S.R., Hines, H.B. and Lawrence, A.L. 1980. Metabolic profiles of penaeid shrimp: dietary lipids and ovarian maturation. <u>Journal of Chromatography</u> 195: 359-368.

Middleditch, B.S., Missler, S.R., Ward, D.G., McVey, J.B., Brown, A. and Lawrence, A.L. 1979. Maturation of penaeid shrimp: dietary fatty acids. <u>Proceedings of the World Mariculture Society</u> 10: 472-476.

Miki, W. 1991. Biological functions and activities of animal carotenoids. <u>Pure and Applied Chemistry</u> 63: 141-146.

Millamena, O.M. 1989. Effect of fatty acid composition of broodstock diet on tissue fatty acid patterns and egg fertilisation and hatching in pond-reared *Penaeus monodon*. <u>Asian Fish. Sci.</u> 2: 127-134.

Millamena, O.M., Pudadera, R.A. and Catacutan, M.R. 1985. Variations in tissue lipid content and fatty acid composition during maturation of unablated and ablated *Penaeus monodon* Fabricius broodstock. In Taki, Y., Primavera, J.H., Llobrera, J.A. (eds.), <u>Proceedings of the first international conference on the culture of penaeid prawns/shrimps</u>, Philippines: Iloilo City. 166 p.

Motoh, H. 1981. Studies on the fishery biology of giant tiger prawn (*Penaeus monodon*) in the Philippines. <u>SEAFDEC Aquaculture Tech. Rep. No. 7</u>.

Muriana, F.J.G., Ruizgutierrez,V., Gallardoguerrero, M.L. and Minguezmosquera, M.I. 1993. A study of the lipids and carotenoprotein in the prawn, *Penaeus japonicus*. Journal of Biochemistry 114: 223-229.

Negre-Sadargues, G., Castillo, R., Petit, H., Sance, S., Gomez-Martinez, R., Milicua, J-C.G., Choubert, G. and Trilles, J-P. 1993. Utilization of synthetic carotenoids by the prawn *Penaeus japonicus* reared under laboratory conditions. Aquaculture, 110: 151-159.

Nelson, K., Heyer, B. and Johnson, E. 1988. Photoperiod-induced changes in female lobsters (*Homarus americanus*). Comp. Biochem. Physiol. B 90: 809-821.

Okada, S., Nur-E.-Bohan, S.A. and Yamaguchi, K.Y. 1994. Carotenoid composition in the exoskeleton of commercial black tiger prawn. Fish. Sci. 60: 213-215.

Otta, S.K., Shubha, G., Joseph, B., Chakraborty, A., Karunasagar, I. and Karunasagar, I. 1999. Polymerase chain reaction (PCR) detection of white spot syndrome virus (WSSV) in cultured and wild crustaceans in India. Diseases of Aquatic Organisms 38: 67-70.

Palacios, E., Racotta, I.S. and Acuacultores de la Paz. 1999. Spawning frequency analysis of wild and pond-reared Pacific white shrimp *Penaeus vannamei* broodstock under large-scale hatchery conditions. Journal of the World Aquaculture Society 30: 180-191.

Palozza, P. and Krinsky, I. 1992. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Archives of Biochemistry and Biophysiology 297: 291-195.

Pan, C.H., Chien, Y.H. and Cheng, J.H. 2001. Effects of light regime, algae in the water, and dietary astaxanthin on pigmentation, growth and survival of black tiger prawn *Penaeus monodon* post-larvae. Zoological Studies 40: 371-382.

Pangantihon-Kuhlmann, M.P., Millamena, O. and Chern, Y. 1998. Effect of dietary astaxanthin and vitamin A on the reproductive performance of *Penaeus monodon* broodstock. Aquat. Living Resour. 11: 403-409.

Petit, H., NegreSadargues, G., Castillo, R. and Trilles, JP. 1997. The effects of dietary astaxanthin on growth and molting cycle of postlarvae stages of the prawn, *Penaeus japonicus* (Crustacea, Decapoda). Comp. Biochem. Physiol. A 117: 539-544.

Petit, H., Sance, S., Negre-Sadargues,G., Castillo,R. and Trilles, J.P. 1991. Ontogeny of carotenoid metabolism in the prawn *Penaeus japonicus* Bate 1888 (Crustacea Penaeidae): a qualitative approach. Comparative Biochemistry and Physiology 99B: 667-671.

Primavera, J.H. 1978. Induced maturation and spawning in five-month-old *Penaeus monodon* Fabricius by eyestalk ablation. Aqauculture 13: 355-359.

Primavera, J.H. 1979. Notes on the courtship and mating behavior in *Penaeus monodon* Fabricius (Decapoda: Natantia). Crustaceana 37: 287-292.

Primavera, J.H. 1988. Maturation, reproduction and broodstock technology. In Aquaculture Department of the Southeast Asian Fisheries Development Center (ed.), Biology and culture of *Penaeus monodon*. pp. 37-58.

Quinito, E.T., Hara, A., Yamauchi, K., Mizushima, T. and Fuji, A. 1989. Identification and characterization of vitellin in a hermaphrodite shrimp, *Pandalus kessleri*. Comp. Biochem. Physiol. B 94: 445-451.

Quinito, E.T., Hara, A., Yamauchi, K. and Fuji, A. 1990. Isolation and characterization of vitellin from the ovary of *Penaeus monodon*. Invertebrate Reproduction and Development 17: 221-227.

Ramos, L., Espejo, M., Samada, S. and Perez, L. 1995. Maturation and reproduction of pond-reared *Penaeus schmitti*. Journal of the World Aquaculture Society 26: 183-187.

Ranby, B. and Rabek, J.F. 1978. Signlet oxygen. Wiley, Chichester, England.

Ribeiro, E.A., Genofre, G.C. and McNamara, J.C. 2001. Identification and quantification of carotenoid pigments during the embryonic development of the freshwater shrimp *Macrobrachium olfersii* (Crustacea, Decapoda). Marine and Freshwater Behaviour and Physiology 34: 105-116.

Sagi, A., Shoukrun, R., Khalaila, I. and Rise, M. 1991. Gonad maturation, morphological and physiological changes during the first reproductive cycle of the crayfish *Cherax quadricarinatus* female. Invertebrate Reproduction & Development 29: 235-242.

Sandifer, P.A., Lawrence, A.L., Harris, S.G., Chamberlain, G.W., Stokes, A.D. and Bray, W.A. 1984. Electrical stimulation of spermatophore expulsion in marine shrimp, *Penaeus* spp. Aquaculture 41: 181-187.

Sangpradub, S., Fast, A.W., Piyatiratitivorakul, S. and Menasveta, P. 1994. Effects of different feeding regimes on ovarian maturation and spawning of pond-reared giant tiger prawn in Thailand. Aquaculture International 2: 49-58.

Simpson, K.L., Katayama, T. and Chichester, C.O. 1981. In Bauernfeind, J.C. (ed), Carotenoids as colorants and vitamin A precursors. New York: Academic Press. pp. 463-538.

Solis, N.B. 1988. Biology and ecology. In Aquaculture Department of the Southeast Asian Fisheries Development Center (ed.), Biology and culture of *Penaeus monodon*. pp. 3-36.

Spaziani, E.P., Hinsch, G.W. and Edwards, S.C. 1991. Possible role of prostaglandin F$_{2a}$ in vitellogenesis in the crayfish (*Procambarus paeninsulanis*). <u>American Zoologist</u> 31: 23A.

Tacon, A.G.J. 1990. <u>Standard methods for the nutrition and feeding of farmed fish and shrimp. Volume I: the essential nutrients</u>. Washington: Argent Laboratories Press.

Tanaka, Y., Matsuguchi, H., Katayama, T., Simpson, K.L. and Chichester, C.O. 1976. The biosynthesis of astaxanthin-XVI. The carotenoids in crustacea. <u>Comparative Biochemistry and Physiology</u> 54B: 391-393.

Tan-Fermin, J.D. and Pudadera, R.A. 1989. Ovarian maturation stages of the wild giant tiger prawn, *Penaeus monodon* Fabricius. <u>Aquaculture</u> 77: 229-242.

Terao, O. 1989. Antioxidant activity of β-carotene related carotenoids in solution. <u>Lipids</u> 24: 659-661.

Teshima, S., Kanazawa, A., Horinouchi, K. and Koshio, S. 1988a. Lipid metabalism in destalked prawn *Penaeus japonicus*: induced maturation and transfer of lipid reserves to the ovaries. <u>Nippon Suisan Gakkaishi</u> 54: 1123-1129.

Teshima, S., Kanazawa, A., Koshio, S. and Horinouchi, K. 1988b. Lipid metabolism in destalked prawn *Penaeus japonicus*: induced maturation and accumulation of lipids in the ovaries. <u>Nippon Suisan Gakkaishi</u> 54: 1115-1122.

Thongrod, S., Tansutapanich, A. and Torrissen, O.J. 1995. Effect of dietary astaxanthin supplementation on accumulation, survival and growth in postlarvae of *Penaeus monodon* Fabricius. In Lavens, P., Jaspers, E. and Roelants, I. (eds.), <u>Larvi '95 – fish & shellfish larviculture symposium</u>. European Aquaculture Society, Special Publication No.24, Gent, Belgium. pp. 251-254.

Torrissen, O.J. 1990. Biological activities of carotenoids in fishes. The current status of fish nutrients in aquaculture. In Takeda, M. and Watanabe T. (eds.), <u>Proceedings of the third international symposium on feeding and nutrition in fish</u>. Tokyo University of Fisheries, Tokyo, Japan. pp. 387-399.

Torrissen, O.J., Hardy, R.W. and Shearer, K.D. 1989. Pigmentation of salmonids: Carotenoid deposition and metabolism. <u>Reviews in Aquatic Science</u> 1: 209-225.

Trujillo, J.R. 1990. <u>Male reproduction in penaeid shrimp: sperm quality and spermatophore production in wild and captive populations</u>. M.S. thesis, Department of Wildlife and Fisheries Sciences, Texas A&M University, TX, USA, p. 91.

Ways, P. and Hanahan, D.J. 1964. Characterization and quantification of red cell lipids in normal man. <u>J. Lipid Res.</u> 5: 318-328.

Weber, S. 1988. Determination of stablilized, added astaxanthin in fish feeds and premixes with HPLC. In Keller, H.E. (ed.), <u>Analytical methods for vitamins and carotenoids in feed</u>, Roche-Publication, No. 2264. pp59-61.

Wouters, R., Gomez, L., Lavens, P. and Calderon, J. 1999. Feeding enriched Artemia biomass to *Penaeus vannamei* broodstock: Its effect on reproductive performance and larval quality. <u>Journal of Shellfish Research</u> 18: 651-656.

Wyban, J., Martinez, G. and Sweeney, J. 1995. Nauplii quality effect of paprika in Penaeus vannamei maturation diet. <u>Fifth international working group on crustacean nutrition symposium</u>. Kagoshima University, Kagoshima, Japan.

Xu, X.L., Ji, W.J., Castell, J.D. and O'Dor, R.K. 1992. Influence of dietary lipid sources on fecundity egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock. <u>Aquaculture</u> 119: 359-370.

Xu, X.L., Ji, W.J., Castell, J.D. and O'Dor, R.K. 1994. Effect of dietary lipids on fecundity, hatchability and egg fatty acid composition of Chinese prawn (*Penaeus chinensis*). Marine Fisheries Research 13: 13-19.

Xu, X.L., Ji, W.J., Castell, J.D. and O'Dor, R.K. 1994. Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*. Aquaculture 127: 29-40.

Yamada, S., Tanaka, Y., Sameshima, M. and Ito, Y. 1990. Pigmentation of prawn (*Penaeus japonicus*) with carotenoids. I. Effect of dietary astaxanthin, beta-carotene and canthaxanthin on pigmentation. Aquaculture 87: 323-330.

Yano, I. 1995. Final oocyte maturation, spawning and mating in penaeid shrimp. Journal of Experimental Marine Biology and Ecology 193: 113-118.

APPENDICES

APPENDIX A

ORIGINAL DATA FROM EXPERIMENT IN CHAPTER III

Table 1A. Growth of adolescent shrimp *P. monodon* during 4 months of experiment.

TRT	REP	MONTH	SEX	WEIGHT
1	1	0	F	24
1	1	0	F	25
1	1	0	F	22
1	1	0	M	22
1	1	0	M	23
1	2	0	F	18
1	2	0	F	26
1	2	0	F	18
1	2	0	M	14
1	2	0	M	16
1	3	0	F	20
1	3	0	F	22
1	3	0	F	24
1	3	0	M	20
1	3	0	M	24
1	4	0	F	12
1	4	0	F	28
1	4	0	F	25
1	4	0	M	18
1	4	0	M	26
2	1	0	F	28
2	1	0	F	18
2	1	0	F	25
2	1	0	M	27
2	1	0	M	16
2	2	0	F	25
2	2	0	F	19
2	2	0	F	19
2	2	0	M	25
2	2	0	M	25
2	3	0	F	22
2	3	0	F	22
2	3	0	F	25
2	3	0	M	18
2	3	0	M	18
2	4	0	F	28
2	4	0	F	20
2	4	0	F	24
2	4	0	M	24
2	4	0	M	27
3	1	0	F	30
3	1	0	F	20
3	1	0	F	16
3	1	0	M	23
3	1	0	M	30
3	2	0	F	27
3	2	0	F	21

Table 1A. (Continued)

```
------------------------------
TRT  REP  MONTH  SEX  WEIGHT
------------------------------
3    2    0      F    24
3    2    0      M    22
3    2    0      M    16
3    3    0      F    27
3    3    0      F    20
3    3    0      F    18
3    3    0      M    25
3    3    0      M    19
3    4    0      F    28
3    4    0      F    11
3    4    0      F    22
3    4    0      M    16
3    4    0      M    18
1    1    1      F    24.35
1    1    1      F    26.45
1    1    1      F    22.4
1    1    1      M    23.7
1    1    1      M    23.85
1    2    1      F    19.8
1    2    1      F    26.7
1    2    1      M    16.55
1    3    1      F    25
1    3    1      M    21.6
1    3    1      M    27.45
1    4    1      F    28.75
1    4    1      F    26.5
1    4    1      M    22.85
1    4    1      M    21.5
2    1    1      F    31.65
2    1    1      F    21.55
2    1    1      F    26.6
2    1    1      M    27.5
2    2    1      F    23.4
2    2    1      F    23.75
2    2    1      F    24.05
2    2    1      M    25.8
2    2    1      M    25.35
2    3    1      F    22.8
2    3    1      F    25.55
2    3    1      F    23.85
2    3    1      M    18.95
2    4    1      F    21.25
2    4    1      F    21.8
2    4    1      F    25.25
2    4    1      M    25.55
2    4    1      M    27.65
3    1    1      F    28.8
3    1    1      F    19.25
3    1    1      M    21.9
3    1    1      M    27.55
3    2    1      F    27.6
3    2    1      F    23.2
3    2    1      M    23.05
3    2    1      M    18.75
3    3    1      F    28
------------------------------
```

Table 1A. (Continued)

TRT	REP	MONTH	SEX	WEIGHT
3	3	1	F	20.35
3	3	1	F	22.55
3	3	1	M	29.6
3	3	1	M	25.15
3	4	1	F	27.9
3	4	1	F	18.2
3	4	1	F	25.5
3	4	1	M	23.1
3	4	1	M	21.15
1	1	2	F	26.98
1	1	2	F	28.39
1	1	2	F	24.67
1	1	2	M	27.62
1	1	2	M	27.1
1	2	2	F	26.41
1	2	2	M	21.27
1	3	2	F	29.18
1	3	2	M	25
1	3	2	M	31.24
1	4	2	F	31.34
1	4	2	F	30.66
1	4	2	M	27.15
1	4	2	M	26.2
2	1	2	F	37.17
2	1	2	F	26.25
2	1	2	F	32.19
2	1	2	M	30.92
2	2	2	F	28.61
2	2	2	M	30.76
2	2	2	M	29.24
2	3	2	F	26.05
2	3	2	F	30.77
2	3	2	F	26.25
2	3	2	M	23.15
2	4	2	F	29.14
2	4	2	F	30.57
2	4	2	M	28.5
2	4	2	M	30.29
3	1	2	F	32.39
3	1	2	F	23.63
3	1	2	M	24.57
3	1	2	M	28.51
3	2	2	F	33.5
3	2	2	F	26.48
3	2	2	M	26.67
3	2	2	M	21.68
3	3	2	F	32.81
3	3	2	F	23.26
3	3	2	F	27.37
3	3	2	M	32.03
3	3	2	M	28.51
3	4	2	F	32.45
3	4	2	F	23.26
3	4	2	F	30.71
3	4	2	M	27.62

Table 1A. (Continued)

TRT	REP	MONTH	SEX	WEIGHT
3	4	2	M	23.68
1	1	3	F	30.01
1	1	3	F	33.58
1	1	3	F	29.74
1	1	3	M	32.12
1	1	3	M	30.54
1	2	3	F	27.06
1	2	3	M	26.45
1	3	3	F	34.47
1	3	3	M	27.61
1	3	3	M	33.9
1	4	3	F	34.32
1	4	3	F	33.83
1	4	3	M	29.55
2	1	3	F	44.83
2	1	3	F	36.16
2	1	3	M	35.43
2	2	3	F	35.56
2	2	3	M	35.17
2	2	3	M	32.31
2	3	3	F	33.5
2	3	3	F	36.87
2	3	3	M	25.32
2	4	3	F	35.51
2	4	3	F	34.14
2	4	3	M	34.4
2	4	3	M	36.87
3	1	3	F	33.5
3	1	3	F	30.27
3	2	3	F	38.67
3	2	3	F	29.78
3	2	3	M	31.79
3	2	3	M	27.12
3	3	3	F	38.78
3	3	3	F	32.49
3	3	3	M	36.85
3	3	3	M	33.22
3	4	3	F	39.55
3	4	3	F	34.1
3	4	3	M	31.79
3	4	3	M	28.55
1	1	4	F	34.56
1	1	4	F	37.44
1	1	4	F	32.04
1	1	4	M	37.22
1	1	4	M	36.14
1	2	4	F	33.18
1	2	4	M	32.4
1	3	4	F	37.46
1	3	4	M	32.2
1	3	4	M	37.82
1	4	4	F	36.18
2	1	4	F	49.5
2	1	4	F	39.94
2	1	4	M	40.32

Table 1A. (Continued)

```
-----------------------------
TRT  REP  MONTH  SEX  WEIGHT
-----------------------------
 2    2     4     F    40.46
 2    2     4     M    41.82
 2    3     4     F    39.88
 2    3     4     F    43.22
 2    3     4     M    29.96
 2    4     4     F    42.02
 2    4     4     F    38.52
 2    4     4     M    38.96
 2    4     4     M    41.88
 3    1     4     F    34.34
 3    2     4     F    45.1
 3    2     4     F    34
 3    2     4     M    35.92
 3    2     4     M    32.34
 3    3     4     F    44.16
 3    3     4     F    38.32
 3    3     4     M    41.18
 3    3     4     M    37.82
 3    4     4     F    44.68
 3    4     4     M    36.96
 3    4     4     M    33.18
-----------------------------
```

```
note: TRT = Treatment: 1 = No added astaxanthin,
          2 = Added 300 mg kg⁻¹ astaxanthin and
          3 = Added 500 mg kg⁻¹ astaxanthin and
      WEIGHT = Shrimp weight (g).
```

Table 2A. Survival rate of adolescent shrimp during 4 months of experiment.

```
---------------------------------
TRT   REP   MONTH   SEX   PER_SUR
---------------------------------
1     1     0       F     100.0
1     2     0       F     100.0
1     3     0       F     100.0
1     4     0       F     100.0
1     1     0       M     100.0
1     2     0       M     100.0
1     3     0       M     100.0
1     4     0       M     100.0
2     1     0       F     100.0
2     2     0       F     100.0
2     3     0       F     100.0
2     4     0       F     100.0
2     1     0       M     100.0
2     2     0       M     100.0
2     3     0       M     100.0
2     4     0       M     100.0
3     1     0       F     100.0
3     2     0       F     100.0
3     3     0       F     100.0
3     4     0       F     100.0
3     1     0       M     100.0
3     2     0       M     100.0
3     3     0       M     100.0
3     4     0       M     100.0
1     1     1       F     100.0
1     2     1       F     66.7
1     3     1       F     33.3
1     4     1       F     66.7
1     1     1       M     100.0
1     2     1       M     50.0
1     3     1       M     100.0
1     4     1       M     100.0
2     1     1       F     100.0
2     2     1       F     100.0
2     3     1       F     100.0
2     4     1       F     100.0
2     1     1       M     50.0
2     2     1       M     100.0
2     3     1       M     50.0
2     4     1       M     100.0
3     1     1       F     66.7
3     2     1       F     66.7
3     3     1       F     100.0
3     4     1       F     100.0
3     1     1       M     100.0
3     2     1       M     100.0
3     3     1       M     100.0
3     4     1       M     100.0
1     1     2       F     100.0
1     2     2       F     33.3
1     3     2       F     33.3
1     4     2       F     66.7
1     1     2       M     100.0
1     2     2       M     50.0
1     3     2       M     100.0
---------------------------------
```

Table 2A. (Continued)

```
--------------------------------
TRT   REP   MONTH   SEX   PER_SUR
--------------------------------
1     4     2       M     100.0
2     1     2       F     100.0
2     2     2       F      33.3
2     3     2       F     100.0
2     4     2       F      66.7
2     1     2       M      50.0
2     2     2       M     100.0
2     3     2       M      50.0
2     4     2       M     100.0
3     1     2       F      66.7
3     2     2       F      66.7
3     3     2       F     100.0
3     4     2       F     100.0
3     1     2       M     100.0
3     2     2       M     100.0
3     3     2       M     100.0
3     4     2       M     100.0
1     1     3       F     100.0
1     2     3       F      33.3
1     3     3       F      33.3
1     4     3       F      66.7
1     1     3       M     100.0
1     2     3       M      50.0
1     3     3       M     100.0
1     4     3       M      50.0
2     1     3       F      66.7
2     2     3       F      33.3
2     3     3       F      66.7
2     4     3       F      66.7
2     1     3       M      50.0
2     2     3       M     100.0
2     3     3       M      50.0
2     4     3       M     100.0
3     1     3       F      66.7
3     2     3       F      66.7
3     3     3       F      66.7
3     4     3       F      66.7
3     1     3       M       0.0
3     2     3       M     100.0
3     3     3       M     100.0
3     4     3       M     100.0
1     1     4       F     100.0
1     2     4       F      33.3
1     3     4       F      33.3
1     4     4       F      33.3
1     1     4       M     100.0
1     2     4       M      50.0
1     3     4       M     100.0
1     4     4       M       0.0
2     1     4       F      66.7
2     2     4       F      33.3
2     3     4       F      66.7
2     4     4       F      66.7
2     1     4       M      50.0
2     2     4       M      50.0
--------------------------------
```

Table 2A. (Continued)

```
------------------------------
TRT  REP  MONTH  SEX  PER_SUR
------------------------------
2    3    4      M    50.0
2    4    4      M    100.0
3    1    4      F    33.3
3    2    4      F    66.7
3    3    4      F    66.7
3    4    4      F    33.3
3    1    4      M    0.0
3    2    4      M    100.0
3    3    4      M    100.0
3    4    4      M    100.0
------------------------------
```

note: TRT = Treatment: 1 = No added astaxanthin,
          2 = Added 300 mg kg^{-1} astaxanthin and
          3 = Added 500 mg kg^{-1} astaxanthin and
     PER_SUR = Percentage of survival.

Table 3A. Amount of total spermatozoa in adolescent male shrimp *P. monodon* after 4 months of experiment.

TRT	REP	NO	SPERM
1	1	1	840909
1	1	1	1000000
1	1	1	795455
1	1	1	522727
1	1	1	1250000
1	1	2	818182
1	1	2	477273
1	1	2	1250000
1	1	2	795455
1	1	2	1136364
1	3	1	431818
1	3	1	659091
1	3	1	1295455
1	3	1	750000
1	3	1	704545
1	3	2	1113636
1	3	2	545455
1	3	2	818182
1	3	2	795455
1	3	2	795455
2	1	1	1513636
2	1	1	1663636
2	1	1	1022727
2	1	1	1909091
2	1	1	1350000
2	2	1	1131818
2	2	1	1009091
2	2	1	1540909
2	2	1	736364
2	2	1	1390909
2	3	1	818182
2	3	1	977273
2	3	1	636364
2	3	1	681818
2	3	1	590909
2	4	1	2454545
2	4	1	2000000
2	4	1	2409091
2	4	1	1181818
2	4	1	727273
2	4	2	954545
2	4	2	909091
2	4	2	1090909
2	4	2	1272727
2	4	2	1136364
3	2	1	1227273
3	2	1	1545455
3	2	1	1409091
3	2	1	727273
3	2	1	1909091
3	2	2	1136364
3	2	2	977273
3	2	2	1159091
3	2	2	1000000

Table 3A. (Continued)

```
---------------------------
TRT  REP  NO   SPERM
---------------------------
3    2    2    977273
3    3    1    2068182
3    3    1    2181818
3    3    1    2136364
3    3    1    2363636
3    3    1    1590909
3    3    2    1863636
3    3    2    1136364
3    3    2    1045455
3    3    2    1136364
3    3    2    1227273
3    4    1    2272727
3    4    1    2000000
3    4    1    863636
3    4    1    1136364
3    4    1    1181818
3    4    2    1590909
3    4    2    1136364
3    4    2    1454545
3    4    2    2454545
3    4    2    1181818
---------------------------
```
note: TRT = Treatment: 1 = No added astaxanthin,
          2 = Added 300 mg kg^{-1} astaxanthin and
          3 = Added 500 mg kg^{-1} astaxanthin and
      SPERM = Number of total permatozoa.

Table 4A. Astaxanthin content in muscle, hepatopancreas and shell of adolescent
shrimp after 4 months of experiment.

```
--------------------------------
TRT   REP   ORG   SEX    ASTA
--------------------------------
1     1     1     F      4.909
1     3     1     F      5.2883
1     1     1     F      5.0862
1     1     1     M      3.9266
1     4     1     M      5.0552
1     3     1     M      5.0967
1     1     2     F      0.1717
1     3     2     F      3.0259
1     1     2     F      1.8913
1     1     2     M      0.397
1     4     2     M      1.9336
1     3     2     M      0.4783
1     1     3     F      22.9978
1     1     3     F      19.4448
1     3     3     F      24.2098
1     4     3     F      29.6347
1     1     3     M      22.8693
1     3     3     M      13.5686
2     1     1     F      8.2701
2     3     1     F      5.5767
2     2     1     F      9.975
2     4     1     F      5.0302
2     1     1     F      6.7644
2     3     1     M      4.7108
2     1     2     F      0.5137
2     3     2     F      3.0412
2     2     2     F      4.3436
2     4     2     F      0.8532
2     1     2     F      4.9686
2     3     2     M      1.4375
2     2     3     F      73.4101
2     1     3     F      25.6032
2     1     3     F      70.16
2     4     3     F      92.6
2     2     3     M      164.7335
2     3     3     M      65.3108
3     2     1     F      15.5754
3     3     1     F      6.2316
3     3     1     M      7.8874
3     3     1     M      8.2335
3     4     1     M      11.3471
3     4     1     M      9.3712
3     2     2     F      5.1846
3     3     2     F      7.3013
3     3     2     M      3.9135
3     3     2     M      3.8508
3     4     2     M      2.8188
3     3     3     F      134.7895
3     2     3     F      74.3297
3     3     3     M      78.9691
3     4     3     M      65.8648
3     4     3     M      122.3112
3     3     3     M      99.692
--------------------------------
```

Table 4A. (Continued)

```
note: TRT = Treatment: 1 = No added astaxanthin,
            2 = Added 300 mg kg⁻¹ astaxanthin and
            3 = Added 500 mg kg⁻¹ astaxanthin,
      ORG = Shrimp tissue: 1 = muscle,
            2 = hepatopancreas and
            3 = shell and
     ASTA = Astaxanthin concentration (mg kg⁻¹).
```

Table 5A. Statistical analysis of weight gain of adolescent shrimp.

Factors		Pr > |T|		
Astaxanthin	i/j	Astaxanthin 0 mg kg^{-1}	Astaxanthin 300 mg kg^{-1}	Astaxanthin 500 mg kg^{-1}
	Astaxanthin 0 mg kg^{-1}	.	0.0348	0.2507
	Astaxanthin 300 mg kg^{-1}	0.0348	.	0.2249
	Astaxanthin 500 mg kg^{-1}	0.2507	0.2249	.
Sex		0.0292		

Table 6A. Statistical analysis of survival rate of adolescent shrimp.

Months	Factors	Pr > F
1	Astaxanthin	0.3690
	Sex	0.6285
	Astaxanthin*Sex	0.0752
2	Astaxanthin	0.2937
	Sex	0.1578
	Astaxanthin*Sex	0.5266
3	Astaxanthin	0.9502
	Sex	0.2749
	Astaxanthin*Sex	0.9502
4	Astaxanthin	0.9345
	Sex	0.3383
	Astaxanthin*Sex	0.8335

Table 7A. Statistical analysis of amount of spermatozoa of adolescent male shrimp.

Factor	Pr > F
Astaxanthin	0.0001

Table 8A. Statistical analysis of astaxanthin contents in muscle, hepatopancreas and shell of adolescent shrimp.

Tissues	Factors	Pr > F
Muscle	Astaxanthin	0.0094
	Sex	0.2653
	Astaxanthin*Sex	0.8096
Hepatopancreas	Astaxanthin	0.0074
	Sex	0.0785
	Astaxanthin*Sex	0.5758
Shell	Astaxanthin	0.0025
	Sex	0.5115
	Astaxanthin*Sex	0.2265

APPENDIX B

ORIGINAL DATA FROM EXPERIMENT IN CHAPTER IV

Table 1B. Growth of broodstock shrimp *P. monodon* during 4 months of experiment.

```
-------------------------
TRT   REP   SEX   WT_GAIN
-------------------------
1     1     F     0.45
1     1     F     3.3
1     2     F     4.8
1     2     F     2.4
1     2     F     4
1     3     F     3.3
1     3     F     2.4
1     3     F     0.35
1     4     F     1.65
1     4     F     3.45
1     4     F     5.8
1     4     F     2.65
1     5     F     0.75
1     5     F     2.65
1     5     F     0.9
1     5     F     2.05
1     6     F     1.4
1     6     F     1.65
1     7     F     1.65
1     7     F     1.65
1     7     F     3.4
1     8     F     0.2
1     8     F     0.3
1     8     F     0.05
1     1     F     4
1     1     F     4.4
1     1     F     5.1
1     1     F     5.15
1     1     F     1.4
1     2     F     1.6
1     2     F     6.65
1     2     F     4.8
1     2     F     7.05
1     2     F     4.15
1     3     F     4.15
1     3     F     5.05
1     3     F     2.45
1     3     F     1.05
1     3     F     5.4
1     3     F     5.7
1     4     F     6.25
1     4     F     4.85
1     4     F     2.25
1     4     F     3.45
1     4     F     5
1     5     F     12
1     5     F     9.1
-------------------------
```

Table 1B. (Continued)

TRT	REP	SEX	WT_GAIN
1	5	F	1.95
1	5	F	3.45
1	5	F	1.25
1	6	F	5
1	6	F	6.2
1	6	F	2.05
1	6	F	0.5
1	6	F	2.65
1	6	F	2.85
1	7	F	1.05
1	8	F	2.85
1	8	F	3.55
1	8	F	2
1	8	F	6.75
1	8	F	0.25
1	1	M	2
1	1	M	4.2
1	1	M	1
1	2	M	0.25
1	2	M	0.7
1	3	M	3
1	3	M	1.2
1	3	M	3.8
1	3	M	1.4
1	3	M	1.7
1	3	M	2.75
1	4	M	0.75
1	4	M	1.5
1	4	M	2.05
1	4	M	2
1	4	M	0.25
1	5	M	2.1
1	5	M	3.55
1	5	M	2.95
1	5	M	4.35
1	5	M	0.65
1	6	M	0.3
1	6	M	2.6
1	6	M	2.45
1	6	M	3.7
1	6	M	0.4
1	7	M	2
1	7	M	2.55
1	7	M	0.4
1	7	M	1.3
1	7	M	2.5
1	7	M	0.5
1	8	M	1
1	8	M	1.25
1	8	M	3.65
1	8	M	0.65
1	8	M	2.6
1	8	M	0.9
2	1	F	4.1
2	1	F	8.75

Table 1B. (Continued)

```
-------------------------
TRT   REP   SEX   WT_GAIN
-------------------------
2     2     F     1.05
2     2     F     2.25
2     3     F     0.2
2     3     F     1
2     3     F     3.65
2     3     F     0.65
2     4     F     0.35
2     4     F     1.3
2     5     F     1.95
2     6     F     0.9
2     6     F     0.15
2     6     F     2.8
2     6     F     3.15
2     7     F     1.1
2     7     F     7.15
2     7     F     2.05
2     7     F     5
2     8     F     2.65
2     8     F     0.45
2     1     F     4.95
2     1     F     4.3
2     1     F     4.25
2     1     F     5.05
2     1     F     5.25
2     2     F     6
2     2     F     1.1
2     2     F     3.8
2     2     F     1.3
2     2     F     1.15
2     2     F     1.05
2     2     F     1.6
2     3     F     6.2
2     3     F     0.75
2     3     F     8
2     4     F     1
2     4     F     3.4
2     4     F     1
2     4     F     0.95
2     4     F     1.6
2     5     F     1
2     5     F     3.4
2     5     F     9.9
2     5     F     6.75
2     5     F     2.9
2     6     F     10
2     6     F     5.15
2     6     F     4
2     6     F     4.05
2     6     F     0.5
2     7     F     3
2     7     F     2.9
2     7     F     2.5
2     7     F     8.95
2     8     F     1
2     8     F     1.15
-------------------------
```

Table 1B. (Continued)

```
-------------------------
TRT   REP   SEX   WT_GAIN
-------------------------
2     8     F     0.45
2     8     F     2.25
2     8     F     2.85
2     8     F     0.8
2     1     M     8
2     1     M     3.65
2     1     M     0.95
2     1     M     2.8
2     1     M     1.6
2     1     M     1.8
2     2     M     3
2     2     M     3.65
2     2     M     3.95
2     2     M     2.35
2     2     M     1.75
2     2     M     0.05
2     2     M     0.6
2     3     M     4
2     3     M     0.15
2     3     M     1.3
2     3     M     0.6
2     3     M     0.9
2     3     M     3.75
2     3     M     1.95
2     4     M     2
2     4     M     6.25
2     4     M     2.4
2     4     M     2.25
2     5     M     2
2     5     M     1.85
2     5     M     2.7
2     6     M     1
2     6     M     6.65
2     6     M     0.85
2     6     M     2.55
2     6     M     1.1
2     7     M     1.15
2     7     M     0.6
2     7     M     0.9
2     7     M     2.2
2     8     M     7
2     8     M     0.15
2     8     M     0.6
2     8     M     0.65
2     8     M     1.6
2     8     M     1.25
2     8     M     0.3
3     1     F     0.5
3     1     F     7.5
3     1     F     3.85
3     2     F     0.95
3     2     F     0.25
3     3     F     1.4
3     3     F     2
3     3     F     4
-------------------------
```

Table 1B. (Continued)

TRT	REP	SEX	WT_GAIN
3	3	F	1.05
3	4	F	4.4
3	4	F	2.6
3	5	F	5.25
3	5	F	1.95
3	5	F	6.75
3	5	F	1.05
3	6	F	1.3
3	6	F	0.75
3	6	F	4.5
3	7	F	1
3	7	F	3.15
3	8	F	1.8
3	8	F	5.25
3	8	F	3.6
3	1	F	9.75
3	1	F	2.2
3	1	F	4.8
3	1	F	0.55
3	2	F	6
3	2	F	5.35
3	2	F	1.4
3	2	F	0.65
3	3	F	0.25
3	3	F	0.15
3	3	F	3.1
3	4	F	1.75
3	4	F	6.9
3	4	F	1.65
3	4	F	0.85
3	4	F	7.55
3	4	F	2.05
3	5	F	1.85
3	5	F	1.2
3	6	F	5.4
3	6	F	1.45
3	6	F	3.25
3	6	F	1.7
3	6	F	1.85
3	7	F	5.8
3	7	F	0.85
3	7	F	3.6
3	8	F	2
3	8	F	5.55
3	8	F	2.75
3	8	F	0.55
3	8	F	3.75
3	8	F	4.55
3	1	M	0.25
3	1	M	2.3
3	1	M	1.05
3	1	M	0.5
3	1	M	2.8
3	1	M	0.6
3	2	M	3

Table 1B. (Continued)

TRT	REP	SEX	WT_GAIN
3	2	M	2.9
3	2	M	3.15
3	2	M	0.4
3	2	M	2
3	2	M	1.25
3	2	M	0.4
3	3	M	1
3	3	M	1.8
3	3	M	0.2
3	3	M	0.3
3	3	M	1.95
3	3	M	2.6
3	4	M	1
3	4	M	0.75
3	4	M	1.95
3	4	M	2.25
3	4	M	1.8
3	5	M	3.85
3	5	M	1.8
3	5	M	0.8
3	5	M	1.85
3	5	M	0.65
3	5	M	0.15
3	6	M	0.2
3	6	M	2.7
3	6	M	1.95
3	6	M	0.8
3	6	M	1.4
3	7	M	5
3	7	M	0.9
3	7	M	0.9
3	7	M	2.25
3	8	M	2
3	8	M	0.75
3	8	M	2.25
3	8	M	2.25
3	8	M	2.2
3	8	M	1.1
4	1	F	2.55
4	1	F	1.4
4	1	F	2.9
4	1	F	1.7
4	2	F	3.3
4	2	F	0.4
4	2	F	8.15
4	2	F	3.6
4	3	F	5.1
4	3	F	2.45
4	3	F	5.9
4	4	F	0.45
4	4	F	0.65
4	4	F	2
4	4	F	3.7
4	5	F	2.2
4	5	F	1.3

Table 1B. (Continued)

TRT	REP	SEX	WT_GAIN
4	5	F	2.95
4	5	F	1.25
4	6	F	0.15
4	6	F	5.7
4	6	F	1.6
4	7	F	0.45
4	7	F	0.1
4	7	F	0.45
4	8	F	3.7
4	8	F	1.9
4	1	F	6
4	1	F	3.95
4	1	F	4.4
4	1	F	0.75
4	2	F	5.3
4	2	F	9.05
4	2	F	3.8
4	2	F	1.05
4	2	F	3.5
4	2	F	3.05
4	3	F	3.15
4	3	F	4.1
4	3	F	4.85
4	3	F	3.35
4	3	F	8.35
4	4	F	0.95
4	4	F	4.45
4	4	F	3.3
4	4	F	1.75
4	4	F	3.55
4	4	F	4.95
4	5	F	1
4	5	F	2.15
4	5	F	1.95
4	5	F	2.5
4	6	F	0.05
4	6	F	2.3
4	6	F	1.25
4	6	F	1.8
4	6	F	3.85
4	7	F	0.7
4	7	F	1.7
4	7	F	0.4
4	7	F	1.9
4	7	F	1.45
4	8	F	1.9
4	8	F	3.35
4	8	F	4.95
4	8	F	0.25
4	1	M	8
4	1	M	2.3
4	1	M	2.5
4	1	M	1.6
4	2	M	4
4	2	M	3.1

Table 1B. (Continued)

```
------------------------
TRT   REP   SEX   WT_GAIN
------------------------
4     2     M     4.3
4     2     M     1.75
4     2     M     2
4     3     M     4
4     3     M     1.65
4     3     M     1.5
4     3     M     1.95
4     3     M     4.15
4     3     M     0.3
4     3     M     1.25
4     4     M     9
4     4     M     0.1
4     4     M     6.2
4     4     M     1.6
4     4     M     0.85
4     4     M     0.75
4     5     M     6
4     5     M     6.85
4     5     M     2.7
4     5     M     5.2
4     5     M     3.4
4     5     M     2.35
4     5     M     2.9
4     6     M     2.5
4     6     M     1.25
4     6     M     1.4
4     6     M     1.75
4     7     M     6
4     7     M     0.4
4     7     M     0.7
4     7     M     0.65
4     7     M     0.15
4     7     M     0.85
4     7     M     0.25
4     8     M     0.75
4     8     M     1.65
4     8     M     1.2
4     8     M     0.95
4     8     M     0.35
------------------------
```

note: TRT = Treatment:
    1 = Added 3% lipid and 100 mg kg^{-1} astaxanthin,
    2 = Added 3% lipid and 500 mg kg^{-1} astaxanthin,
    3 = Added 8% lipid and 100 mg kg^{-1} astaxanthin and
    4 = Added 8% lipid and 100 mg kg^{-1} astaxanthin and
WT_GAIN = Shrimp weight gain(g month^{-1}).

Table 2B. Amount of total eggs in broodstock shrimp *P. monodon* after 4 months of experiment.

```
-------------------------------
TRT LIPID  ASTA REP    EGG
-------------------------------
1    3     100   8    66000
1    3     100   8    84000
1    3     100   8    97000
1    3     100   2    121000
1    3     100   2    94000
1    3     100   2    86000
1    3     100   1    87000
1    3     100   1    116000
1    3     100   1    127000
2    3     500   8    134000
2    3     500   8    120000
2    3     500   8    102000
2    3     500   7    91000
2    3     500   7    87000
2    3     500   7    112000
2    3     500   6    140000
2    3     500   6    125000
2    3     500   6    166000
2    3     500   7    149000
2    3     500   7    165000
2    3     500   7    129000
2    3     500   1    185000
2    3     500   1    164000
2    3     500   1    161000
3    8     100   6    134000
3    8     100   6    167000
3    8     100   6    101000
3    8     100   6    85000
3    8     100   6    94000
3    8     100   6    107000
3    8     100   4    146000
3    8     100   4    98000
3    8     100   4    148000
3    8     100   4    123000
3    8     100   4    141000
3    8     100   4    96000
3    8     100   7    143000
3    8     100   7    134000
3    8     100   7    116000
4    8     500   4    164000
4    8     500   4    204000
4    8     500   4    179000
4    8     500   4    147000
4    8     500   4    165000
4    8     500   4    113000
4    8     500   6    184000
4    8     500   6    195000
4    8     500   6    166000
4    8     500   8    158000
4    8     500   8    194000
4    8     500   8    235000
4    8     500   3    227000
4    8     500   3    216000
4    8     500   3    187000
-------------------------------
```

Table 2B. (Continued)

```
------------------------------
TRT LIPID   ASTA REP    EGG
------------------------------
4      8    500   3    170000
4      8    500   3    198000
4      8    500   3    237000
------------------------------
```
note: TRT = Treatment:
          1 = Added 3% lipid and 100 mg kg^{-1} astaxanthin,
          2 = Added 3% lipid and 500 mg kg^{-1} astaxanthin,
          3 = Added 8% lipid and 100 mg kg^{-1} astaxanthin and
          4 = Added 8% lipid and 100 mg kg^{-1} astaxanthin,
   LIPID = Lipid levels: 3 = 3% lipid and 8 = 8% lipid,
   ASTA = Astaxanthin levels: 100 = 100 mg kg^{-1} astaxanthin and
        500 = 500 mg kg^{-1} astaxanthin and
   EGG = Total shrimp eggs (eggs shrimp^{-1}).

Table 3B. Amount of total spermatozoa in broodstock shrimp *P. monodon* after 4 months of experiment.

TRT	LIPID	ASTA	NO	SPERM
1	3	100	1	608025
1	3	100	1	1981482
1	3	100	1	762345
1	3	100	1	1904321
1	3	100	1	666666
1	3	100	1	1941359
1	3	100	1	564815
1	3	100	1	765432
1	3	100	1	1074074
1	3	100	1	1132716
1	3	100	1	1123457
1	3	100	1	731481
1	3	100	1	1179012
1	3	100	2	743828
1	3	100	2	2277779
1	3	100	2	682100
1	3	100	2	1941359
1	3	100	2	626543
1	3	100	2	1700618
1	3	100	2	515432
1	3	100	2	811728
1	3	100	2	1117284
1	3	100	2	1175927
1	3	100	2	1086420
1	3	100	2	783951
1	3	100	2	1209876
1	3	100	3	682100
1	3	100	3	2148149
1	3	100	3	780864
1	3	100	3	1648148
1	3	100	3	608025
1	3	100	3	2126543
1	3	100	3	549383
1	3	100	3	830247
1	3	100	3	1080248
1	3	100	3	1141976
1	3	100	3	1138889
1	3	100	3	799383
1	3	100	3	1234568
2	3	500	1	1171296
2	3	500	1	2169444
2	3	500	1	1643210
2	3	500	1	2247531
2	3	500	1	858951
2	3	500	1	1588889
2	3	500	1	1904630
2	3	500	1	1245987
2	3	500	1	1083024
2	3	500	1	1789197
2	3	500	1	1167902
2	3	500	1	1602470
2	3	500	1	1792593
2	3	500	1	1731482
2	3	500	1	2522531

Table 3B. (Continued)

TRT	LIPID	ASTA	NO	SPERM
2	3	500	2	1225617
2	3	500	2	2230556
2	3	500	2	1324074
2	3	500	2	2298458
2	3	500	2	950618
2	3	500	2	1741667
2	3	500	2	1541358
2	3	500	2	1157717
2	3	500	2	1181481
2	3	500	2	1870679
2	3	500	2	1072839
2	3	500	2	1524383
2	3	500	2	1931790
2	3	500	2	1823148
2	3	500	2	2549691
2	3	500	3	1147532
2	3	500	3	2203395
2	3	500	3	1683951
2	3	500	3	2166050
2	3	500	3	818210
2	3	500	3	1721297
2	3	500	3	1592285
2	3	500	3	1218827
2	3	500	3	1076235
2	3	500	3	1829939
2	3	500	3	1307099
2	3	500	3	1476852
2	3	500	3	2162655
2	3	500	3	1880864
2	3	500	3	2858642
3	8	100	1	1169907
3	8	100	1	1578240
3	8	100	1	1348148
3	8	100	1	1970370
3	8	100	1	1053240
3	8	100	1	2044908
3	8	100	1	680556
3	8	100	1	1549074
3	8	100	1	1173149
3	8	100	1	1477778
3	8	100	1	2064353
3	8	100	1	1247685
3	8	100	1	2216667
3	8	100	1	1267130
3	8	100	1	1843982
3	8	100	2	1160186
3	8	100	2	1461575
3	8	100	2	1322222
3	8	100	2	1918518
3	8	100	2	1095371
3	8	100	2	2171297
3	8	100	2	622223
3	8	100	2	1701389
3	8	100	2	1050000
3	8	100	2	1438889

Table 3B. (Continued)

TRT	LIPID	ASTA	NO	SPERM
3	8	100	2	2193981
3	8	100	2	1315740
3	8	100	2	2100000
3	8	100	2	1208796
3	8	100	2	1801853
3	8	100	3	1092129
3	8	100	3	1412963
3	8	100	3	1273611
3	8	100	3	1879629
3	8	100	3	962501
3	8	100	3	2190741
3	8	100	3	725927
3	8	100	3	1643055
3	8	100	3	1007871
3	8	100	3	1523148
3	8	100	3	2200463
3	8	100	3	1221759
3	8	100	3	2174537
3	8	100	3	1228241
3	8	100	3	1821297
4	8	500	1	1824074
4	8	500	1	2598765
4	8	500	1	1759260
4	8	500	1	1296297
4	8	500	1	2243828
4	8	500	1	1166667
4	8	500	1	1913580
4	8	500	1	2179013
4	8	500	1	2123457
4	8	500	1	2493827
4	8	500	1	1617284
4	8	500	1	1419753
4	8	500	1	1867284
4	8	500	1	1447532
4	8	500	1	2049383
4	8	500	2	1651235
4	8	500	2	2354939
4	8	500	2	1876544
4	8	500	2	1429013
4	8	500	2	2003087
4	8	500	2	1219136
4	8	500	2	2003087
4	8	500	2	2293211
4	8	500	2	2040123
4	8	500	2	2120370
4	8	500	2	1657407
4	8	500	2	1413581
4	8	500	2	2046297
4	8	500	2	1395062
4	8	500	2	2129630
4	8	500	3	1688271
4	8	500	3	2679012
4	8	500	3	1632716
4	8	500	3	1336421
4	8	500	3	2475309

Table 3B. (Continued)

```
--------------------------------
TRT LIPID   ASTA   NO    SPERM
--------------------------------
4     8      500    3    1314815
4     8      500    3    1888889
4     8      500    3    2237655
4     8      500    3    1984568
4     8      500    3    2706791
4     8      500    3    1808642
4     8      500    3    1324074
4     8      500    3    1941359
4     8      500    3    1453704
4     8      500    3    2339507
--------------------------------
```
note: TRT = Treatment:

      1 = Added 3% lipid and 100 mg kg^{-1} astaxanthin,

      2 = Added 3% lipid and 500 mg kg^{-1} astaxanthin,

      3 = Added 8% lipid and 100 mg kg^{-1} astaxanthin and

      4 = Added 8% lipid and 100 mg kg^{-1} astaxanthin,

  LIPID = Lipid levels: 3 = 3% lipid and 8 = 8% lipid,

  ASTA = Astaxanthin levels: 100 = 100 mg kg^{-1} astaxanthin and

      500 = 500 mg kg^{-1} astaxanthin and

  SPERM = Total shrimp spermatozoa (individuals shrimp^{-1}).

Table 4B. Astaxanthin accumulation in muscle, hepatopancreas, ovary and shell of broodstock shrimp *P. monodon*.

TRT	LIPID	ASTA	ORG	SEX	AST_CONC
1	3	100	1	F	2.137
1	3	100	1	F	2.607
1	3	100	1	F	2.368
1	3	100	1	F	1.423
1	3	100	1	F	4.221
1	3	100	1	F	1.436
1	3	100	1	M	3.326
1	3	100	1	M	1.991
1	3	100	1	M	2.271
1	3	100	1	M	2.772
1	3	100	2	F	1.118
1	3	100	2	F	5.127
1	3	100	2	F	1.958
1	3	100	2	F	2.826
1	3	100	2	F	5.406
1	3	100	2	F	1.16
1	3	100	2	M	0.218
1	3	100	2	M	0.005
1	3	100	2	M	0.087
1	3	100	3	F	1.992
1	3	100	3	F	33.468
1	3	100	3	F	14.306
1	3	100	4	F	95.741
1	3	100	4	F	56.258
1	3	100	4	F	148.616
1	3	100	4	F	116.486
1	3	100	4	F	50.045
1	3	100	4	F	44.052
1	3	100	4	M	92.672
1	3	100	4	M	86.738
1	3	100	4	M	77.221
1	3	100	4	M	84.057
2	3	500	1	F	11.255
2	3	500	1	F	3.414
2	3	500	1	F	3.702
2	3	500	1	F	3.028
2	3	500	1	F	3.079
2	3	500	1	M	3.245
2	3	500	1	M	3.528
2	3	500	1	M	2.783
2	3	500	1	M	2.513
2	3	500	2	F	5.372
2	3	500	2	F	1.788
2	3	500	2	F	3.306
2	3	500	2	F	4.898
2	3	500	2	F	4.994
2	3	500	2	M	0.051
2	3	500	2	M	0.043
2	3	500	2	M	1.111
2	3	500	2	M	3.096
2	3	500	3	F	107.21
2	3	500	3	F	47.524
2	3	500	3	F	46.914
2	3	500	4	F	102.797

Table 4B. (Continued)

TRT	LIPID	ASTA	ORG	SEX	AST_CONC
2	3	500	4	F	51.953
2	3	500	4	F	70.551
2	3	500	4	F	85.49
2	3	500	4	F	84.653
2	3	500	4	M	105.234
2	3	500	4	M	98.977
2	3	500	4	M	51.247
2	3	500	4	M	62.175
3	8	100	1	F	1.773
3	8	100	1	F	2.692
3	8	100	1	F	0.04
3	8	100	1	F	0.269
3	8	100	1	F	1.661
3	8	100	1	M	0.041
3	8	100	1	M	2.682
3	8	100	1	M	3.615
3	8	100	1	M	2.048
3	8	100	2	F	0.091
3	8	100	2	F	1.681
3	8	100	2	F	0.077
3	8	100	2	F	0.023
3	8	100	2	M	0.202
3	8	100	2	M	0.071
3	8	100	2	M	0.149
3	8	100	2	M	0.302
3	8	100	3	F	34.053
3	8	100	3	F	0.119
3	8	100	4	F	121.699
3	8	100	4	F	95.23
3	8	100	4	F	64.222
3	8	100	4	F	56.431
3	8	100	4	M	69.114
3	8	100	4	M	58.132
3	8	100	4	M	37.547
3	8	100	4	M	90.776
3	8	100	4	M	30.568
4	8	500	1	F	4.53
4	8	500	1	F	0.137
4	8	500	1	F	6.378
4	8	500	1	F	6.546
4	8	500	1	F	8.53
4	8	500	1	F	7.351
4	8	500	1	M	4.989
4	8	500	1	M	3.034
4	8	500	1	M	0.099
4	8	500	1	M	9.261
4	8	500	2	F	3.78
4	8	500	2	F	9.979
4	8	500	2	F	17.699
4	8	500	2	F	0.032
4	8	500	2	F	5.347
4	8	500	2	F	4.101
4	8	500	2	M	3.455
4	8	500	2	M	1.067
4	8	500	2	M	1.659

Table 4B. (Continued)

TRT	LIPID	ASTA	ORG	SEX	AST_CONC
4	8	500	2	M	2.262
4	8	500	3	F	167.503
4	8	500	3	F	95.669
4	8	500	3	F	110.381
4	8	500	3	F	103.989
4	8	500	4	F	117.366
4	8	500	4	F	56.332
4	8	500	4	F	65.332
4	8	500	4	F	93.776
4	8	500	4	M	21.478
4	8	500	4	M	85.103
4	8	500	4	M	106.918
4	8	500	4	M	70.913

note: TRT = Treatment:
      1 = Added 3% lipid and 100 mg kg^{-1} astaxanthin,
      2 = Added 3% lipid and 500 mg kg^{-1} astaxanthin,
      3 = Added 8% lipid and 100 mg kg^{-1} astaxanthin and
      4 = Added 8% lipid and 100 mg kg^{-1} astaxanthin,
LIPID = Lipid levels: 3 = 3% lipid and 8 = 8% lipid,
ASTA = Astaxanthin levels: 100 = 100 mg kg^{-1} astaxanthin and
      500 = 500 mg kg^{-1} astaxanthin,
ORG = Shrimp tissues: 1 = Muscle, 2 = Hepatopancreas,
      3 = Ovary and 4 = shell and
AST_CONC = Astaxanthin concentration in shrimp tissue
      (mg kg^{-1}).

Table 5B. Fatty acid contents in muscle, hepatopancreas and ovary of broodstock
shrimp *P. monodon* after 4 months of experiment.

```
-----------------------------------------------------------------------
NO     TRT   HUFA   ASTA  SEX   ORG   N0140 N5141 N0150 N5151 N0160
-----------------------------------------------------------------------
d602   1     3      100   F     1     0.1   0     0.1   0     3.9
d603   1     3      100   F     2     6.9   0.7   2.4   0.3   81
d604   1     3      100   F     3     1.4   0.1   0.5   0.1   25.2
d605   1     3      100   F     1     0.1   0     0.1   0     3
d606   1     3      100   F     2     3.2   0.5   1     0.1   34.5
d607   1     3      100   F     3     1.9   0.1   0.6   0.1   28.6
d608   1     3      100   F     1     0.1   0     0.1   0     2.1
d609   1     3      100   F     2     0.3   0     0.2   0     8.5
d610   1     3      100   F     1     0.1   0     0.1   0     3.2
d611   1     3      100   F     2     8.9   0.9   3     0.4   82.3
d612   1     3      100   F     1     0.1   0     0.1   0     3
d613   1     3      100   F     2     3.3   0.7   1     0.1   34.7
d614   1     3      100   M     1     0.1   0     0.1   0     2.7
d615   1     3      100   M     2     0.4   0     0.2   0     8.8
d616   1     3      100   M     1     0.1   0     0.1   0     2.9
d617   1     3      100   M     2     2.77  0.3   0.83  0.1   28.1
d618   1     3      100   M     1     0.1   0     0     0     2.3
d619   1     3      100   M     2     2.1   0.6   0.7   0.1   21.8
d620   2     3      500   F     1     0.1   0     0     0     2
d621   2     3      500   F     2     6.3   0.9   2.2   0.3   79.7
d622   2     3      500   F     1     0.1   0     0.1   0     2.7
d623   2     3      500   F     2     0.2   0.2   0.1   0     5.9
d624   2     3      500   F     3     1.9   0.1   0.5   0.1   27.4
d625   2     3      500   F     1     0.1   0     0.1   0     2.8
d626   2     3      500   F     2     0.7   0.5   0.4   0     14.2
d627   2     3      500   F     3     1.8   0.1   0.5   0.1   24.9
d628   2     3      500   F     1     0.1   0     0.1   0     3.3
d629   2     3      500   F     2     3.33  0.27  1.13  0.13  31.67
d630   2     3      500   M     1     0.1   0     0.1   0     2.8
d631   2     3      500   M     2     2.65  0.45  1     0.15  31.1
d632   2     3      500   M     1     0.1   0     0.1   0     2.6
d633   2     3      500   M     2     4.05  0.5   1.25  0.15  38.9
d634   2     3      500   M     1     0.1   0     0     0     2.1
d635   2     3      500   M     2     0.5   0.5   0.4   0     7.8
d636   2     3      500   F     1     0.1   0     0.1   0     3.1
d637   2     3      500   F     2     2.63  0.3   0.9   0.1   28.17
d638   2     3      500   F     1     0.1   0     0.1   0     3.1
d639   2     3      500   F     2     3.43  0.3   1.13  0.13  34.23
d546   3     8      100   F     1     0.1   0     0.1   0     2.9
d547   3     8      100   F     2     2.1   0.4   0.9   0.9   27.4
d548   3     8      100   F     1     0.1   0     0.1   0.6   3.1
d549   3     8      100   F     2     6.3   1.5   2.4   2.7   77.4
d550   3     8      100   F     3     1.4   0.1   0.4   1.1   20.1
d551   3     8      100   F     1     0.1   0     0.1   0.7   3
d552   3     8      100   F     2     7.5   0.5   2.9   0.4   77.7
d553   3     8      100   F     3     1.7   0.1   0.7   1.1   31.5
d554   3     8      100   F     1     0.1   0     0.1   0.6   2.2
d555   3     8      100   F     2     3.5   1.4   1.4   4.2   46.2
d556   3     8      100   F     1     0     0     0.1   0.6   2.6
d557   3     8      100   F     2     2.8   2.8   1.4   6.3   61.6
d558   3     8      100   M     1     0.1   0     0.1   0     2.5
d559   3     8      100   M     2     5.6   0     1.5   0.2   38.5
d560   3     8      100   M     1     0.1   0     0.1   0     2.5
d561   3     8      100   M     2     5.5   0     1.5   0.2   37.4
-----------------------------------------------------------------------
```

Table 5B. (Continued)

```
------------------------------------------------------------------
NO    TRT  HUFA  ASTA SEX   ORG   N0140 N5141 N0150 N5151 N0160
------------------------------------------------------------------
d566   3    8    100   M    1     0.1   0     0.1   0     3.6
D567   3    8    100   M    2     5.2   0     1.7   0.2   45.1
d568   3    8    100   M    1     0.1   0     0.1   0     2.8
d569   3    8    100   M    2     7.7   0     2.2   0.2   53.5
d570   4    8    500   F    1     0.1   0     0     0     2.7
d571   4    8    500   F    2     4.9   0     1.4   0.2   35.3
d572   4    8    500   M    1     0.1   0     0.1   0     3.5
d573   4    8    500   M    2     7.8   0.5   2.9   0.4   84.6
d574   4    8    500   M    1     0.1   0     0.1   0     2.6
d575   4    8    500   M    2     7.2   0     2     0.3   46.9
d576   4    8    500   M    1     0.1   0     0.1   0     2
d577   4    8    500   M    2     2.5   0.7   0.9   0.1   26.2
d586   4    8    500   F    1     0.1   0     0.1   0     3.8
d587   4    8    500   F    2     1.5   0.9   1     0.1   37.1
d588   4    8    500   F    3     1.9   0.2   0.6   0.1   32.8
d589   4    8    500   F    1     0.1   0     0.1   0     4.2
d590   4    8    500   F    2     7.3   0.7   2.3   0.4   60.3
d591   4    8    500   F    3     2.2   0.2   0.8   0.1   33.5
d592   4    8    500   F    1     0.1   0     0.1   0     2.2
d593   4    8    500   F    2     5.6   1.6   2.4   0.4   57.2
d594   4    8    500   F    1     0.1   0     0.1   0     4
d595   4    8    500   F    2     1.9   0     1     0     48.1
d596   4    8    500   F    3     1.2   0.1   0.6   0.1   36
d597   4    8    500   F    1     0.1   0     0.1   0     3.5
d598   4    8    500   F    2     7.3   0.6   3     0.7   89.2
d599   4    8    500   F    1     0.1   0     0.1   0     3.5
d600   4    8    500   F    2     11    0.8   3.9   0.5   104.7
------------------------------------------------------------------
```

Table 5B. (Continued)

NO	N7161	N0170	N7171	N0180	N9181	N7181	N6182T	N6182C	N0190	N6183
d602	0.4	0.4	0	2.9	2.7	0.5	0	1.3	0.1	0
d603	10.5	2.8	1.9	15.2	34.6	7.8	0.4	9.1	0.7	0.3
d604	3.2	1.8	0.1	9.9	16.8	3	0.1	3.8	0.3	0.1
d605	0.3	0.4	0	2.5	2.1	0.4	0	1.4	0.1	0
d606	3.2	1.9	1.4	11.2	16.8	3.5	0.2	7.2	0.4	0.1
d607	3.9	2.2	0.4	12.2	19	3.1	0.1	5.4	0.3	0.1
d608	0.2	0.2	0	2.2	2.3	0.2	0	1.5	0.1	0
d609	3.3	0.5	0.2	5.2	6.7	2.5	0.1	2.4	0.1	0
d610	0.3	0.3	0	2.7	2.3	0.4	0	1.3	0.1	0
d611	11.5	3.6	0.8	17.4	49.7	10.7	0.6	18.3	1.1	0.6
d612	0.2	0.3	0	2.5	2.1	0.3	0	1.4	0.1	0
d613	3.4	1.7	1.1	10.5	18.3	3.7	0.2	10	0.4	0.1
d614	0.2	0.3	0.1	2.8	2.2	0.3	0	1.3	0.1	0
d615	0.8	0.8	0.2	5.6	4.4	0.9	0	1.3	0.1	0
d616	0.2	0.3	0	2.4	2.3	0.4	0	1.9	0.1	0
d617	3.23	0.97	0	5.3	20.0	33.53	0.17	10.97	0.33	0.13
d618	0.2	0.3	0	2.4	2.2	0.3	0	1.6	0.1	0
d619	2.2	1.2	0.6	8.2	13.5	2.8	0.1	8.1	0.3	0.1
d620	0.1	0.3	0	2.3	1.8	0.2	0	0.7	0.1	0
d621	7.8	2.6	0	14.2	48	9.2	0.5	25.2	1	0.5
d622	0.2	0.3	0.1	2.4	2.3	0.4	0	1	0.1	0
d623	0.8	0.4	0.4	4.4	1.1	0	1.5	0.1	0	0
d624	4.7	1.6	0.3	8.4	22	3.7	0.1	5.4	0.2	0.1
d625	0.3	0.3	0	2.5	2.2	0.3	0	1.7	0.1	0
d626	1.1	0.9	1	6.5	6.1	1.7	0.1	2.2	0.2	0
d627	4.2	1.7	0.3	9.1	20	3.4	0.2	5.3	0.3	0.1
d628	0.3	0.3	0	2.6	2.3	0.4	0	1.5	0.1	0
d629	3.7	1.13	0.2	5.87	16	3.33	0.17	8.47	0.33	0.13
d630	0.2	0.3	0.1	2.5	2	0.4	0	1.4	0.1	0
d631	2.9	1.5	0	7.8	12.4	3.45	0.45	5.75	0.5	0.25
d632	0.2	0.3	0	2.4	2.1	0.3	0	1.6	0.1	0
d633	4.2	1.65	0.25	8.6	22.9	4.5	0.2	12.6	0.45	0.15
d634	0.2	0.2	0	2.2	1.9	0.2	0	1.3	0.1	0
d635	0.6	0.8	0.7	5.8	4.7	1.1	0	2.2	0.1	0
d636	0.3	0.3	0	2.5	2.3	0.4	0	1.5	0.1	0
d637	3.63	1	0.23	5.03	19.5	3.67	0.17	10.0	70.33	0.2
d638	0.2	0.3	0	2.5	2.3	0.4	0	1.8	0.1	0
d639	4.33	1.23	0.23	5.73	22.5	74	0.17	12.9	0.37	0.17
d546	0.2	0.3	0	2.5	2.3	0.4	0	1.1	0.1	0
d547	2.4	1.6	0.4	8.7	13.7	3.2	0.2	4.3	0.3	0.1
d548	0.3	0.3	0.1	2.5	2.5	0.3	0	1.5	0.1	0
d549	6.3	4.2	0.9	26.7	31.2	7.8	0.6	7.2	0.9	0.3
d550	3	1.3	0.3	7.4	14.8	2.4	0.1	2.3	0.2	0.1
d551	0.3	0.2	0	2.3	2.3	0.4	0	1.6	0.1	0
d552	12.4	0.6	0.9	13.4	54.5	9.2	0.6	17.7	1	0.7
d553	4.8	1.9	0.4	10.4	23.7	4.1	0.2	6.7	0.4	0.2
d554	0.2	0.3	0	2.4	2.2	0.3	0	1.3	0.1	0
d555	3.5	6.3	1.4	39.2	32.2	7.7	0	11.2	0.7	0
d556	0.1	0.3	0.1	2.6	1.9	0.3	0	1.2	0.1	0
d557	4.2	7.7	0	49	23.1	6.3	0.7	4.2	0.7	0
d558	0.2	0.3	0.1	2.4	2.2	0.3	0	1.3	0.1	0
d559	6.1	2.1	0.5	11.5	25.6	5.4	0.3	10.8	0.6	0.3
d560	0	0.3	0.1	2.6	2.5	0.3	0	1.6	0.1	0
d561	6.4	2	0.3	11.5	26.9	5.4	0.3	11.6	0.5	0
d566	0.3	0.3	0	2.9	2.5	0.5	0	1.9	0.1	0

Table 5B. (Continued)

NO	N7161	N0170	N7171	N0180	N9181	N7181	N6182T	N6182C	N0190	N6183
d567	5.5	2.3	0.4	12.5	23.3	5.5	0.2	9.8	0.5	0.2
d568	0.3	0.3	0.1	2.5	2.1	0.4	0	1.1	0.1	0
D569	8.7	2.4	0.5	12.9	32.8	7.1	0.3	14.5	0.7	0.3
d570	0.2	0.3	0	2.7	2.1	0.3	0	1.5	0.1	0
d571	5.7	2.1	0.4	11.8	22.6	4.9	0.2	9.6	0.5	0.2
d572	0.3	0.3	0.1	2.8	2.6	0.4	0	1.6	0.1	0
d573	10.2	3.3	1.1	16.3	48.3	10.3	0.6	17.7	1	0.6
d574	0.2	0.3	0.1	2.4	2.2	0.4	0	1.2	0.1	0
d575	8.8	2.4	0	12	33.8	7.4	0.3	14.9	0.6	0.3
d576	0.2	0.2	0	2	1.6	0.2	0	0.8	0.1	0
d577	2.3	1.4	0.4	8.6	6.6	2.4	0.1	0.8	0.3	0.1
d586	0.4	0.3	0.1	2.9	3.2	0.5	0	1	0.1	0
d587	2.3	1.7	1.1	9.9	16.6	4.5	0.2	2.1	0.3	0.1
d588	5.4	2	0.5	10.8	26.7	4.6	0.2	3.4	0.4	0.2
d589	0.4	0.3	0.1	3.1	3.1	0.5	0	2.2	0.1	0
d590	10.1	2.6	0.9	14.8	40	8.4	0.5	0	0.8	0.4
d591	5.6	2.3	0.4	12.3	22.6	4.1	0.2	0	0.4	0.2
d592	0.2	0.3	0	2.2	1.8	0.3	0	0	0.1	0
d593	7.2	4.4	0.8	26	38.4	8	0.4	13.6	0.8	0.4
d594	0.4	0.4	0.1	3	3.1	0.5	0	0.8	0.1	0
d595	3.3	2.2	0.7	10.9	15.3	4.2	0.3	1	0.5	0.3
d596	5	2.5	0.5	12.4	25.3	5.1	0.1	2.3	0.5	0.2
d597	0.3	0.3	0	2.7	2.6	0.5	0.1	1.9	0.1	0
d598	12.3	2.9	0.9	14.3	57.3	12.2	0.9	24.2	1.2	0.9
d599	0.3	0.3	0.1	2.7	2.6	0.4	0	1.5	0.1	0
d600	15.3	4	0.7	17.2	61.7	13.5	12	23.1	1.6	1.1

Table 5B. (Continued)

NO	N9191	N3183	N3184	N0200	N9201	N7201	N0210	N6203	N6204	N3203
D602	0	0.1	0.2	0	0.1	0	0	0	1.9	0
D603	0	0.6	1.5	0	5.4	0.5	0.2	0.2	7.8	0.4
D604	0.2	0.1	0.6	0	1.1	0.1	0	0	6.2	0.1
D605	0	0.1	0.1	0	0.1	0	0	0	2	0
D606	0.1	0.4	0.8	0	2.2	0.2	0.1	0.1	4.9	0.2
D607	0.1	0.3	0.7	0	1.1	0.1	0	0.1	8.1	0.1
D608	0	0	0.2	0	0.1	0	0	0	1.6	0
D609	0	0.1	0.2	0	0.6	0	0	0	3.4	0.1
D610	0	0.1	0.1	0	0.1	0	0	0	1.4	0
D611	0.4	1.8	2.5	0.1	4.5	0.7	0.3	0.4	7	0.5
D612	0	0	0.1	0	0.1	0	0	0	1.4	0
D613	0.1	0.6	0.9	0	1.8	0.2	0	0.1	5	0.2
D614	0	0	0.3	0	0.1	0	0	0	2.2	0
D615	0	0.1	0.2	0	0.5	0	0	0	2.7	0
D616	0	0.1	0.1	0	0.1	0	0	0	1.1	0
D617	0.1	0.7	0.77	0.03	1.3	0.23	0.1	0.13	1.8	0.13
D618	0	0.1	0.2	0	0.1	0	0	0	1.3	0
D619	0.1	1.1	1	0	1.5	0.1	0	0.1	4.3	0.2
D620	0	0	0.1	0	0.1	0	0	0	1.8	0
D621	0.4	1.7	2.1	0.1	3.9	0.5	0.4	0.3	5.7	0.4
D622	0	0	0.2	0	0.1	0	0	0	1.9	0
D623	0.1	0.2	0	0.6	0	0	0	0	3.4	0
D624	0.2	0.2	0.7	0	1.1	0.2	0.1	0.1	6.5	0.1
D625	0	0	0.2	0	0.1	0	0	0	1.5	0
D626	0	0.1	0.3	0.1	1.2	0.1	0	0	3	0.1
D627	0.2	0.2	0.8	0	1.3	0.2	0	0.1	5.8	0.1
D628	0	0.1	0.1	0	0.1	0	0	0	1.4	0
D629	0.1	0.6	0.73	0.03	1.83	0.2	0.07	0.13	3.33	0.13
D630	0	0.1	0.1	0	0.1	0	0	0	1.4	0
D631	0.3	0.4	0.55	0	1.9	0.15	0.05	0.1	4.25	0.2
D632	0	0.1	0.1	0	0.1	0	0	0	1.2	0
D633	0.15	0.85	1.05	0.05	1.95	0.3	0.1	0.15	3.3	0.15
D634	0	0	0.2	0	0.1	0	0	0	1.5	0
D635	0	0.1	0.3	0	0.8	0	0	0	2.1	0
D636	0	0.1	0.1	0	0.1	0	0	0	1.2	0
D637	0.17	0.6	0.73	0.03	1.47	0.2	0.1	0.13	2.2	0.13
D638	0	0.1	0.1	0	0.1	0	0	0	1.2	0
D639	0.1	0.87	0.83	0.03	1.5	0.27	0.1	0.17	2.27	0.17
D546	0	0.1	0.1	0	0.1	0	0	0	1.3	0
D547	0.2	0.4	0.5	0	1.8	0.3	0.1	0.1	3.9	0.2
D548	0	0.1	0.2	0	0.2	0	0	0	1.8	0
D549	0.6	0.6	1.5	0	4.8	0.6	0.3	0	8.7	0.3
D550	0.2	0.2	0.6	0	1	0.1	0	0.1	6.1	0.1
D551	0	0.1	0.2	0	0.1	0	0	0	1.2	0
D552	0.6	1.4	1.4	0	5.8	0.9	0.3	0.5	4.3	0.6
D553	0.2	0.4	0.8	0	1.3	0.2	0	0.1	5.7	0.2
D554	0	0.1	0.2	0	0.1	0	0	0	1.4	0
D555	0.7	0.7	1.4	0	2.8	0	0	0	17.5	0.7
D556	0	0.1	0.2	0	0.1	0	0	0	1.6	0
D557	0.7	0	1.4	0	5.6	0	0	0	25.9	0.7
D558	0	0.1	0.2	0	0.1	0	0	0	1.3	0
D559	0.2	1	1.4	0	2.1	0.4	0.2	0.2	4	0.3
D560	0	0.1	0.2	0	0.1	0	0	0	1.7	0
D561	0.2	1.1	1.3	0	2	0.3	0.2	0.2	4.9	0.3
D566	0	0.1	0.2	0	0.1	0	0	0	1.8	0

Table 5B. (Continued)

NO	N9191	N3183	N3184	N0200	N9201	N7201	N0210	N6203	N6204	N3203
D567	0.2	1	1.4	0	2.6	0.2	0.1	0.2	6.5	0.3
D568	0	0.1	0.1	0	0.1	0	0	0	2	0
D569	0.2	1.5	2	0	3	0.3	0.2	0.2	6.3	0.3
D570	0	0.1	0.2	0	0.1	0	0	0	1.4	0
D571	0.2	1	1.3	0	2	0.3	0.1	0.2	5.4	0.2
D572	0	0.1	0.1	0	0.1	0	0	0	1.4	0
D573	0.5	1.7	1.6	0	5.2	0.8	0.1	0	6.4	0.6
D574	0	0	0.1	0	0.1	0	0	0	1.4	0
D575	0.2	1.5	1.6	0.1	2.4	0.3	0.1	0.3	5.3	0.3
D576	0	0	0.1	0	0.1	0	0	0	1.4	0
D577	0.2	0.1	0.6	0	2.5	0.2	0.1	0.1	4.4	0.2
D586	0	0	0.1	0	0.1	0	0	0	2	0
D587	0.2	0.1	0.5	0	2.1	0.3	0	0	3.4	0.2
D588	0.3	0.2	0.7	0	1.4	0.2	0	0	7.1	0.2
D589	0	0.1	0.2	0	0.1	0	0	0	1.5	0
D590	0.3	1.4	1.9	0	3.4	0.5	0.1	0.2	5.7	0.3
D591	0.2	0.4	0.7	0	1.1	0.2	0	0	6.4	0.2
D592	0	0	0.1	0	0.1	0	0	0	1.3	0
D593	0.4	0.8	2	0	3.6	0.4	0	0.4	17.2	0.4
D594	0	0	0.1	0	0.1	0	0	0	1.9	0
D595	0.3	0.1	0.8	0	4	0.3	0.3	0.1	5.1	0.3
D596	0.3	0.1	0.8	0	2	0.4	0	0	8.9	0.5
D597	0	0.1	0.1	0	0.1	0	0	0	1.5	0
D598	0.5	1.6	2.2	0.2	5	1	0	0.6	7.1	0.7
D599	0	0.1	0.1	0	0.1	0	0	0	1.5	0
D600	0.6	2.4	2.9	0.2	5.7	1	0.4	0.6	6.5	0.8

Table 5B. (Continued)

NO	N3204	N0220	N3205	N9221	N7221	N0230	N3215	N9231	N6224	N3223
D602	0	0.2	3.1	0	0	0	0	0	0	0
D603	0.4	0.9	13.5	1.6	0.3	0.2	0.3	0	1	0
D604	0.1	0.3	11.1	0	0.2	0	0.1	0	0.2	0
D605	0	0.1	2.8	0	0	0	0	0	0	0
D606	0.2	0.4	7.5	0.5	0.2	0.1	0.1	0	0.3	0
D607	0.1	0.4	10.9	0	0.1	0	0.1	0	0.4	0
D608	0	0.3	2.6	0	0	0	0	0	0	0
D609	0	0.2	5.2	0	0.2	0	0.1	0	0	0
D610	0	0.1	2.9	0	0	0	0	0	0	0
D611	0.9	1	15.9	0.9	0.4	0.3	0.6	0	0.8	0
D612	0	0.1	2.7	0	0	0	0	0	0	0
D613	0.2	0.4	9.3	0.4	0.2	0.1	0.2	0	0.3	0
D614	0	0.3	2.5	0	0	0	0	0	0	0
D615	0	0.2	3	0	0.1	0	0	0	0	0
D616	0	0.1	2.5	0	0	0	0	0	0	0
D617	0.33	0.33	5.27	0.2	0.13	0.1	0.23	0	0.23	0
D618	0	0.2	2.6	0	0	0	0	0	0	0
D619	0.2	0.4	7.1	0.2	0.2	0.1	0.2	0	0.2	0
D620	0	0.2	2.5	0	0	0	0	0	0	0
D621	0.7	0.8	14.6	0.9	0.3	0.2	0.7	0	0.6	0
D622	0	0.2	3	0	0	0	0	0	0	0
D623	0	0	4.5	0	0.1	0	0	0	0	0
D624	0.1	0.3	13.2	0.1	0.2	0	0.2	0	0.2	0
D625	0	0.2	2.8	0	0	0	0	0	0	0
D626	0	0.2	4.9	0.1	0.1	0	0	0	0.1	0
D627	0.2	0.4	9.7	0.1	0.1	0.1	0.1	0	0.2	0
D628	0	0.1	2.5	0	0	0	0	0	0	0
D629	0.3	0.33	6.77	0.43	0.1	0.07	0.2	0	0.4	0
D630	0	0.1	2.6	0	0	0	0	0	0	0
D631	0.25	0.35	6.65	0.55	0.15	0.1	0.15	0	0.4	0
D632	0	0.1	2.6	0	0	0	0	0	0	0
D633	0.35	0.5	7.45	0.4	0.15	0.15	0.25	0	0.35	0
D634	0	0.2	2.5	0	0	0	0	0	0	0
D635	0	0.1	2.1	0	0.2	0	0	0	0.1	0
D636	0	0.1	2.8	0	0	0	0	0	0	0
D637	0.27	0.3	5.4	0.27	0.1	0.07	0.23	0	0.27	0
D638	0	0.1	2.5	0	0	0	0	0	0	0
D639	0.4	0.3	6.7	0.33	0.17	0.1	0.3	0	0.3	0
D546	0	0.1	2.5	0	0.1	0	0	0	0	0
D547	0.2	0.4	5	0.4	0.2	0.1	0	0.2	0.3	0
D548	0	0.2	3	0	0.1	0	0	0	0	0
D549	0.3	0.9	14.7	1.2	0.3	0.3	0	0.3	0.3	0
D550	0.1	0.4	11.4	0.2	0.1	0	0	0.1	0.2	0
D551	0	0.1	2.3	0	0.1	0	0	0	0	0
D552	0.9	0.9	8.2	0.9	0.3	0.2	0.7	0	1	0
D553	0.3	0.3	12.7	0	0.1	0	0	0.2	0.3	0
D554	0	0.1	2.6	0	0.1	0	0	0	0	0
D555	0	1.4	18.9	0	0.7	0	0.7	0	0	0
D556	0	0.1	2.5	0	0.1	0	0.1	0	0	0
D557	0	1.4	32.2	1.4	0.7	0	0.7	0	0.7	0
D558	0	0.1	2.3	0	0.1	0	0.1	0	0	0
D559	0.5	0.6	7.8	0.4	0.2	0.2	0.3	0	0.4	0
D560	0	0.3	2.5	0	0.1	0.1	0	0	0	0
D561	0.5	0.5	8.7	0.4	0.2	0.2	0.4	0	0.4	0
D566	0	0.3	2.8	0	0.1	0	0	0	0	0

Table 5B. (Continued)

```
-------------------------------------------------------------------
NO    N3204 N0220 N3205 N9221 N7221 N0230 N3215 N9231 N6224 N3223
-------------------------------------------------------------------
D567  0.5   0.6   11.1  0.6   0.1   0.1   0.3   0     0.5   0
D568  0     0.1   2.8   0     0     0     0     0     0     0
D569  0.7   0.7   13.8  0.7   0.2   0.2   0.5   0     0.7   0
D570  0     0.1   2.7   0     0     0     0     0     0     0
D571  0.4   0.6   10    0.4   0.1   0.1   0.3   0     0.4   0
D572  0     0.1   3.7   0     0     0     0     0     0     0
D573  1     1     15.7  1     0.3   0.2   0.7   0     1     0
D574  0     0.1   2.5   0     0     0     0     0     0     0
D575  0.6   0.7   12.1  0.4   0.2   0.1   0.5   0     0.6   0
D576  0     0.1   2.2   0     0     0     0     0     0     0
D577  0.1   0.5   5.3   1     0.2   0.1   0.1   0     0.3   0.1
D586  0     0.1   3.1   0     0     0     0     0     0     0
D587  0     0.2   5.1   0.3   0.2   0     0     0     0.1   0
D588  0.1   0.4   10.7  0.1   0     0     0     0     0.2   0
D589  0     0.2   3     0     0     0     0     0     0     0
D590  0.7   0.6   13.5  0.3   0     0.2   0.5   0     0.6   0
D591  0.2   0.3   13.8  0     0     0     0.1   0     0.2   0
D592  0     0.1   2.3   0     0     0     0     0     0     0
D593  0.4   1.6   21.2  0.4   0.4   0.8   0.4   0     0.8   0
D594  0     0.2   2.9   0     0     0     0.1   0     0     0
D595  0.1   0.5   7     1.1   0.2   0.1   0.2   0     0.4   0
D596  0.1   0.4   13.1  0.8   0.2   0.1   0.2   0     0.4   0
D597  0.1   0     2.7   0     0     0     0     0     0.1   0
D598  1.2   0.7   16.6  0.9   0.3   0.2   0     0     0.9   0
D599  0     0.1   2.8   0     0     0     0     0     0     0
D600  1.3   1     17.3  1.2   0.4   0.3   1     0     1.1   0
-------------------------------------------------------------------
```

Table 5B. (Continued)

NO	N6225	N3224	N0240	N3225	N9241	N3226
D602	0.2	0	0	0.1	0	1.1
D603	3.1	0	0	0.3	0	2.3
D604	1	0	0	0	0	0.8
D605	0.2	0	0	0	0	1.2
D606	1.1	0	0	0.2	0	0.8
D607	1.4	0	0	0	0	1.1
D608	0.1	0	0.1	0	0	1.1
D609	0.4	0	0	0	0	0.3
D610	0.2	0	0	0	0	1.1
D611	4.2	0	0	0.5	0	3.6
D612	0.2	0	0	0.1	0	3.8
D613	1.2	0	0	1.1	0.5	21.3
D614	0.1	0	0	0.2	0	2.5
D615	0.2	0	0	0.1	0	4.6
D616	0.1	0	0	0.1	0	3.6
D617	1.03	0.03	0.03	1.23	0.27	16.13
D618	0.1	0	0	0.1	0.1	2.9
D619	1.4	0	0	1	0.3	18.2
D620	0.1	0	0	0	0	2
D621	2.6	0	0.1	3	0.8	26.9
D622	0.1	0	0	0	0	2.8
D623	0.2	0	0	0	0	4.9
D624	0.8	0	0	0.8	0	20.3
D625	0.1	0	0	0.1	0.1	3.2
D626	0.5	0	0	0.2	0.3	8.9
D627	1.1	0	0	0.9	0	22.1
D628	0.1	0	0	0.1	0	2.8
D629	1.47	0	0.03	1.53	0	24.37
D630	0.2	0	0	0.1	0	4
D631	1.4	0	0.05	0.85	0.3	21.95
D632	0.1	0	0	0.1	0	3.4
D633	1.45	0	0.05	1.35	0.45	23.05
D634	0.1	0	0	0	0.1	2.6
D635	0.2	0	0	0.2	0.2	4.3
D636	0.1	0	0	0.1	0	2.9
D637	1.1	0	0.03	1.23	0.3	19.17
D638	0.1	0	0	0.1	0	3.6
D639	1.27	0	0.03	1.5	0.3	22.03
D546	0.2	0	0	0.1	0	3.9
D547	0.8	0	0.2	0.6	0.4	10.7
D548	0.1	0	0.1	0.1	0.1	2.8
D549	1.8	0	0.3	0.9	0.9	25.8
D550	0.9	0	0	0.6	0.1	18.5
D551	0.2	0	0	0.1	0	3.9
D552	4.1	0.1	0.1	3.9	0.9	58.8
D553	1.4	0	0	1.3	0	34.3
D554	0.1	0	0	0.1	0.1	2.7
D555	1.4	0	0	1.4	0.7	30.1
D556	0.2	0	0	0.1	0.1	3.9
D557	2.1	0	0	0.7	0.7	51.8
D558	0.2	0	0	0.1	0.1	3.7
D559	1.9	0	0.4	1.6	0.8	24
D560	0.2	0	0.1	0.1	0.2	3.5
D561	2.1	0	0.3	1.9	0.7	29.3
D566	0.1	0	0	0.1	0.1	3.8

Table 5B. (Continued)

```
-----------------------------------------
NO    N6225 N3224 N0240 N3225 N9241 N3226
-----------------------------------------
D567  2.6   0     0     2     0.7   37
D568  0.1   0     0     0     0.1   3.2
D569  3.5   0     0.1   2.8   1     48.2
D570  0.1   0     0     0.1   0     3.5
D571  2.2   0     0     1.8   0.7   29.4
D572  0.2   0     0.1   0     0     4.2
D573  4.6   0     0.1   4     1.1   68.9
D574  0.2   0     0.1   0     0     3.6
D575  3.2   0     0.1   3     0.9   43.2
D576  0.1   0     0     0     0.1   3.2
D577  0.9   0     0     0.5   0.4   13.4
D586  0.1   0     0     0.1   0     3.8
D587  0.6   0     0     0.3   0.3   40.8
D588  1     0     0     0.9   0.1   26.9
D589  0.2   0     0     0.1   0.1   4.4
D590  3.4   0     0     0.3   0     43
D591  1.4   0     0     0     0     32.2
D592  0.1   0     0     0     0     3.7
D593  2.8   0     0     0.4   0     51.8
D594  0.2   0     0     0     0     4.1
D595  1.3   0     0     0.8   0.4   22.1
D596  1.7   0     0     1.1   0.1   36.5
D597  0     0     0     0.1   0     3.4
D598  3.9   0     0     0.3   0     43.9
D599  0.2   0     0     0     0     4
D600  5.7   0.1   0     0.4   0     45.3
-----------------------------------------
```

```
note: TRT = Treatment:
          1 = Added 3% lipid and 100 mg kg$^{-1}$  astaxanthin,
          2 = Added 3% lipid and 500 mg kg$^{-1}$ astaxanthin,
          3 = Added 8% lipid and 100 mg kg$^{-1}$ astaxanthin and
          4 = Added 8% lipid and 100 mg kg$^{-1}$ astaxanthin,
      LIPID = Lipid levels: 3 = 3% lipid and 8 = 8% lipid,
      ASTA = Astaxanthin levels: 100 = 100 mg kg$^{-1}$ astaxanthin and
          500 = 500 mg kg$^{-1}$ astaxanthin,
      ORG = Shrimp tissues: 1 = Muscle, 2 = Hepatopancreas and
          3 = Ovary and
      N0140 = 14:0 (mg kg$^{-1}$ day weight),
      N5141 = 14:1(n-5) (mg kg$^{-1}$ day weight),
      N0150 = 15:0 (mg kg$^{-1}$ day weight),
      N5151 = 15:1(n-5) (mg kg$^{-1}$ day weight),
      N0160 = 16:0 (mg kg$^{-1}$ day weight),
      N7161 = 16:1(n-7) (mg kg$^{-1}$ day weight),
      N0170 = 17:0 (mg kg$^{-1}$ day weight),
      N7171 = 17:1(n-7) (mg kg$^{-1}$ day weight),
      N0180 = 18:0 (mg kg$^{-1}$ day weight),
      N9181 = 18:1(n-9) (mg kg$^{-1}$ day weight),
      N7181 = 18:1(n-7) (mg kg$^{-1}$ day weight),
      N6182T = 18:2(n-6)t (mg kg$^{-1}$ day weight),
      N6182C = 18:2(n-6)c (mg kg$^{-1}$ day weight),
      N0190 = 19:0 (mg kg$^{-1}$ day weight),
      N6183 = 18:3(n-6) (mg kg$^{-1}$ day weight),
      N9191 = 19:1(n-9) (mg kg$^{-1}$ day weight),
      N3183 = 18:3(n-3) (mg kg$^{-1}$ day weight),
      N3184 = 18:4(n-3) (mg kg$^{-1}$ day weight),
      N0200 = 20:0 (mg kg$^{-1}$ day weight),
```

Table 5B. (Continued)

```
N9201 = 20:1(n-9) (mg kg⁻¹ day weight),
N7201 = 20:1(n-7) (mg kg⁻¹ day weight),
N0210 = 21:0 (mg kg⁻¹ day weight),
N6203 = 20:3(n-6) (mg kg⁻¹ day weight),
N6204 = 20:4(n-6) (mg kg⁻¹ day weight),
N3203 = 20:3(n-3) (mg kg⁻¹ day weight),
N3204 = 20:4(n-3) (mg kg⁻¹ day weight),
N0220 = 22:0 (mg kg⁻¹ day weight),
N3205 = 20:5(n-3) (mg kg⁻¹ day weight),
N9221 = 22:1(n-9) (mg kg⁻¹ day weight),
N7221 = 22:1(n-7) (mg kg⁻¹ day weight),
N0230 = 23:0 (mg kg⁻¹ day weight),
N3215 = 21:5(n-3) (mg kg⁻¹ day weight),
N9231 = 23:1(n-9) (mg kg⁻¹ day weight),
N6224 = 22:4(n-6) (mg kg⁻¹ day weight),
N3223 = 22:3(n-3) (mg kg⁻¹ day weight),
N6225 = 22:5(n-6) (mg kg⁻¹ day weight),
N3224 = 22:4(n-3) (mg kg⁻¹ day weight),
N0240 = 24:0 (mg kg⁻¹ day weight),
N3225 = 22:5(n-3) (mg kg⁻¹ day weight),
N9241 = 24:1(n-9) (mg kg⁻¹ day weight) and
N3226 = 22:6(n-3) (mg kg⁻¹ day weight).
```

Table 6B. Statistical analysis of weight gain of broodstock shrimp *P. monodon* after 4 months of experiment.

Groups	Factors	Pr > F
All	Fish oil	0.2942
	Astaxanthin	0.5002
	Fish oil*Astaxanthin	0.3753
	Sex	0.0001
	Fish oil*Sex	0.4048
	Astaxanthin*Sex	0.0403
	Fish oil*Astaxanthin*Sex	0.5220
Astaxanthin 100 mg kg^{-1}	Sex	0.0001
Astaxanthin 500 mg kg^{-1}	Sex	0.0718
Female	Astaxanthin	0.4390
Male	Astaxanthin	0.0129

Table 7B. Statistical analysis of amount of eggs and spermatozoa of broodstock shrimp *P. monodon* after 4 months of experiment.

Parameters	Factors	Pr > F
Amount of egg	Fish oil	0.0001
	Astaxanthin	0.0001
	Fish oil*Astaxanthin	0.1016
Amount of spermatozoa	Fish oil	0.0001
	Astaxanthin	0.0001
	Fish oil*Astaxanthin	0.3012

Table 8B. Statistical analysis of astaxanthin content in muscle, hepatopancreas, ovary and shell of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.8853
	Astaxanthin	0.0038
	Fish oil*Astaxanthin	0.2451
	Sex	0.4972
	Fish oil*Sex	0.6869
	Astaxanthin*Sex	0.1804
	Fish oil*Astaxanthin*Sex	0.9841
Hepatopancreas	Fish oil	0.5260
	Astaxanthin	0.1110
	Fish oil*Astaxanthin	0.1282
	Sex	0.0102
	Fish oil*Sex	0.4373
	Astaxanthin*Sex	0.0390
	Fish oil*Astaxanthin*Sex	0.2869
Ovary	Fish oil	0.3190
	Astaxanthin	0.0150
	Fish oil*Astaxanthin	0.3277
Shell	Fish oil	0.4100
	Astaxanthin	0.9838
	Fish oil*Astaxanthin	0.5380
	Sex	0.3309
	Fish oil*Sex	0.3238
	Astaxanthin*Sex	0.6984
	Fish oil*Astaxanthin*Sex	0.7114

Table 9B. Statistical analysis of interaction of astaxanthin content in hepatopancreas
of broodstock shrimp *P. monodon* after 4 months of experiment.

Groups	Factors	Pr > F
Astaxanthin 100 mg kg^{-1}	Sex	0.2733
Astaxanthin 500 mg kg^{-1}	Sex	0.0100
Female	Astaxanthin	0.0363
Male	Astaxanthin	0.5367

Table 10B. Statistical analysis of 18:2(n-6) content in muscle, hepatopancreas and
ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.4436
	Astaxanthin	0.4160
	Fish oil*Astaxanthin	0.8105
	Sex	0.5903
	Fish oil*Sex	0.7032
	Astaxanthin*Sex	0.5491
	Fish oil*Astaxanthin*Sex	0.9138
Hepatopancreas	Fish oil	0.3425
	Astaxanthin	0.7693
	Fish oil*Astaxanthin	0.8660
	Sex	0.7056
	Fish oil*Sex	0.5316
	Astaxanthin*Sex	0.7318
	Fish oil*Astaxanthin*Sex	0.7789
Ovary	Fish oil	0.2258
	Astaxanthin	0.5111
	Fish oil*Astaxanthin	0.2375

Table 11B. Statistical analysis of 18:3(n-3) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.4875
	Astaxanthin	0.0853
	Fish oil*Astaxanthin	0.1486
	Sex	0.9944
	Fish oil*Sex	0.4875
	Astaxanthin*Sex	0.8382
	Fish oil*Astaxanthin*Sex	0.6178
Hepatopancreas	Fish oil	0.1058
	Astaxanthin	0.8369
	Fish oil*Astaxanthin	0.5050
	Sex	0.7536
	Fish oil*Sex	0.3307
	Astaxanthin*Sex	0.4654
	Fish oil*Astaxanthin*Sex	0.7132
Ovary	Fish oil	0.4882
	Astaxanthin	0.7238
	Fish oil*Astaxanthin	0.7238

Table 12B. Statistical analysis of 20:4(n-6) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.8354
	Astaxanthin	0.2304
	Fish oil*Astaxanthin	0.7120
	Sex	0.6186
	Fish oil*Sex	0.4483
	Astaxanthin*Sex	0.2990
	Fish oil*Astaxanthin*Sex	0.3139
Hepatopancreas	Fish oil	0.0190
	Astaxanthin	0.2582
	Fish oil*Astaxanthin	0.6335
	Sex	0.0717
	Fish oil*Sex	0.3527
	Astaxanthin*Sex	0.2293
	Fish oil*Astaxanthin*Sex	0.7153
Ovary	Fish oil	0.9642
	Astaxanthin	0.7052
	Fish oil*Astaxanthin	0.1293

Table 13B. Statistical analysis of 20:5(n-3) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.7011
	Astaxanthin	0.4763
	Fish oil*Astaxanthin	0.2343
	Sex	0.3857
	Fish oil*Sex	0.3050
	Astaxanthin*Sex	0.6978
	Fish oil*Astaxanthin*Sex	0.6780
Hepatopancreas	Fish oil	0.0096
	Astaxanthin	0.5347
	Fish oil*Astaxanthin	0.9309
	Sex	0.0846
	Fish oil*Sex	0.9533
	Astaxanthin*Sex	0.3922
	Fish oil*Astaxanthin*Sex	0.9887
Ovary	Fish oil	0.3608
	Astaxanthin	0.6784
	Fish oil*Astaxanthin	0.9881

Table 14B. Statistical analysis of 22:6(n-3) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.0004
	Astaxanthin	0.0301
	Fish oil*Astaxanthin	0.2633
	Sex	0.0681
	Fish oil*Sex	0.0505
	Astaxanthin*Sex	0.2060
	Fish oil*Astaxanthin*Sex	0.5117
Hepatopancreas	Fish oil	0.0001
	Astaxanthin	0.1669
	Fish oil*Astaxanthin	0.8225
	Sex	0.6904
	Fish oil*Sex	0.8134
	Astaxanthin*Sex	0.7759
	Fish oil*Astaxanthin*Sex	0.5373
Ovary	Fish oil	0.0062
	Astaxanthin	0.0232
	Fish oil*Astaxanthin	0.1223

Table 15B. Statistical analysis of saturated fatty acid (Σ Saturated) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.8307
	Astaxanthin	0.5374
	Fish oil*Astaxanthin	0.9711
	Sex	0.2961
	Fish oil*Sex	0.5613
	Astaxanthin*Sex	0.8610
	Fish oil*Astaxanthin*Sex	0.9270
Hepatopancreas	Fish oil	0.2280
	Astaxanthin	0.6913
	Fish oil*Astaxanthin	0.6341
	Sex	0.7968
	Fish oil*Sex	0.8914
	Astaxanthin*Sex	0.6972
	Fish oil*Astaxanthin*Sex	0.1711
Ovary	Fish oil	0.6260
	Astaxanthin	0.5055
	Fish oil*Astaxanthin	0.3632

Table 16B. Statistical analysis of monounsaturated fatty acid (Σ Monoenes) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.9268
	Astaxanthin	0.3949
	Fish oil*Astaxanthin	0.9103
	Sex	0.4480
	Fish oil*Sex	0.5353
	Astaxanthin*Sex	0.7409
	Fish oil*Astaxanthin*Sex	0.9296
Hepatopancreas	Fish oil	0.0130
	Astaxanthin	0.9042
	Fish oil*Astaxanthin	0.5943
	Sex	0.1907
	Fish oil*Sex	0.6299
	Astaxanthin*Sex	0.8074
	Fish oil*Astaxanthin*Sex	0.9506
Ovary	Fish oil	0.2800
	Astaxanthin	0.2229
	Fish oil*Astaxanthin	0.2861

Table 17B. Statistical analysis of n-6 polyunsaturated fatty acid (Σ n-6 PUFA) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.6589
	Astaxanthin	0.1119
	Fish oil*Astaxanthin	0.9396
	Sex	0.8809
	Fish oil*Sex	0.8327
	Astaxanthin*Sex	0.3149
	Fish oil*Astaxanthin*Sex	0.3871
Hepatopancreas	Fish oil	0.0445
	Astaxanthin	0.8908
	Fish oil*Astaxanthin	0.9083
	Sex	0.2863
	Fish oil*Sex	0.7997
	Astaxanthin*Sex	0.8085
	Fish oil*Astaxanthin*Sex	0.8321
Ovary	Fish oil	0.4486
	Astaxanthin	0.7241
	Fish oil*Astaxanthin	0.9292

Table 18B. Statistical analysis of n-3 polyunsaturated fatty acid (Σ n-3 PUFA) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.0011
	Astaxanthin	0.0833
	Fish oil*Astaxanthin	0.4665
	Sex	0.2030
	Fish oil*Sex	0.1051
	Astaxanthin*Sex	0.2370
	Fish oil*Astaxanthin*Sex	0.6657
Hepatopancreas	Fish oil	0.0001
	Astaxanthin	0.4166
	Fish oil*Astaxanthin	0.9062
	Sex	0.8413
	Fish oil*Sex	0.9920
	Astaxanthin*Sex	0.9607
	Fish oil*Astaxanthin*Sex	0.6469
Ovary	Fish oil	0.0096
	Astaxanthin	0.0357
	Fish oil*Astaxanthin	0.1600

Table 19B. Statistical analysis of n-3 highly unsaturated fatty acid (Σ n-3 HUFA) content in muscle, hepatopancreas and ovary of broodstock shrimp *P. monodon* after 4 months of experiment.

Tissues	Factors	Pr > F
Muscle	Fish oil	0.0012
	Astaxanthin	0.0442
	Fish oil*Astaxanthin	0.5566
	Sex	0.2099
	Fish oil*Sex	0.1332
	Astaxanthin*Sex	0.2724
	Fish oil*Astaxanthin*Sex	0.6664
Hepatopancreas	Fish oil	0.0001
	Astaxanthin	0.3948
	Fish oil*Astaxanthin	0.8664
	Sex	0.8494
	Fish oil*Sex	0.9476
	Astaxanthin*Sex	0.9298
	Fish oil*Astaxanthin*Sex	0.5954
Ovary	Fish oil	0.0092
	Astaxanthin	0.0342
	Fish oil*Astaxanthin	0.1577

Table 20B. Fatty acid composition of the experimental diet and squid (means ± s.d., mg g^{-1} dry weight).

Fatty acid	Pelleted diets				Squid
	LFLA	LFHA	HFLA	HFHA	
14:0	4.3 ± 0.2	4.2 ± 0.9	6.1 ± 0.1	5.8 ± 0.1	1.3 ± 0.2
16:0	24.5 ± 1.7	23.1 ± 5.0	29.1 ± 2.0	31.1 ± 0.4	15.3 ± 0.3
16:1(n-7)	4.4 ± 0.2	4.4 ± 1.0	6.6 ± 0.1	6.3 ± 0.0	0.3 ± 0.1
18:0	6.5 ± 0.4	6.8 ± 1.6	8.9 ± 0.0	8.8 ± 0.4	4.5 ± 0.4
18:1(n-9)	17.1 ± 0.5	16.0 ± 1.9	18.4 ± 0.5	19.0 ± 0.3	1.6 ± 0.0
18:1(n-7)	3.0 ± 0.1	3.2 ± 0.4	4.8 ± 0.4	3.7 ± 0.1	0.8 ± 0.1
18:2(n-6)	13.0 ± 0.4	11.2 ± 0.4	12.2 ± 0.7	10.6 ± 0.0	0.1 ± 0.1
19:0	0.3 ± 0.0	0.5 ± 0.1	1.2 ± 0.1	0.5 ± 0.0	0.1 ± 0.0
18:3(n-3)	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.2	1.4 ± 0.0	0.1 ± 0.1
18:4(n-3)	1.5 ± 0.1	1.5 ± 0.3	2.0 ± 0.1	2.0 ± 0.1	0.1 ± 0.1
20:0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	nd[a]
20:1(n-9)	0.6 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.0	0.9 ± 0.3
20:3(n-6)	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	nd
20:4(n-6)	1.5 ± 0.0	1.8 ± 0.3	2.3 ± 0.1	2.3 ± 0.1	3.1 ± 0.2
20:4(n-3)	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.0 ± 0.1
20:5(n-3)	7.3 ± 0.3	8.2 ± 0.8	10.0 ± 0.4	9.9 ± 0.3	5.0 ± 0.3
21:5(n-3)	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.3	0.3 ± 0.0	0.1 ± 0.1
22:5(n-6)	1.0 ± 0.0	1.4 ± 0.2	1.9 ± 0.2	1.8 ± 0.1	1.3 ± 0.1
24:0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	nd
22:5(n-3)	1.2 ± 0.0	1.4 ± 0.1	1.8 ± 0.2	1.7 ± 0.0	0.4 ± 0.1
22:6(n-3)	16.3 ± 0.6	20.6 ± 2.7	27.0 ± 2.3	26.8 ± 0.8	21.0 ± 1.0
Σ Saturated[b]	38.0 ± 2.3	37.2 ± 7.8	49.2 ± 1.9	49.8 ± 0.4	22.4 ± 0.4
Σ Monoenes	26.4 ± 0.6	26.4 ± 3.0	33.5 ± 0.4	32.0 ± 0.1	4.1 ± 0.3
Σ n-6 PUFA	15.8 ± 0.4	14.8 ± 0.1	17.5 ± 0.1	15.4 ± 0.0	4.8 ± 0.2
Σ n-3 PUFA	28.0 ± 0.9	33.7 ± 4.1	43.0 ± 3.5	42.6 ± 1.3	26.8 ± 1.3
Σ n-3 HUFA[c]	25.4 ± 0.8	31.0 ± 3.7	39.5 ± 3.2	39.2 ± 1.2	26.6 ± 1.3

[a]nd: not detected.

[b]Sums include minor fatty acids not shown in table.

[c]≥20:3(n-3).

Table 21B. Fatty acid compositions (means ± s.d., mg g^{-1} dry weight) in muscle, hepatopancreas and ovary of female broodstock *P. monodon*.

Fatty acids	Muscle				Hepatopancreas				Ovary			
	Fish oil (%)		Astaxanthin (mg kg^{-1})		Fish oil (%)		Astaxanthin (mg kg^{-1})		Fish oil (%)		Astaxanthin (mg kg^{-1})	
	3	8	100	500	3	8	100	500	3	8	100	500
14:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	3.6 ± 2.8	5.1 ± 2.9	4.5 ± 2.7	4.3 ± 3.1	1.7 ± 0.2	1.7 ± 0.4	1.6 ± 0.2	1.8 ± 0.4
16:0	2.9 ± 0.5	3.1 ± 0.7	2.9 ± 0.5	3.1 ± 0.7	39.5 ± 28.6	60.2 ± 23.4	53.1 ± 26.4	48.1 ± 29.2	26.5 ± 1.8	30.8 ± 6.2	26.4 ± 4.9	30.9 ± 4.6
16:1(n-7)	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	4.8 ± 3.5	7.1 ± 4.4	6.1 ± 3.9	6.0 ± 4.4	4.0 ± 0.6	4.8 ± 1.0	3.7 ± 0.8	5.0 ± 0.6
18:0	2.5 ± 0.2	2.6 ± 0.3	2.5 ± 0.2	2.6 ± 0.3	9.2 ± 4.7	20.2 ± 12.7	19.6 ± 14.3	11.3 ± 6.1	9.9 ± 1.7	10.7 ± 2.0	10.0 ± 2.0	10.6 ± 1.8
18:1(n-9)	2.2 ± 0.2	2.5 ± 0.5	2.3 ± 0.2	2.4 ± 0.5	21.8 ± 16.2	33.9 ± 16.8	28.1 ± 15.4	28.1 ± 19.2	19.5 ± 2.2	22.6 ± 4.6	18.6 ± 3.8	23.3 ± 2.7
18:1(n-7)	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	4.6 ± 3.3	7.5 ± 3.1	6.2 ± 2.8	6.0 ± 4.0	3.3 ± 0.3	4.1 ± 1.0	3.1 ± 0.7	4.2 ± 0.7
18:2(n-6)	1.4 ± 0.3	1.3 ± 0.6	1.4 ± 0.2	1.3 ± 0.6	10.0 ± 7.3	11.2 ± 10.5	9.5 ± 5.5	11.5 ± 11.0	5.1 ± 0.8	3.1 ± 2.5	4.7 ± 1.9	3.4 ± 2.2
19:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.3	0.8 ± 0.4	0.6 ± 0.3	0.6 ± 0.4	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
18:3(n-3)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.7 ± 0.6	0.9 ± 0.7	0.7 ± 0.6	0.9 ± 0.7	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
18:4(n-3)	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	1.0 ± 0.8	1.5 ± 0.7	1.2 ± 0.6	1.3 ± 0.9	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
20:0	Nd[a]	nd	nd	nd	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	nd	nd	nd	nd
20:1(n-9)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 1.7	3.9 ± 1.5	3.5 ± 1.9	2.7 ± 1.7	1.2 ± 0.1	1.4 ± 0.4	1.1 ± 0.1	1.4 ± 0.4
20:3(n-6)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	0.1 ± 0.0	0.0 ± 0.1	0.1 ± 0.0	0.0 ± 0.1
20:4(n-6)	1.6 ± 0.3	1.5 ± 0.2	1.6 ± 0.3	1.5 ± 0.3	4.4 ± 1.9	9.2 ± 7.1[s]	8.8 ± 7.3	5.4 ± 3.9	6.7 ± 1.0	6.8 ± 1.3	6.5 ± 1.1	6.9 ± 1.2
20:4(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.5 ± 0.5	0.3 ± 0.3	0.4 ± 0.4	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
20:5(n-3)	2.7 ± 0.2	2.7 ± 0.3	2.7 ± 0.2	2.7 ± 0.2	8.6 ± 4.2	14.1 ± 7.9	13.0 ± 8.2	10.3 ± 5.7	11.2 ± 1.5	12.3 ± 1.3	11.5 ± 0.8	12.1 ± 1.8
21:5(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.4 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:5(n-6)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	1.6 ± 1.2	2.5 ± 1.5	2.0 ± 1.3	2.1 ± 1.6	1.1 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.4
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:5(n-3)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.9 ± 0.9	1.0 ± 1.0	1.0 ± 1.1	0.9 ± 0.9	0.4 ± 0.5	0.8 ± 0.5	0.5 ± 0.6	0.7 ± 0.4
22:6(n-3)	2.3 ± 1.1	3.7 ± 0.5	2.5 ± 1.3	3.4 ± 0.7	12.2 ± 10.5	37.8 ± 14.2	20.6 ± 21.3	29.4 ± 14.6	11.1 ± 11.7	29.7 ± 7.2	13.7 ± 16.0	27.6 ± 6.8
Σ Saturated[b]	6.2 ± 0.7	6.5 ± 1.0	6.2 ± 0.7	6.5 ± 0.9	56.4 ± 38.4	92.9 ± 32.5	83.8 ± 39.4	69.0 ± 39.6	41.2 ± 3.4	46.5 ± 8.9	40.9 ± 7.3	46.7 ± 6.7
Σ Monoenes	3.0 ± 0.4	3.6 ± 0.6	3.3 ± 0.5	3.3 ± 0.7	36.0 ± 25.6	57.8 ± 25.5	49.4 ± 24.6	45.7 ± 30.2	28.9 ± 3.3	34.7 ± 6.7	28.2 ± 5.7	35.3 ± 4.3
Σ n-6 PUFA	3.1 ± 0.3	3.0 ± 0.6	3.1 ± 0.3	3.0 ± 0.6	16.6 ± 9.9	24.1 ± 14.0	21.2 ± 9.7	20.0 ± 14.8	13.3 ± 1.7	11.7 ± 2.6	12.9 ± 2.7	12.0 ± 2.1
Σ n-3 PUFA	5.3 ± 1.0	6.7 ± 0.6	5.6 ± 1.2	6.4 ± 0.8	24.1 ± 13.5	56.6 ± 20.6	37.5 ± 27.7	43.8 ± 21.3	24.0 ± 12.6	44.2 ± 8.6	26.91 ± 7.7	41.9 ± 7.9
Σ n-3 HUFA[c]	5.1 ± 1.0	6.5 ± 0.6	5.3 ± 1.2	6.2 ± 0.8	22.4 ± 12.7	54.2 ± 19.9	35.6 ± 27.4	41.6 ± 20.0	23.1 ± 12.5	43.3 ± 8.5	26.01 ± 7.5	40.9 ± 7.9

[a]nd: not detected.
[b]Sums include minor fatty acids not shown in table.
[c]≥20:3(n-3).

Table 22B. Fatty acid compositions (means ± s.d., mg g^{-1} dry weight) in muscle and hepatopancreas of male broodstock *P. monodon*.

Fatty acids	Muscle				Hepatopancreas			
	Fish oil (%)		Astaxanthin (mg kg^{-1})		Fish oil (%)		Astaxanthin (mg kg^{-1})	
	3	8	100	500	3	8	100	500
14:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.1 ± 1.4	5.9 ± 1.9	4.2 ± 2.5	4.1 ± 2.9
16:0	2.6 ± 0.3	2.8 ± 0.6	2.8 ± 0.4	2.6 ± 0.5	22.8 ± 12.5	47.5 ± 18.5	33.3 ± 15.0	39.2 ± 25.8
16:1(n-7)	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	2.3 ± 1.4	6.9 ± 2.6	4.7 ± 2.7	4.8 ± 3.8
18:0	2.4 ± 0.2	2.5 ± 0.3	2.6 ± 0.2	2.4 ± 0.3	6.9 ± 1.5	12.2 ± 2.3	9.6 ± 3.2	9.9 ± 3.7
18:1(n-9)	2.1 ± 0.1	2.2 ± 0.3	2.3 ± 0.2	2.1 ± 0.3	13.0 ± 7.6	28.2 ± 12.6	20.9 ± 9.4	21.5 ± 17.1
18:1(n-7)	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	2.7 ± 1.4	6.2 ± 2.4	4.4 ± 2.1	4.9 ± 3.4
18:2(n-6)	1.5 ± 0.2	1.4 ± 0.4	1.5 ± 0.3	1.3 ± 0.3	7.0 ± 4.7	11.7 ± 5.6	9.8 ± 4.2	9.3 ± 7.2
19:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0.5 ± 0.3
18:3(n-3)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.5 ± 0.4	1.1 ± 0.5	0.9 ± 0.4	0.8 ± 0.7
18:4(n-3)	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.4	1.4 ± 0.4	1.2 ± 0.6	0.9 ± 0.6
20:0	nd[a]	nd	nd	nd	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:1(n-9)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.3 ± 0.6	2.8 ± 1.1	1.9 ± 0.8	2.5 ± 1.5
20:3(n-6)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
20:4(n-6)	1.5 ± 0.4	1.6 ± 0.3	1.6 ± 0.4	1.4 ± 0.1	3.1 ± 1.1	5.4 ± 1.0	4.4 ± 1.7	4.3 ± 1.5
20:4(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	0.4 ± 0.4
20:5(n-3)	2.5 ± 0.1	2.7 ± 0.5	2.6 ± 0.2	2.7 ± 0.5	5.3 ± 2.2	10.6 ± 3.6	8.1 ± 3.6	8.2 ± 4.9
21:5(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.3
22:5(n-6)	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.9 ± 0.6	2.7 ± 1.2	1.8 ± 1.1	2.0 ± 1.6
24:0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.0
22:5(n-3)	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.8 ± 0.5	2.3 ± 1.1	1.5 ± 0.9	1.7 ± 1.5
22:6(n-3)	3.2 ± 0.6	3.6 ± 0.4	3.3 ± 0.5	3.5 ± 0.6	14.7 ± 8.3	37.7 ± 18.1	25.3 ± 14.4	29.1 ± 23.4
Σ Saturated[b]	5.7 ± 0.5	6.1 ± 0.9	6.1 ± 0.7	5.7 ± 0.9	34.4 ± 16.0	71.4 ± 23.8	51.3 ± 22.3	57.7 ± 34.6
Σ Monoenes	2.8 ± 0.2	3.1 ± 0.5	3.1 ± 0.2	2.8 ± 0.4	21.0 ± 11.4	47.2 ± 19.1	33.9 ± 15.8	36.5 ± 26.6
Σ n-6 PUFA	3.1 ± 0.3	3.1 ± 0.5	3.3 ± 0.4	2.8 ± 0.3	11.4 ± 5.7	20.8 ± 7.7	16.6 ± 6.7	16.3 ± 10.3
Σ n-3 PUFA	6.1 ± 0.6	6.6 ± 0.8	6.3 ± 0.5	6.4 ± 1.0	22.4 ± 12.0	54.5 ± 24.1	37.9 ± 20.2	41.6 ± 31.7
Σ n-3 HUFA[c]	5.8 ± 0.6	6.3 ± 0.8	6.0 ± 0.5	6.2 ± 1.0	21.2 ± 11.3	51.9 ± 23.3	35.9 ± 19.2	39.9 ± 30.5

[a]nd: not detected.
[b]Sums include minor fatty acids not shown in table.
[c]≥20:3(n-3).

APPENDIX C

ASTAXANTHIN DETERMINATION

Determination of astaxanthin in feeds by HPLC (Weber, 1988)

Range

Above 1 mg/kg.

Standard solution

Astaxanthin (1.5 µg ml^{-1}): Dissolve approximate 3 mg pure astaxanthin cyst in 10 ml chloroform in a 100-ml volumetric flask and make up to volume with n-hexane. Mix 5 ml of this dilution with 4 ml of chloroform and dilute to 100 ml with n-hexane. To calculate the exact content of this standard solution the absorbance is measured in a spectrophotometer at 470 nm (at the maximum).

E(1%/1cm) = 2100

= Standard absorbance of 1% astaxanthin solution

(weight/volume) in a 1-cm cuvette at 470 nm in n-hexane solvent.

The purity of the standard is checked by HPLC. This standard solution may be stored for 2 days if kept protected from light in a refrigerator.

Procedure

Preparation of samples

Grind pellets in a coffee grinder. Mash feed and powdery premixes can be used as such. Mix well.

Extraction

Declared content above 20 mg astaxanthin/kg:

Weight approximate 10 g of feed or 1 g of premix into a weighed 100-ml volumetric flask, add approximate 100 mg MAXATASE and approximate 80 ml of distilled water. Place the flask for 30 minutes in an ultrasonic water-bath at 50 ℃. Cool to ambient temperature and fill to the mark with distilled water. Weigh again, shake and weigh approximate 10 g of the mixture into a 250-ml volumetric flask. Add 100 ml absolute ethanol, shake, make up almost to volume with dichloromethane and shake. Leave in a dark place until room temperature is re-established. Make up to volume with dichloromethane and mix well.

Declared content lower than 20 mg astaxanthin/kg:

Weigh approximate 50 g of final feed into a tared round-bottom flask, and approximate 200 mg MAXATASE and approximate 150 ml of distilled water, then weigh again. Place the flask for 30 minutes in an ultrasonic water-bath at 50 ℃. Cool to ambient temperature and weigh approximate 10 g of the mixture into a 250-ml volumetric flask. Add 100 ml absolute ethanol, shake, make up almost to volume with dichloromethane and shake. Leave in a dark place until room temperature is re-established. Make up to volume with dichloromethane and mix well.

Purification of the extract by open-column chromatography on silica gel

Preparation of the silica gel column:

Fill a chromatography tube with approximate 10 ml n-hexane/ether (1:1), add 5 g silica gel and bring the silica gel into suspension by a jet of n-hexane/ether (1:1) from a wash bottle. Let the silica gel sediment and keep it covered with solvent all the time.

Chromatography on the silica gel:

Transfer an aliquot (e.g. 25 ml) of the extract (from "Extraction") onto the silica gel and elute with 100-ml n-hexane/ether (1:1). Collect the eluate in a 250-ml round-bottom flask and remove the solvent in a rotary evaporator. Dissolve the dry residue in 5 or 10 ml n-hexane containing 14% acetone.

High-performance liquid chromatography

Pretreatment of the stationary phase in the column:

A solution of phosphoric acid in methanol (1g/100 ml) is pumped through the column filled with silica gel for 1 hour at a flow rate of 1 ml per minute.

HPLC procedure:

Pump mobile phase through the column for at least 30 minutes to equilibrate the system. Determine the peak area (peak height) of the standard by repeated injection of 10 µl of standard solution. The retention time should be about 11 minutes.

Inject 10 to 50 µl of sample solution. Use the net peak heights, or preferably the peak areas, recorded by an integrator, for the calculation.

Specifications for high-performance liquid chromatography

- Column: stainless steel, length 25 cm, inner diameter 4 mm

- Stationary phase: silica gel (e.g. HIBAR prepacked column filled with LiChrosorb Si 60, 5 μm, Merck, Darmstadt, FRG): pretreated with phosphoric acid

- Mobile phase: n-hexane containing 14% acetone, isocratic

- Flow rate: 1.5 ml min^{-1}

- Pressure: approximate 80 bar

- Temperature: ambient

- Injection volume: 10 to 50 μl

- Detection: VIS-detection at 470 nm

- Retention time: canthaxanthin: about 4 minutes;

    astaxanthin (all-E): about 11 minutes;

    (Z)-isomers of astaxanthin: about 12 and 13 minutes

- Run time: 15 minutes

Calculation

The sum of the resulting peak areas form 11 to 13 minutes retention time, calculated by the integrator (or corresponding peak heights obtained on a record of the chromatogram), is compared with the corresponding peak area (height) for the standard solution.

mg total astaxanthin per kg of sample =

Sum of the peak areas (test) x XF x Standard conc. (μg ml^{-1})

Peak area (standard) x Test portion (g)

XF = dilution factor

= theoretical volume in which the sample is dissolved

$$XF = \frac{V_{fin} \times 250 \times W_1 \times 10}{V_{eva} \times W_2 \times V_{inj}}$$

V_{fin} = Volume of the final test solution in n-hexane-acetone

V_{eva} = Volume of the evaporated ether solution

W_1 = Weight of the aqueous suspension

W_2 = Weight of the suspension transferred into the 250-ml flask

V_{inj} = Volume injected

If the extraction method is used for a content lower than 20 mg kg^{-1} (according to "Extraction"): $W_1 = W_2 = 1$

Determination of astaxanthin esters in animal tissues (Weber, 1988)

Acetone extraction:

Cut the tissue in pieces of about 1 cm in length using a pair of scissors or a knife. Weigh 10 to 20 g of the minced tissue into a 200 ml leaker and add

approximate 5 g of magnesium sulfate hydrate (22.5-25% H_2O e.g. Merck No. 5885) for removing water. Add approximate 40 ml of acetone and homogenize the mixture with a rotation homogenizer. Filter the homogenate with partial vacuum through sintered glass. Scrape off the residue and homogenize again with a fresh portion of acetone. repeat this procedure until the filtrate is colourless. Combine the filtrates in a volumetric flask and make up to volume with acetone.

Evaporate half of this extract under partial vacuum at 50 ℃ by means of a rotary evaporator. Dry the residue by repeated addition and evaporation of small of ethanol. Take up the dry residue in an appropriate volume of n-hexane containing 14% of acetone and inject an aliquot of this extract into the HPLC system.

Saponification:

The other half of the acetone extract is saponified. For that purpose transfer the remaining half of the acetone extract into a 250 ml round-bottom flask and evaporate to dryness under partial vacuum at 50 ℃. Redissolve the residue in 60 ml ethanol, 10 ml t-butyl methyl ether, and 5 ml 50% aqueous potassium hydroxide solution and reflux for 30 minutes (water bath, approximate 80 ℃). After saponification, add approximate 20 ml of distilled water to the solution and cool down to ambient temperature.

Ether extraction:

Rinse the content of the flask into a 500 ml separating funnel with approximate 100 ml distilled water and with the first 100 ml of diethyl ether required for the extraction. Shake carefully and de-aerate the funnel several times. As soon as the overpressure is removed shake the content of the funnel vigorously. After complete

separation of the phases transfer the aqueous one into a second separating funnel and extract again with fresh 100 ml diethyl ether as described above. Repeat the extraction a third time. Wash the combined organic phases with distilled water until their pH is neutral (check with pH-paper). Evaporate the neutral ether extract with a rotary evaporator under partial vacuum at 50 ºC. Remove the last traces of water by addition and evaporation of some ethanol. Take up the dry residue in an appropriate volume of n-hexane containing 14% of acetone and inject an aliquot of this extract into the HPLC system.

Calculation:

In the HPLC chromatogram of the non-saponified extract the peaks of the astaxanthin esters occur with a retention time of 1.25 – 4.65 minutes. The peak areas in this chromatographic range are summarized and quantified with the response factor of free astaxanthin. The result gives the "non-corrected amount of esterified astaxanthin", calculated as free astaxanthin.

Peaks, which occur in HPLC chromatogram of the saponified extract with the retention time of astaxanthin exters (1.25 – 4.65 minutes) are in no case due to astaxanthin esters, since astaxanthin is readily liberated from the esters and oxidized to astacene (t_R = 5.1 minute) by the saponification process. These peaks are rather due to carotene (t_R = 1.30 minute) and the alkali-stable keto-carotenoids (echinenone t_R = 2.0 minute, canthaxanthin t_R = 4.1 minute). Therefore, such peaks are quantified with the response factor of free astaxanthin and subtracted from the "non-corrected amount of esterified astaxanthin", determined by HPLC of the non-saponified acetone extract.

Esters of hydroxy-xanthophylls (as those of lutein and zeaxanthin) also cochromatographed with the esters of astaxanthin. If these esters are present in the tissue, the peaks of the free xanthophylls detected in the HPLC chromatogram of the saponified extract will be larger than the corresponding peaks produced by the non-saponified extract. In this case, the xanthophyll esters are quantified from the area difference between the peaks in the chromatograms of the saponified and non-saponified extract using the response factor of free astaxanthin, and subtracted from the "non-corrected amount of esterified astaxanthin".

In summary, the amount of astaxanthin esters is calculated as the "non-corrected" amount of esterified astaxanthin" minus the amount of cochromatographing alkali-stable carotenoids and minus the amount of other xanthophyll esters.
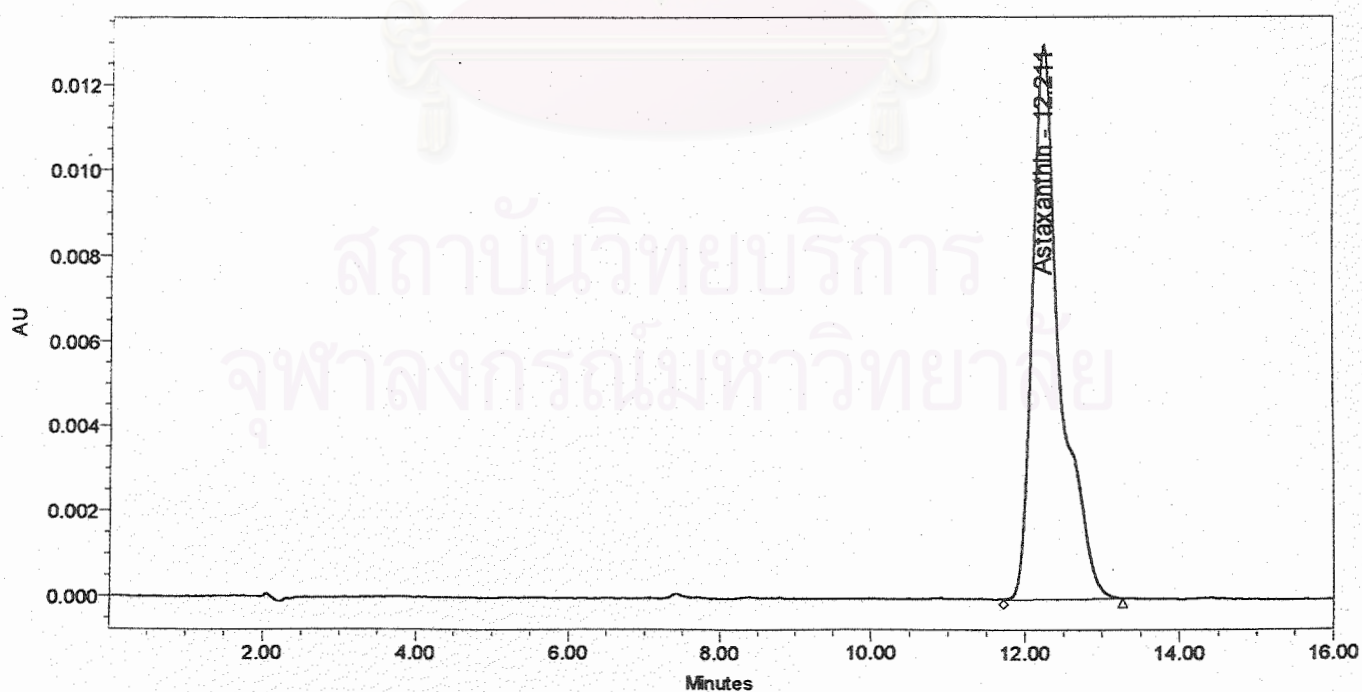
Figure 1C. Chromatogram of standard astaxanthin.

APPENDIX D

FATTY ACIDS DETERMINATION

Determination of fatty acids in feeds and shrimp tissues by GC (Christie, 1989)

FAME-preparation by direct esterification

Amount of product used for FAME analysis between 50-100 mg (dry) and 200-500 mg (wet) sample. Amount of product used for dry weight measurement: 3 x 50 mg (dry), 3 x 100 mg. The detail of product amount and internal standard were showed in **Table 1D**.

Table 1D. Amount of product and internal standard were used for FAME analysis.

Samples	Amount of product	Internal Standard
Artemia	0.20 g - 0.25 g	100 µl 20:2n6 - 5 mg ml^{-1}
Algae	0.10 g - 0.15 g	100 µl 22:2n6 - 15 mg ml^{-1}
Rotifers	0.35 g - 0.40 g	100 µl 20:2n6 - 5 mg ml^{-1}
Emulsion	~ 0.050 g	100 µl 22:2n6 - 15 mg ml^{-1}
Oil	~ 0.010 g	100 µl 22:2n6 - 15 mg ml^{-1}
Fish meal	0.05 g - 0.07 g	100 µl 20:2n6 - 5 mg ml^{-1}
Macrobrachium Larvae	0.15 g - 0.20 g	100 µl 20:2n6 - 5 mg ml^{-1}
Macrobrachium Eggs	0.04 g - 0.05 g	100 µl 20:2n6 - 5 mg ml^{-1}

FAME analysis procedure

- Put the sample in a 35 ml glass tube with a teflon lined screw cap

- Add 5 ml of methanol/toluene mixture (3:2 v/v) Add exactly 0.100 ml of the internal
standard solution (containing 4.78255 mg ml^{-1} 20:2(n-6) or 4.79995 mg 22:2(n-6)

fatty acid dissolved in iso-octane). Add 5 ml of freshly prepared

acetylchloride/methanol mixture (1:20 v/v) as the esterification reagent.

- Flush the tube with nitrogen gas and close tightly.

- Shake tube carefully, make sure that the product doesn't stick too high up the wall of

  the glass tube (to avoid inclompete reaction).

- Put the glass tube in a boiling waterbath (100 °C) for one hour, shaking the tubes

  regularly (every 10 min) - but carefully.

- After one hour, cool down tubes, add 5 ml of distilled water and 5 ml hexane.

- Centrifuge the tube for five minutes, and transfer the upper (hexane) layer into a

  teflon tube. Repeat the hexane extraction two more times with 3 ml of hexane.

- Dry the combined hexane phases by filtering in a pearshaped flask of a known

  weight over an anhydrous sodiumsulphate filter. Evaporate the solvents on a

  rotavapor at 35 °C, flush the remaining solvents with nitrogen gas, and weigh the

  pearshaped flask again.

- The FAME's are finally dissolved in 0.5 ml iso-octane and transferred in a 2 ml

  glass vial with teflon lined screw cap. The vial is flushed with nitrogen and the

  sample is stored in a freezer at -30 °C untill injection.

- For the actual GC anlysis, inject 0.25:l of a dilution in iso-octane, containing 2 mg

  FAME's ml^{-1}. The dillution can be calculated from the difference between the two

  weighings of the pear-shaped flask; The individual FAME-amounts are calculated

  using the known amount of the internal standard as a reference.

Fatty acid composition determination

The total lipids of muscle, hepatopancreas and ovary were extracted according to Folch *et al*. (1957) modified by Ways and Hanahan (1964) using chloroform and methanol (2:1, v/v).

Fatty acid composition of total lipid was determined following the method of Christie (1989) by gas chromatography. Fatty acid methyl esters (FAME) were prepared via a modified procedure of Lepage and Roy (1984). This method implicates a direct acid catalysed transesterification without prior extraction of total fat, on dry sample amounts ranging from 10 to 150 mg. Ten percent of a internal standard 20:2 (n-6) was added prior to the reaction. FAME were extracted with hexane. After evaporation of the solvent the FAME were prepared for injection by redissolving them in iso-octane (2 mg ml^{-1}). Quantitative determination was done by a Chrompack CP9001 gas chromatograph equipped with an autosampler and a TPOCI (temperature programmable on-column injector). Injection (0.2 μl) was done on column into a very polar 50 m capillary column, BPX 70 (SGE Australia), with a diameter of 0.32 mm and a layer thickness of 0.25 μm connected to a 2.5 m methyl deactivated pre-column. The carrier gas was H$_2$, at a pressure of 100 kPa and the detection mode FID. The oven was programmed to increase the initial temperature of 85 ˚C to 150 ˚C at a rate of 30 ˚C min^{-1}, from 150 ˚C to 152 ˚C at 0.1 ˚C min^{-1}, from 152 ˚C to 172 ˚C at 0.65 ˚C min^{-1}, from 172 ˚C to 187 ˚C at 25 ˚C min^{-1} and to stay at 187 ˚C for 7 min. The injector was heated from 85 ˚C to 190 ˚C at 5 ˚C sec^{-1} and stayed at 190 ˚C for 30 min. Identification was based on a standard reference mixture (GLC 68B, NU-Chek Prep, Inc., USA). Integration and calculation were done on computer with the software program "Maestro" (Chrompack).
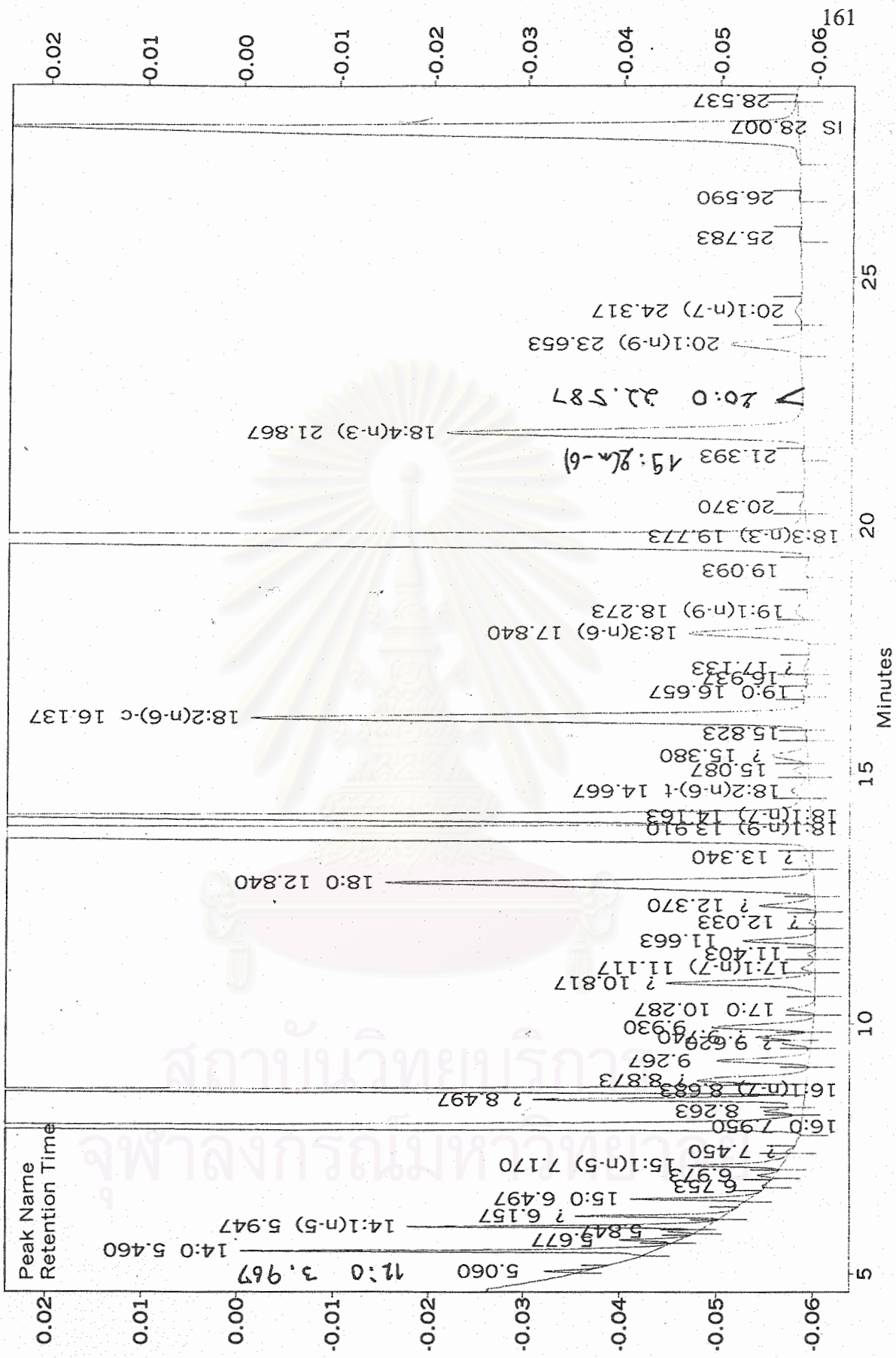
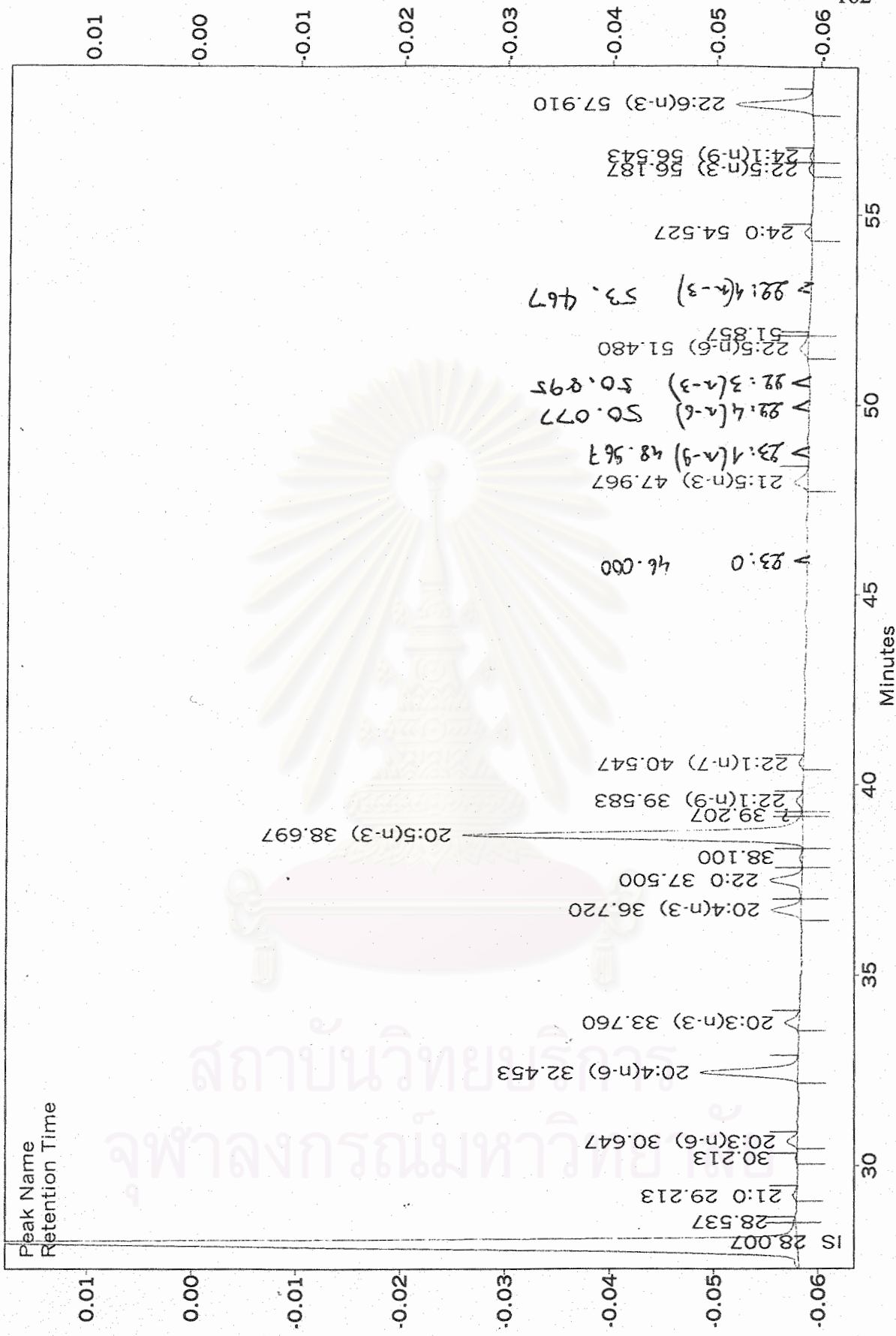**Figure 1D.** Chromatogram of standard fatty acid.

Figure 1D. (Continued)

BIOGRAPHY

Mr. Chalee Paibulkichakul was born on September 13, 1972 in Nakorn Ratchasima Province. He graduated with a Bachelor Degree of Science (Agriculture) from King Monkut Institute of Technology Ladkrabang in 1992 and Master Degree of Science (Marine Science) from Chulalongkorn University in 1996. He studied for the Degree of Doctor of Philosophy in Marine Science at Department of Marine Science, Faculty of Science, Chulalongkorn University since 1998. After graduation, he will serve as a lecturer at Faculty of Marine Technology, Burapa University, Chantaburi Campus.

International oral presentation:

- Effect of astaxanthin on maturation performance in adolescent pond-reared black tiger shrimp *Penaeus monodon*. The Fourth Asia-Pacific Marine Biotechnology Conference. USA: Hawaii. April 22-26, 2002.