CHAPTER III

PROXIMATE COMPOSITION OF FARMED AND WILD SANDWORMS

3.1 Introduction

Marine animals are widely used as food around the world. According to United Nations Food and Agriculture Organization (FAO), Thailand was in the top five producers of global aquaculture production according to 1,144,000 tons of total aquaculture products in 2005. One of the important marine aquaculture business in Thailand is shrimp farming and penaeid shrimps is the most important commercial in both domestic and export markets.

In penaeid shrimps dietary requirements are generally higher in sexually maturing adults (broodstock) than in non-reproductive adults and juveniles (Wouters *et al.*, 2001b). However, the nutritional requirements of broodstock are less understood than the requirements of the other phases of production. Many researchers try to find out the nutritional requirements and to develop a suitable artificial diet for maturation of broodstock (Coman *et al.*, 2007; Marsden *et al.*, 1997; Meunpol *et al.*, 2005). However, the successful diet for broodstock maturation still relies on the nutrition from natural food organisms, such as squid, annelid worms, bivalves, crustaceans and fish (Coman *et al.*, 2007). Because in the wild adult shrimps they eat a variety of invertebrate such as gastropods, bivalves, crustaceans and polychaetes (Wouters *et al.*, 2001a). For this reason, shrimp hatcheries are still using natural organisms as live feed for broodstock, one of those is the sandworm which contains highly unsaturated fatty acids and protein.

Nowadays sandworm (polychaete) is used as live feed for shrimp broodstock to obtain better maturation and oocyte and sperm production especially when worms are in a reproductive stage (Wouters *et al.*, 2001a). The common polychaetes used for shrimp hatcheries in Thailand are sandworm (*Perinereis* sp.) and mudworm (*Marphysa* sp.) (Meunpol *et al.*, 2005). The marine worms for broodstock feed were collected from natural populations on sediment shore and they may be a carrier of pathogens to shrimp broodstock, such as white spot syndrome virus WSSV (Vijayan *et al.*, 2005). Furthermore, the worm collecting activities aggravated environment due to an over-harvesting and destruction of worm habitat and wild worms are depleting. For these reasons, Chunhabundit (1991) collected sandworm (*Perinereis nuntia* Savigny) from natural habitat and grew them with semi-sterile technique and developed to a farming scale in order to reduce the impact of over-harvesting and pathogen-carrier. The SPF (Specific Pathogen Free) farmed sandworms are supplied to shrimp hatcheries especially for broodstock shrimp to avoid from getting pathogens from live feed organisms. Moreover, farmed sandworm is suitable for economic important fish and ornamental fish due to nutritional values and non-pathogen live feed (Sales and Janssens, 2003) and another big market for farmed sandworm is the fishing bait business especially in Asia and Europe (Scaps 2004; Costa *et al.*, 2006). Another potential application was the extraction of active compounds of sandworm which could be used in many applications (Pan *et al.*, 2004; Zhang *et al.*, 2007).

However, farmed sandworm fed with shrimp feed in a commercial farming system differed from wild sandworm which fed by scavenging. In other marine organism such as fish, farmed and wild animals were different in nutritional values depending on a variety of factors including age, diet and environment and many researchers tried to adjust nutritional value of farmed animals to equivalent or better

than those of wild animals (Mnari *et al.*, 2007; Olsson *et al.*, 2003). However, little is known about nutritional value of farmed and wild sandworms. The purpose of this study was to determine the nutritional values of farmed and wild sandworms (*Perinereis nuntia* Savigny). These data will be used as a guideline for quality control of farmed sandworm as live feed for aquatic animals.

3.2 Materials and methods

3.2.1 Equipments

Auto pipette	Gilson, USA
Gas chromatography	Agilent Technology 6890 N, USA
Gas chromatography column	Innowax, USA
Digester	Buchi 425, Switzerland
Distillater	Buchi 315, Switzerland
Freezer –70 °C	Sanyo Ultra Low, Japan
Freeze dryer	Eyela FD550, Japan
Hot air oven	Contherm, New Zealand
Muffle furnace	Carbolite CWF 1200, England
Refrigerator	Sanden Intercool, Thailand
Water bath	Aquatherm, USA

3.2.2 Chemicals

Boric acid	AR grade	Merck, USA
Boron Trifluoride	AR grade	Sigma, USA
Bromocresol green	AR grade	Sigma, USA

Copper sulphate	AR grade	Merck, USA
Ethanol	AR grade	Merck, USA
Heptane	AR grade	Merck, USA
Hydrochloric acid	AR grade	Merck, USA
Methanol	AR grade	Merck, USA
Methyl red	AR grade	Sigma, USA
Petroleum ether	AR grade	Merck, USA
Potassium sulphate	AR grade	Sigma, USA
Sodium chloride	AR grade	Merck, USA
Sodium hydroxide	AR grade	Merck, USA
Standard FAMEs	AR grade	Aldrich, USA
Sulfuric acid	AR grade	Merck, USA

3.2.3 Animals

Live wild sandworm (average weight 2.73 g/worm) was obtained from Bangphra beach, Chonburi province, Thailand in August 2006 and starved for 2 days then it was put on ice and frozen at -70 °C. Four months old farmed sandworm was obtained from a commercial sandworm farm in Samutsongkram province (average weight 0.73 g/worm). Farmed sandworm was fed with a commercial shrimp diet that contained 38% protein and 5% lipid. Upon arrival, farmed sandworm was starved for 2 days then put on ice and frozen at -70 °C until use. Both groups of sandworm were freeze-dried for proximate composition except moisture was used fresh sample and ash was used dry sample from moisture procedure.

3.2.4 Proximate composition analysis

3.2.4.1 *Protein content by Kjeldahl method*

Two hundred milligrams of freeze dried sandworms or 1 ml of distilled water for blank were placed in the digestion flask. 7 g of catalyst (K_2SO_4 and $CuSO_4$) and 15 ml of conc. H_2SO_4 were then added. The digestion flask was placed into a digestion block and heated gently until boil. Digestion was completed when heat for 45 min and clear solution. After cooling, 50 ml of distilled water was added and then added 40% NaOH enough solution to make a strongly alkaline. The digestion flask was connected to distill bulb on condenser and tip of condenser was immersed in 100 ml of 4% boric acid with 3 drops of indicator (bromocresol green and methyl red, Appendix A) in receiving flask. The mixture was distilled until the solution in receiving flask was raisen to 200 ml and indicator colour was changed from purple to green, receiving flask was removed and titrated with known exactly 0.1 N HCl until end point. The percentage of protein was calculated as equation (1)

%Protein = [normality of hydrochloric acid (N) x volume of hydrochloric acid (ml) x 6.25 x 1.4]/weight of dried sandworms (g) .....(1)

3.2.4.2 *Fat content by Soxhlet extraction*

Two grams of freeze dried sandworms were placed in an extraction thimble, covered with cotton wool and placed all in the middle part of Soxhlet apparatus. The known accurate weight round bottom flask was filled with 200 ml petroleum ether then placed as lower part. The condenser, upper part was attached to pump then three parts of apparatus were assembled. The round bottom flask was placed in water bath at 60 °C. The condensing vapor was filled the middle part and

carried the dissolved fat into the flask by a siphoning. Sandworms were extracted for 8 hr, the apparatus was disconnected and evaporated solvent out from the flask until constant weight. The %fat content was calculated as equation (2).

%Fat = [weight of flask and fat (g) – weight of flask (g)] x 100/
weight of sandworms (g) .....(2)

3.2.4.3 *Moisture and ash contents*

Two grams of fresh sandworms were placed on know accurate weight filter paper. The sandworms were heated in hot air oven at 100 °C for 6 hr, cooled down in desiccators and weighed. It was heated and cooled again every 2 hr until constant weight. The %moisture content was calculated as equation (3).

%Moisture = [weight of fresh sandworms (g)–weight of dry sandworms (g)] x
100/weight of fresh sandworms (g) .....(3)

For ash content, 2 grams of dry sandworms from above procedure were placed into know accurate weight porcelain crucible. The sandworms were heated in muffle furnace at 550 °C for 6 hr, cooled down in desiccators and weighed. It was heated and cooled again every 2 hr until constant weight. The %ash content was calculated as equation (4)

%Ash = [Weight of ash (g)/weight of dry sandworms (g)] x 100 .....(4)

3.2.4.4 *Energy content*

The energy content was estimated according to FAO and calculated as : protein = 4.27 kcal/g and lipid = 9.02 kcal/g (Rosa and Nunes, 2003).

3.2.5 Fatty acid composition

0.02-0.04 gram of lipid extract was placed into a round bottom flask and added 4 ml of 5N NaOH in ethanol, heated for 15 min. The flask was added 4 ml of 14% BF_3/methanol, heated 15 min and added 1 ml of heptane, heated 1 min. After cooling, fatty acid methyl esters (FAMEs) were extracted by addition saturated NaCl into flask until full and collected the upper phase (FAMEs in heptane) to a screw cap tube. Heptane phase was evaporated and dissolved in new heptane before apply to GC.

FAMEs were determined on a Aligent Technologies 6890 N gas chromatography equipped with INNOWAX column, with a temperature gradient (10 °C/min to 180 °C, 5 °C/min to 200 °C, 0.5 °C/min to 205 °C and hold for 2 min, 5 °C/min to 250 °C and hold 5 min) and flame ionization detector. Helium was used as a carrier gas. Individual FAMEs were identified by comparison with known standards. The fatty acid compositions were shown as percentage in total fatty acids as equation (5).

%Fatty acid in total fatty acid = (area fatty acid x 100)/total area .....(5)

3.2.6 Cholesterol content

Cholesterol was analyzed at the Institute of Food Research and Product Development (IFRPD), Kasetsart University, Thailand based on AOAC 2000 method 994.10:Cholesterol in Foods applicable to determination of cholesterol in food and food product using gas chromatography.

3.2.7 Amino acid cmposition

Amino acid were analyzed by Ajinomoto Co., (Thailand) Ltd. base on ISO 13903:the determination of free amino acid in feeding stuffs, using amino acid analyzer equipment.

3.2.8 Minerals and vitamins contents

Minerals and vitamins were analyzed at the Institute of Food Research and Product Development (IFRPD) Kasetsart University, Thailand. Calcium was analyzed based on AOAC 2000 method 984.27, while other minerals were analyzed based on method 999.10:Determination of mineral in Food using Atomic Absorption Spectrophotometer. Vitamin A (beta-carotene) was extracted with hexane (Munzuroglu *et al.*, 2003). Vitamin E and D3 were extracted with acetone : chloroform solution (Qian *et al.*, 1998). Vitamin B1 (thiamin) was extracted with acid phosphatase and papain then oxidized with potassium ferricyanide (Batifoulier *et al.*, 2005). Vitamin C was extracted with metaphosphoric acid and acetic acid, reduction of dehydroacetic acid to ascorbic acid by dithiothretol (Sanchez – Moreno *et al.*, 2003). All vitamins were identified by HPLC and compared with known standards.

3.2.9 Statistical analysis

Results were expressed as the mean ± SD of three separate contents except for amino acid, vitamins and cholesterol. T-test in SPSS for window was used to determine difference of mean between the two groups of sandworm; significance was accepted at 5% level ($p \leq 0.05$).

3.3 Results and discussion

The proximate compositions of farmed and wild sandworms are given in Table 3.1. The value obtained for protein, fat contents in farmed and wild sandworms were not significantly ($P \geq 0.05$) different (51.24 ± 0.69%, 17.80 ± 1.39% and 52.82 ± 0.13%, 17.39 ± 0.45% respectively). The moisture content of wild sandworm (81.29 ± 0.37%) was significantly higher than that of farmed sandworm (76.27 ± 0.40%) while the ash content was in contrary (9.44 ± 0.66% in farmed and 6.71 ± 0.008% in wild). Gross energy in both groups of sandworm were not significantly different (379.38 ± 15.36% in farmed and 382.41 ± 3.68% in wild). The proximate composition of wild sandworm was different from other previous reports; Luis and Passos (1995) reported protein and fat content in marine polycheate, *Nereis diversicolor* were 60% and 4.4% respectively. While Meunpol (2005) reported protein and fat content in wild sand polycheate, *Perinereis* sp. were 63.87% and 14.19%. This may be explained by the difference in diet, environment and methodology. In this study, both groups of worm were starved for 2 days to reduce the interference from diet in the digestive system.

Table 3.1 The proximate composition of farmed and wild sandworms *P. nuntia*

Composition	Farmed	Wild
Protein (%)	51.24 ± 0.69 [a]	52.82 ± 0.13 [a]
Fat (%)	17.80 ± 1.39 [a]	17.39 ± 0.45 [a]
Moisture (%)	76.27 ± 0.40 [a]	81.29 ± 0.37 [b]
Ash (%)	9.44 ± 0.66 [a]	6.71 ± 0.01 [b]
Energy (kcal / 100 g dry weight)	379.38 ±15.36 [a]	382.41 ± 3.68 [a]

Data are mean ± SD with different letters in the same row are significantly different at $P \leq 0.05$.

Fatty acid profile is very important for polychates, for example fatty acid profile in life feed especially in polychaete such as bloodworms and sandworms have been used successfully as a feed source to induce maturation in peneaid shrimp (Lytle *et al.*, 1990; Wouters *et al.*, 2001a). The fatty acid profile of farmed and wild sandworms is listed in Table 3.2. The fatty acid analyses were grouped as saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) while di-, tri-, tetra-, penta- and hexa – onic fatty acid were grouped as polyunsaturated fatty acid (PUFA). The major SFA, MUFA and PUFA identified in both groups of sandworm were C16:0 (palmitic acid), C18:1 (oleic acid) and C18:2 (linoleic acid) respectively. The percentages of total SFA and MUFA of wild sandworm were higher than those of farmed sandworm and were significantly different, but total PUFA in farmed was higher than that in

wild animals. Moreover, AA and DHA contents were not significantly different in both groups of sandworm while EPA in wild animal was significantly higher than that in farmed animal. The other wild polychaetes were reported for fatty acid profile such as *Nereis diversicolor*. It was found that the major SFA, MUFA and PUFA are the same as this report but higher in total PUFA (average 45%) and found the average AA (3.16%) and DHA (0.80%) were in the same order of this study while EPA (7.88%) was different (Luis and Passos, 1995). In contrast, Meunpol (2005) reported that wild *Perinereis* sp. contained EPA (3.94%) which is comparable to this study but AA (6.40%) and DHA (0.54%) were different. The difference in PUFA profile may come from season, habitat, environment and food web of each polychaetes (Luis and Passos, 1995; Meunpol *et al.*, 2005). Furthermore, farmed sandworm is able to biosynthesize de novo AA and DHA even fed with diet contained only EPA. They may be capable of elongation and desaturation of shorter chain unsaturated fatty acids (Costa *et al.*, 2000; Aranyakananda *et al.*, 2005). However, the result above showed that EPA content in wild sandworms was significantly higher than farmed sandworm and EPA is a precursor of prostaglandin in crustacean which required for final maturation and spawning (Yano, 1995). Therefore, diet for farmed sandworm could be improved for higher EPA content in sandworm which may give better maturation of shrimp.

Cholesterol content is also shown in Table 3.2. The result indicated that cholesterol in wild sandworm was higher than that in farmed animal (97.86 and 68.38 mg/100g respectively). In most marine animals, cholesterol was important in oocyte and sperm development (Benkendorff *et al.*, 2005; Lee *et al.*, 2005; Palacios *et al.*, 2000, 2007) and mature animals store cholesterol in tissue for these activities (Buckup

et al., 2008). Therefore, wild sandworm which was older and more mature than farmed sandworm was found to contain higher cholesterol.

Table 3.2 Fatty acid composition (% of total fatty acid) and cholesterol content of farmed and wild sandworms *P. nuntia*

Fatty acid	Farmed	Wild
C 14:0	0.85 ± 0.05 [a]	2.15 ± 0.15 [b]
C 15:0	0.37 ± 0.003 [a]	0.57 ± 0.05 [b]
C 16:0	30.86 ± 1.47 [a]	33.24 ± 1.44 [a]
C 17:0	1.19 ± 0.17 [a]	1.56 ± 0.06 [b]
C 18:0	7.78 ± 0.50 [a]	8.57 ± 0.80 [a]
Total SFA	**41.06 ± 1.75 [a]**	**46.11 ± 2.16 [b]**
C 16:1	3.89 ± 0.24 [a]	6.04 ± 0.40 [b]
C 18:1	8.34 ± 0.10 [a]	13.10 ± 0.47 [b]
C 20:1	3.99 ± 0.36 [a]	1.74 ± 1.60 [b]
Total MUFA	**19.80 ± 0.28 [a]**	**21.77 ± 0.38 [b]**
C 18:2	9.37 ± 0.50 [a]	7.82 ± 0.75 [b]
C 18:3	0.78 ± 0.03 [a]	ND
C 20:2	6.65 ± 1.40 [a]	2.27 ± 0.23 [b]
C 20:4 (AA)	3.34 ± 0.12 [a]	3.08 ± 0.18 [a]
C 20:5 (EPA)	2.97 ± 0.20 [a]	4.17 ± 0.18 [b]
C 22:6 (DHA)	1.54 ± 0.44 [a]	1.07 ± 0.22 [a]
Total PUFA	**24.68 ± 1.38 [a]**	**18.42 ± 1.17 [b]**
Cholesterol (mg/100 g)	68.38	97.86

ND = Not determined

Data are mean ± SD with different letters in the same row are significantly different at $P \leq 0.05$ except cholesterol.

No previous published data on minerals content in polychaete was reported. The minerals content of farmed and wild sandworms were significantly different (Table 3.3). Micro minerals, iron and copper contents of farmed sandworm were significantly higher than those of wild sandworm while manganese and zinc contents in wild sandworm were significantly higher than farmed sandworm. Macro minerals, calcium and magnesium contents of farmed sandworm were significantly higher than in wild sandworm while phosphorus content was in the contrary. There was no significantly different in potassium content. Furthermore these results shown that potassium was the major mineral in both groups of worm because potassium is a essential mineral for osmoregulation in cell. The different of minerals content in farmed and wild sandworms was resulted from diet and environment as other marine organisms (Sriket *et al.*, 2007). The farmed sandworm was cultivated in closed system with semi-sterile sea water while wild sandworm was grown in open system which high mineral rotation of sea water in the ocean.

Table 3.3 Minerasl content in farmed and wild sandworms *P. nuntia*

Minerals (mg/Kg)	**Farmed**	**Wild**
Micro-minerals		
Iron (Fe)	189.03 ± 18.53 [a]	45.23 ± 4.27 [b]
Copper (Cu)	8.89 ± 0.53 [a]	5.34 ± 0.32 [b]
Manganese (Mn)	1.82 ± 0.14 [a]	2.78 ± 0.19 [b]
Zinc (Zn)	9.43 ± 0.26 [a]	56.93 ± 4.53 [b]
Macro-minerals		
Magnesium (Mg)	1430.43 ± 123.29 [a]	387.13 ± 27.89 [b]
Phosphorus (P)	952.83 ± 81.52 [a]	1185.63 ± 91.70 [b]
Potassium (K)	1583.35 ± 156.25 [a]	1646.49 ± 163.44 [a]
Calcium (Ca)	430.81 ± 36.86 [a]	331.13 ± 32.90 [b]

Data are mean ± SD with different letters in the same row are significantly different at $P \leq 0.05$.

The amino acid profile of farmed and wild sandworms is summarized in Table 3.4. The results indicated that the most abundant amino acid in both groups of sandworm was glutamic acid followed by aspartic acid and alanine whereas the less abundant was cystine. The amino acid profile of farmed and wild sandworms was not too much different. It was appeared to be unaffected by diet and environment. As found in another marine organism, amino acid profile in wild and farmed yellow perch fish (*Perca flavescens*) was not different (Gonzalez *et al.*, 2006). However, there was no report on optimal amino acid profile for shrimp broodstock diets (Wouter *et al.*, 2001a.).

The content of vitamin A (Table 3.5) was quite different, wild animal was higher than farmed animal (11.00 and 0.26 μg/g respectively). Variation in the content of carotenoid may from the differences in dietary pigments and habitat. While contents of vitamin C, D3 and E in farmed sandworm (45.20, 0.169 and 29.50 μg/g) were higher than wild sandworm (26.20, 0.093 and 14.70 μg/g) and vitamin B1 was found to be in the same order in both groups of sandworm (0.10 μg/g). No information is available on the contents of vitamin in polychaetes. These research results revealed high content of vitamins C and E in both groups of worm. They may play an important role like in other marine animal. In peneaid shrimp, vitamin C has an important role in immune response (Lee and Shiau, 2002) and in sea bass fish vitamin E is involved in reproductive process in broodstock (Guerriero *et al.*, 2004). While in marine crab, vitamins C and E play an antioxidant role when exposed to free radical (Vijayavel *et al.*, 2004). Same as in *Lipopeneaus vannamei*, both vitamins in cell act as antioxidants protecting the cell from free radical (Wouter *et al.*, 2001b). Therefore, vitamins C and E in sandworms may play important roles for immune response, reproductive process and strong antioxidant.

Table 3.4 Amino acid profile (% of dry tissue) of farmed and wild sandworms *P. nuntia*

Amino acid	Farmed	Wild
Alanine	3.46	3.85
Arginine	3.08	3.39
Aspartic acid	4.63	4.45
Cystine	0.59	0.59
Glutamic acid	7.09	7.62
Glycine	3.17	3.10
Histidine	1.47	1.29
Isoleucine	1.68	1.84
Leucine	3.24	3.51
Lysine	3.03	3.38
Methionine	1.28	1.41
Phenylalanine	1.73	1.74
Proline	2.31	1.78
Serine	1.87	2.00
Threonine	2.05	2.22
Tyrosine	1.57	1.53
Valine	1.88	2.01

Table 3.5 Vitamins content in farmed and wild sandworms *P. nuntia*

Vitamins (μg/g)	Farmed	Wild
Fat soluble vitamins		
Vitamin A (beta-carotene)	0.26	11.00
Vitamin D3	0.17	0.09
Vitamin E	29.50	14.70
Water soluble vitamins		
Vitamin B1	0.10	0.10
Vitamin C	45.20	26.20

Data are mean of duplicate.

3.4 Conclusion

Proximate composition of farmed and wild sandworm was concluded in Table 3.6. Protein and fat contents were not significantly different in both groups of sandworm. However, significantly different was found in fatty acid profile which higher PUFA in farmed than in wild sandworm whereas SFA and MUFA were in contrary. The mineral contents were significantly different in both groups of worm except potassium. Besides, cholesterol and vitamin A were found to be higher in wild than in farmed animal while vitamins C, D3 and E were in contrary. The different in compositions between farmed and wild sandworms may from diets and habitat of worms. Amino acid profile and vitamin B1 in both groups of sandworm were not significantly different. These results revealed important nutritional values of farmed sandworm which could be used directly or improved to be suitable for broodstock

shrimp and help reduce the destruction of environment from worms collecting activities.

Table 3.6 Conclusion of proximate composition in farmed and wild sandworms

Significant different	**Not Significant different**
Moisture (W) Ash (F) EPA (W) SFA (W) MUFA (W) PUFA (F) Mineral except potassium (F and W)	Protein Fat AA DHA Potassium
Different	**Not different**
Vitamin A (beta-carotene) (W)	Amino acid Vitamin B1

F = higher in farmed sandworm

W = higher in wild sandworm