ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Animal Sciences 6 (2): 65-75, 2012
ISSN 1819-1878 / DOI: 10.3923/ajas.2012.65.75

Fatty Acid Profile and Lipid Composition of Farm-raised and Wild-caught Sandworms, *Perinereis nuntia*, the Diet for Marine Shrimp Broodstock

[1]Maneerat Limsuwatthanathamrong, [2]Sarintip Sooksai, [3]Suraphol Chunhabundit, [2]Sajee Noitung, [1,2]Nattaya Ngamrojanavanich and [1,2]Amorn Petsom

[1]Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand
[2]Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, 10330, Thailand
[3]Aquatic Resources Research Institute, Chulalongkorn University, Bangkok, 10330, Thailand

Corresponding Authors: Nattaya Ngamrojanavanich and Sarintip Sooksai, Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, 10330, Thailand Tel: +662-218-8073 Fax: +662-253-3543

ABSTRACT

The total fat content (% (w/w)) of eight-month-old farm-raised sandworms (21.0%), *Perinereis nuntia* which were in the beginning stage of reproductive cell synthesis, was slightly (1.2-fold) higher than those aged 2, 4 and 6 months (17.4%) and ~1.5-fold higher than that found in wild-caught sandworms (13.4%). The ratio of saturated: monounsaturated: polyunsaturated fatty acid (SFA: MUFA: PUFA) in farm-raised worms were ~1.3:1:1, while for wild-caught sandworms these ratios were significantly different at 4:2.2:1 and 2.3:1.5:1 for summer and winter season caught sandworms, respectively. The ratio of arachidonic acid: eicosapentaenoic acid: docosahexaenoic acid (ARA: EPA: DHA) of farm-raised *P. nuntia* of 4, 6 and 8 months age were all similar, at ~1.6:1.3:1, but significantly varied between summer (1.7:1:0) and winter (0.9:1:0.4) wild-caught sandworms. The ratio of n-3: n-6 fatty acids in farm-raised sandworms did not significantly differ between age groups (1:3) or summer wild-caught sandworms (1:3.2) but was much higher in winter season wild-caught sand worms (1:1.7). The different fatty acid profiles between farm-raised and wild-caught sandworms may arise from their respective diets and habitats. The major lipid component was phospholipids, especially Phosphatidylcholine (PC). The most abundant constituent of PC in farm-raised and wild-caught sandworms was PC C16:0/C18:1, but the second and third most abundant PC differed, being PC C16:0/C18:2 and PC C18:0/C18:2 in farm-reared sand worms and PC C16:0/C20:4 and PC C18:0/C20:4 in wild-caught ones. The cholesterol level in wild-caught sandworms (493 mg/100 g dw) was 1.5-fold higher than that of farm-raised sandworms (323 mg/100 g dw).

Key words: Fatty acid profile, *Perinereis nuntia*, phospholipid, polychaete, sandworm

INTRODUCTION

Polychaetes, *Perinereis* sp., are used extensively as live feed for shrimp broodstocks to obtain better maturation and oocyte or sperm production, especially when worms are in the reproductive stage (Wouters *et al*., 2001), due to their qualities in enhancing prawn reproductive performances (Middleditch *et al*., 1979; Lytle *et al*., 1990). The most common polychaetes species that are used

in shrimp hatcheries in Thailand are the sandworms (*Perinereis* sp.) and mudworms (*Marphysa* sp.) (Meunpol *et al*., 2005). Broodstock fed with sandworms yield both a higher number of eggs and a better egg-hatching rate when compared to other commercially available diets (Millamena and Pascual, 1990). One of the reasons that make sandworms of high nutritional quality is that they contain a high content of lipid with a PUFAs profile that is suitable for gonadogenesis of marine shrimps. Concerns in extrapolating these data to early developing stages are (a) marine fish larvae grow much more rapidly than juvenile stages so that the (n-3) PUFA requirement of larvae is likely to exceed that of juveniles, (b) marine fish larvae commonly ingest natural diets rich in phospholipids rather than triacylglycerols, (c) the ratio of DHA: EPA [C22:6 (n-3): C20:5 (n-3)] in the phospholipid naturally consumed by larvae is generally ca. 2:1 whereas the corresponding ratio in triacylglycerol fish oils is generally less than or equal to 1:1 (Sargent *et al*., 1997).

Lipids play an important role in the physiology of most animals. They are an essential source of nutrients and energy reserves during periods of poor feeding and negative energy balance, such as hibernation (mammals), sexual development and breeding (starfish, many vertebrates) and migration (birds, fish and insects) (Giese, 1966). During periods of sufficient feeding and sexual immaturity, several marine invertebrates, including polychaetes, accumulate lipid stores, often as glycerides which are then depleted during maturation and/or starvation (Farmanfarmaian *et al*., 1958; Boolootian, 1963; Giese, 1966; Fish, 1967). Wild-caught sandworms for broodstock feed are typically collected from natural populations on the sediment shore and, as such, they may be carriers of pathogens to shrimp broodstock, such as microsporidia, viruses and bacteria (Vijayan *et al*., 2005). Furthermore, the sandworm collecting activities aggravate the environment due to over-harvesting and destruction of the worm's native habitat, leading to both the depletion of wild sandworm populations and the reduction of wild-caught sandworm availability. For these reasons, Chunhabundit (1991) collected sandworms (*Perinereis nuntia* Savigny) from their natural habitat and cultivated them using a semi-sterile technique and developed this culture system to a farming scale, in order to reduce the impact of over-harvesting and pathogen transmission into broodstocks. However, farm-raised sandworms in a commercial farming system which are fed with shrimp feed, differ markedly from wild-caught sandworms, which feed by scavenging. In other marine organisms, such as fish, farmed and wild animals are known to differ in nutritional values depending on a variety of factors, including their age, diet and environment, leading to attempts to try to improve nutritional value of farmed animals (Olsson *et al*., 2003; Mnari *et al*., 2007). However, no information on the proximate lipid compositions and the values of lipid compositions in wild-caught compared to farm-raised sandworms (*P. nuntia*) is currently available. Therefore, the purpose of this study was to determine the lipid and fatty acid values in farm-raised sandworms at different developmental ages (2, 4, 6 and 8 months) and in wild-caught sandworms that were caught in the summer and the winter seasons. The data will be used as a guideline for the quality control of farmed sandworms as live feed for aquatic animals.

MATERIALS AND METHODS

Experimental animals: At least one hundred grams each of farm-raised sandworms (*P. nuntia*) of 2, 4, 6 and 8 months age was harvested from commercial sandworm farms in Chonburi, Samutsakorn, Chumporn and Rayong provinces, Thailand and three individual cultures were used for analysis. Live wild-caught sandworms of the same species (*P. nuntia*) were collected in the winter and summer seasons from the shore line at Chonburi and Rayong provinces, Thailand and

frozen at -70°C until use and two individual collections were used for analysis. Both groups of sandworms were dried in a hot air oven at 100±5°C until at constant weight.

Fat extraction and fatty acid composition: Two grams of dried sandworms was soxhlet extracted with petroleum ether for at least 10 h at 65°C and then evaporated to dryness (AOAC, 1995). A portion (0.04 g) of extract was derivatized to Fatty Acid Methyl Esters (FAMEs) (IUPAC, 1979) and 50 mL of an internal standard (12.34 mg mL^{-1} of heptadecanoic acid methyl ester; C17:0 methyl ester) was added to 200 mL of each sample for quantitative analyses.

Gas chromatographic (GC) analysis: For identification, FAMEs were determined on an Agilent Technologies 6890 N gas chromatography equipped with INNOWAX capillary column (30 m×0.3 mm i.d., 0.25 μm film thickness) and a Flame Ionization Detector (FID) system. The injector and detector temperatures were set at 150 and 250°C, respectively. Helium was used as the carrier gas at a flow rate of 2.3 mL min^{-1}. The GC chromatographic temperature program was set as follows: initial temperature of 150°C, increasing to 180°C at 10°C min^{-1}, then to 200°C at 5°C min^{-1}, to 205°C at 0.5°C min^{-1} and held at 205°C for 2 min and finally to 250°C at 5°C min^{-1} and then held for 5 min (total runtime of 33 min). Individual FAMEs were identified by comparison with reference standards.

Phospholipid extraction and purification: Lipids were extracted with 2:1 (v/v) ratio of dichloromethane: methanol according to Bligh and Dyer (1959). The dichloromethane phase was harvested and evaporated under a flow of nitrogen, reconstituted with 100 μL of 2:1 (v/v) dichloromethane: methanol and then further diluted with 900 μL of 4:1 (v/v) hexane: isopropanol, of which 5 μL was injected into the HPLC-MS system.

HPLC-MS system: The lipids were separated on a diol column, Nucleosil 100-OH (Macherey-Nagel, Germany), (250 mm×3.0 mm i.d., 5 μm. particles size) with an HP 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA). A linear solvent gradient was used according to the method described (Wang *et al*., 2004) with the following slight modification. A 4:1 (v/v) hexane: isopropanol mixture was used as mobile phase A and mobile phase B was 89.3:1:0.2:0.5 (v/v/v/v) isopropanol: water: formic acid: ammonia. Separation was obtained by using a gradient elution starting at 30% B, increasing to 60% in 22 min, then maintained for another 2 min. After that, mobile phase B was linearly increased to 80% over 11 min and then maintained at 20:80 (v/v) A:B for an additional 28 min. Finally, solvent B was quickly decreased to 30% over 2 min and the column was re-equilibrated for about 10 min before the next sample injection. The flow rate was 0.50 mL min^{-1} and the column temperature was 35°C. The volume of sample injection was 5 μL.

The HPLC system was coupled on-line to an Esquire HCT Ion trap mass spectrometer (ESI-MS) (Bruker Daltonics, GmbH, Germany) with an electrospray ionization source. The analyte eluted from the HPLC column directly entered the MS through a steel ES ionization needle set at 4.5 kV in the negative ion mode. The dry nitrogen gas flow rate was approximately 8.0 L min^{-1} at 300°C. All ion source and ion optic parameters were optimized with respect to the negative ion of the phospholipids standards. The MS data were collected under full scan mode (500-1,000 m z^{-1} at a rate of five spectra for each time point). All chromatograms and spectra of the phospholipids were analyzed using the Data analysis™ software version 3.2 (Bruker Daltonik GmbH, Germany). In this study, absolute phospholipid concentrations cannot be shown due to the differences in the

ionization efficiency and instrument response of the different head groups and also of the unsaturated degree of acyl chains in the phospholipid structure (Koivusalo *et al*., 2001).

Sterol extraction and silyl derivatization: Five grams of dried sandworms were soxhlet extracted with 200 mL of a 2:1 (v/v) dichloromethane: methanol mixture at approximately 5-6 cycles per hour for 24 h and then evaporated to dryness (Li *et al*., 2007). A 0.1 g portion of the extract was transferred to a glass vial, two mL of acetone was added and then a silyl derivatization reaction was performed with 250 μL of BSTFA. The derivatizing reagent and acetone were evaporated under a gentle flow of dry nitrogen and then 2.5 mL of hexane was added to the vial. The concentrated extract was subjected to a florisil column and eluted with hexane. The eluent was concentrated to 1 mL under a gentle flow of dry nitrogen.

Sterol analysis: Sterols were analyzed with an Agilent Technologies 6890 N gas chromatography equipped with a HP-5 column and a FID system. The injector and detector temperatures were set at 290°C and 250°C, respectively. Helium was used as the carrier gas. The flow rate of the carrier gas was 2.3 mL min^{-1}. The capillary column used was a 30 m×0.25 mm i.d., 0.25 μm film thickness. The GC chromatographic temperature program was set as follows: initial temperature was 80°C, held for 1 min, increased to 200°C at 30°C min^{-1}, increased to 205°C at 0.5°C min^{-1}, increased to 260°C at 30°C min^{-1}, increased to 270°C at 4°C min^{-1}, increased to 320°C at 20°C min^{-1} and then held for 10 min (total runtime 31.83 min). Individual sterols were identified by comparison with known standards (Cholesterol, Progesterone, Stigmasterol).

Statistical analysis: Analysis of variance (one-way ANOVA) was used to determine statistical differences between different age cohorts of farm-raised and different seasons of wild-caught sandworms. The Duncan test was performed for multiple comparisons. All references to significant differences are at the 5% level or lower ($p \leq 0.05$).

RESULTS

Fatty acid composition: The total fat content (% (w/w)) in farm-raised sandworms of 2, 4, 6 and 8 months age, and in summer and winter caught wild-sandworms, are summarized in Table 1. The total fat content of farm-raised sandworms was significantly (1.37-fold) higher than that of wild-caught sandworms. The only other significant difference was with eight-month-old farm-raised sandworms which were in the beginning stage of reproductive cell synthesis which had a slightly (1.2-fold) higher total fat content than younger (2-, 4- and 6-months old) sandworms from the same rearing facilities (Table 1).

Fatty acids were grouped as Saturated (SFA), Monounsaturated (MUFA) and Polyunsaturated (PUFA) fatty acids, their yield (mg per g of dry weight) and ratio of fatty acid compositions in both farm-raised and wild-caught sandworms are summarized in Table 2. Focusing on the SFA component, the total SFA trend broadly followed that of total fat content, in that the significantly different ranked order of content was eight-month-old farm-raised>4- and 6-month old farm-raised = summer wild caught>immature (two-month old) farm-raised>winter wild caught sand worms. The clear major SFA component of both farm-raised and wild-caught sandworms was C16:0 (palmitic acid) and it was the significant variations in palmitic acid levels that lead to the differences in total SFA above, except that eight-month-old farm-raised sandworms also had significantly higher C18:0 levels, the second most common SFA in all sandworm samples.

Table 1: Comparison of the total fat level (% (w/w)) in different age cohorts (2, 4, 6 and 8 months) of farm-raised *P. nuntia* sandworms and in summer and winter season wild-caught *P. nuntia*

Farm-raised (age, months)	Fat (%)	Wild-caught (season)	Fat (%)
2	17.3±1.0^{b}	Winter	13.8±1.9^{a}
4	17.4±2.1^{b}	Summer	13.0±3.9^{a}
6	17.4±1.8^{b}	-	-
8	21.0±0.7^{c}	-	-
Average	18.3±2.1^{bc}	Average	13.4±2.8^{a}

Data are shown as the Mean±SD. Within and between columns, values with different superscript letters are significantly different ($p \leq 0.05$)

Table 2: Comparison of fatty acid compositions (mg g^{-1} of dry weight) and the ratio of fatty acid class of farm-raised *P. nuntia* sandworms of 2, 4, 6 and 8 months age and wild-caught *P. nuntia* in summer and winter

		Fatty acid (mg g^{-1} of dry weight)					
		Farm-raised				Wild-caught	
Fatty acid	Feed	2 m	4 m	6 m	8 m	summer	winter
Saturated							
12:0	0.00	0.07±0.00^{b}	0.08±0.08^{b}	0.05±0.00^{b}	0.12±0.02^{b}	0.00^{a}	0.58±0.57^{c}
14:0	2.23	0.98±0.06^{a}	1.10±0.13^{a}	1.12±0.07^{a}	1.41±0.09^{b}	1.41±0.51^{b}	1.62±0.19^{b}
15:0	0.48	0.00^{a}	0.46±0.05^{b}	0.49±0.05^{b}	0.58±0.02^{bc}	0.70±0.18^{c}	0.54±0.06^{bc}
16:0	20.58	30.6±1.7^{a}	35.2±4.6^{a}	35.6±4.0^{a}	44.1±1.2^{b}	37.8±7.7^{ab}	28.0±3.2^{a}
18:0	6.12	9.06±0.5^{b}	10.5±1.4^{b}	10.1±0.8^{b}	11.7±0.3^{c}	7.31±1.96^{a}	6.72±1.07^{a}
Total SFA	29.4	40.7^{a}	47.3^{b}	47.4^{b}	57.9^{c}	47.2^{b}	37.4^{a}
Monounsaturated							
16:1 n-7	2.83	5.30±0.30^{a}	5.85±0.74^{a}	5.28±0.77^{a}	6.70±0.59^{b}	5.85±1.33^{a}	5.58±0.86^{a}
18:1 trans	0.00	3.15±0.18^{a}	4.77±0.55^{b}	4.72±1.10^{b}	5.40±0.43^{c}	4.14±0.92^{b}	3.20±0.61^{a}
18:1 n-9	25.07	24.1±1.4^{b}	13.10±1.50^{a}	14.30±1. 90^{a}	22.50±2.40^{b}	6.51±2.68^{c}	5.55±1.61^{c}
18:1 n-7	2.15	0.00^{a}	8.25±1.14^{b}	7.78±0.91^{b}	10.1±0.8^{b}	8.86±2.35^{b}	7.57±0.79^{b}
20:1 n-9	1.19	0.00^{a}	5.68±0.74^{c}	5.25±0.59^{c}	5.45±0.50^{c}	0.00^{a}	2.83±1.55^{b}
Total MUFA	31.2	32.6^{b}	37.7^{b}	37.3^{b}	50.1^{c}	25.4^{a}	24.7^{a}
Polyunsaturated							
18:2 n-6	24.58	17.30±1.00^{b}	14.80±1.80^{a}	14.20±0.90^{a}	19.40±2.20^{b}	4.10±1.55^{c}	3.78±1.52^{c}
20:2 n-6	0.00	3.45±0.20^{b}	7.35±0.86^{c}	7.31±0.93^{c}	9.21±0.80^{c}	0.00^{a}	2.67±1.46^{b}
20:4 n-6	0.87	4.12±0.23^{ab}	4.58±1.10^{b}	4.54±0.53^{b}	6.30±0.30^{c}	4.79±0.16^{b}	3.92±0.24^{a}
18:3 n-3	2.67	1.31±0.07^{b}	1.27±0.16^{b}	1.30±0.08^{b}	1.76±0.11^{b}	0.00^{a}	0.00^{a}
20:5 n-3	2.45	3.24±0.18^{b}	4.05±0.53^{bc}	3.56±0.42^{b}	4.85±0.56^{c}	2.77±0.16^{a}	4.30±0.28^{bc}
22:5 n-3	0.57	0.00^{a}	0.86±0.11^{b}	0.85±0.07^{b}	0.00^{a}	0.00^{a}	0.00^{a}
22:6 n-3	5.26	1.99±0.11^{b}	2.88±0.37^{bc}	2.77±0.25^{bc}	3.89±0.12^{c}	0.00^{a}	1.76±0.19^{b}
Total PUFA	36.4	31.4^{a}	35.8^{a}	34.6^{a}	45.5^{b}	11.7^{a}	16.4^{b}
SFA:MUFA:PUFA	0.8:0.9:1	1.3:1:1	1.3:1.1:1	1.4:1.1:1	1.3:1.1:1	4:2.2:1	2.3:1.5:1
ARA:EPA:DHA	0.3:1:2	1.3:1:0.6	1.1:1:0.7	1.2:1:0.7	1.3:1:0.8	1.7:1:0	0.9:1:0.4
n-3: n-6	1:2.3	1:3.8	1:2.9	1:3.1	1:3.3	1:3.2	1:1.7
LC: VLC	8.3:1	6.1:1	2.8:1	2.9:1	2.9:1	7.8:1	3.7:1

Data are shown as the Mean±S.D. Within a row, values with different superscript letters are significantly different ($p \leq 0.05$)

Analysis of the MUFA component revealed a slightly different trend in the total MUFA content in that the reduced levels in immature farm-reared sandworms compared to the mature sandworms was not, however, statistically significant, and that there were no differences between summer and

winter wild-caught sandworms leading to a ranked order of eight-month-old farm-raised >> all other farm-reared>>wild caught sandworms. The major MUFA component of farm-raised sandworms, C18:1 n-9 (oleic acid), clearly differed from that of wild-caught sandworms which were more broadly distributed with C18:1 n-7 (vaccenic acid) being slightly more prevalent than oleic acid. Interestingly, the immature farm reared sandworms lacked the larger (C18:1 n-7 and C20:1 n-9) MUFA, and had a significantly higher C18:1 n-9 composition instead.

With respect to the PUFA component, the total PUFA levels showed a slight variation on but somewhat, the same broad trend in that the ranked (significant differences) order was eight-month-old farm-raised >> all other farm-reared >> winter wild-caught > summer wild-caught sandworms. Within farm-raised sandworms C18:2 n-6 (linoleic acid) was clearly the major PUFA. However, the PUFA composition within the wild-caught sandworms was evenly spread with three PUFA slightly dominating (C18:2 n-6, C20:4 n-6 and C20:5 n-3) which varied between summer (C20:4 n-6>C20:5 n-3) and winter (C20:5 n-3>C20:4 n-6).

Focusing upon the fatty acid class composition by dry weight (Table 2), the total fatty acid content of eight-month-old farm-raised sandworms was significantly higher than that found in younger (~1.2-fold) or immature (~1.5-fold) farm-reared sand worms as well as in summer (~1.5-fold) or winter (~2-fold) wild-caught sandworms of the same species (*P. nuntia*). The ratio of SFA: MUFA: PUFA of all farm-raised sandworms were about 1.3:1.1:1, compared to 4:2.2:1 and 2.3:1.5:1 for summer and winter wild-caught sandworms, respectively, with their much lower relative levels of MUFA and PUFA.

The ratio of ARA: EPA: DHA of farm-raised sandworms were about 1.2:1:0.7 compared to 1.7:1:0 and 0.9:1:0.4 for wild-caught *P. nuntia* sandworms in summer and winter, respectively, reflecting the significantly lower levels of DHA in wild-caught sandworms and that the ratio of ARA: EPA in wild-caught sandworms in summer (1.73) was higher than in winter (0.91). While significant differences in the level on n-3 were found between immature and mature farm-reared sandworms and also between summer and winter wild-caught sandworms for n-3 and n-6, the ratio of n-3: n-6 did not vary much between all age cohorts of farm-raised *P. nuntia* and the summer wild-caught ones (~1:3.1), those caught in winter were significantly different (1:1.7). Finally, the ratio of LC: VLC differed significantly between immature and mature farm-raised sandworms and between summer and winter wild-caught *P. nuntia*, with summer and winter caught sandworms resembling immature and mature farm-raised sandworms, respectively.

Phospholipids: The data derived from all age classes of farm-reared sandworms and both summer and winter wild-caught sandworms revealed that Phosphatidylcholine (PC) was the major class of phospholipids in all sandworms. The fatty acid components within the PC pool of each category of sandworms were identified by ESI-MS. The derived data for the three most common PC species from all the chromatograms of each farm-raised and wild-caught sandworm are summarized in Table 3. The most abundant PC species in all sandworms assayed was PC C16:0/C18:1 except for summer wild-caught ones where this was the third most common PC, after PC C18:0/C20:4 and PC C16:0/C20:4. However, the composition of the second and third most prevalent PC's showed a clear split between and within farm-reared (mature vs. immature) and wild-caught (summer vs. winter) *P. nuntia* sandworms.

Sterol composition: The steroid compositions of farm-raised and wild-caught sandworms were analyzed by GC and cholesterol was clearly the major component in the steroid extract of both

Table 3: Comparative of the fatty acid composition of the first, second and third most abundant PC species in farm-raised and wild-caught *P. nuntia* sandworms

	PC species		
Sandworm	First	Second	Third
2 m	C16:0/C18:1	C18:0/C18:2	C16:0/C18:2
4 m	C16:0/C18:1	C16:0/C18:2	C18:0/C18:2
6 m	C16:0/C18:1	C16:0/C18:2	C18:0/C18:2
8 m	C16:0/C18:1	C16:0/C18:2	C18:0/C18:2
summer	C18:0/C20:4	C16:0/C20:4	C16:0/C18:1
winter	C16:0/C18:1	C18:0/C20:4	C16:0/C20:4

Table 4: Comparison of the cholesterol level (mg per 100 g dry weight) in farm-raised *P. nuntia* sandworms of different ages (2, 4, 6 and 8 months) and in summer and winter of wild-caught *P. nuntia*

Farm-raised (age, months)	Cholesterol (mg/100 g dw)	Wild-caught (season)	Cholesterol (mg/100 g dw)
2	252.5^{a}	Winter	522.3^{c}
4	375.9^{b}	Summer	465.9^{c}
6	312.6^{b}	-	-
8	341.2^{b}	-	-
Average	323.9^{b}±15.1		493.3^{c}±19.9

Data are shown as the Mean±SEM. Within a column, values with different superscript letters are significantly different ($p \leq 0.05$)

farm-reared and wild-caught sandworms, with the data (as mg of cholesterol per 100 g dry weight) summarized in Table 4. The cholesterol level was not significantly different between the different age cohorts of mature sandworms, including the eight-month-old ones, but was significantly (~1.3-fold) lower in immature sandworms. However, the cholesterol level of wild-caught sandworms was significantly higher (~1.4- and ~1.5-fold in summer and winter) than that of the mature farm-raised sandworms. No other steroid components were detected in this study.

DISCUSSION

Fatty acid composition: The total fat content level in the farm-raised sandworms was significantly higher than that in wild-caught sandworms. The difference of fat contents might result from their different diets and environment (Sriket *et al*., 2007). However, it is of slight note that there was no significant difference in the total fat contents between sandworms caught in winter when compared to those caught in the summer. The 1.2-fold higher total fat content of eight-month-old farm-raised sandworms, compared to the other younger sandworms likely reflects both that they were at the beginning stage of reproductive cell synthesis and the availability of a plentiful rich diet and so in contrast to diet restricted wild sandworms, they were able to accumulate lipids in response to developmental hormones.

Whilst the major SFA of sandworms was C16:0, the major MUFA and PUFA of farm-raised sandworms differed from those of wild-caught sandworms with C18:1 n-9 and C18:2 n-6 clearly being the major MUFA and PUFA components of farm-raised sandworms, whilst for wild-caught sandworms the constituents were more evenly represented with C18:1 n-7 as the major MUFA and no clear main PUFA component. Somewhat, comparable data have been reported for *Nereis diversicolor*, where the major SFA, MUFA and PUFA were C16:0, C18:1, C18:2 and C20:5 (EPA) (Luis and Passos, 1995). The most abundant fatty acids in farm-raised sandworms were C16:0, C18:1 n-9 and C18:2 n-6, compared to C16:0, C18:1 n-7 and C18:0 in wild caught sandworms. The

high content of C18:1 n-9 and C18:2 n-6 of farm-raised sandworms may come from their diet which, being for farmed animals, has been improved by supplementation with vegetable oil containing C18:1 n-9 and C18:2 n-6.

Fatty acids are an important factor to consider when providing a maturation diet for shrimps. For example, altered fatty acid profiles in their live feed, especially in polychaetes, such as bloodworms and sandworms, has been used successfully to induce maturation in peneaid shrimps (Lytle *et al*., 1990; Wouters *et al*., 2001). The higher total fatty acid content of eight-month-old farm-raised sandworms, improved MUFA and PUFA proportions as well as net levels and a more optimal level of DHA, in farm-raised sandworms compared to wild-caught sandworms, illustrates a potential significant improvement in their use as live feed supplements for shrimp broodstock and general shrimp livestock rearing. Moreover, the components are more stable, avoiding the fluctuations seen between summer and winter collected wild *P. nuntia* sandworms. These seasonal variations have been noted in other marine organisms, such as the higher ratio of ARA: EPA of tropical marine fish species over cold water fish species (JIRCAS, 2006). At a composition level, the fatty acid profiles of farm-raised sandworms did not significantly differ between the age cohorts, whilst those for wild-caught sandworms differed between the summer and winter seasons. Such differences likely arise from the differences in the diets and habitats of the worms and it is known that the fatty acid composition of marine animals is influenced by several factors, including the mode of feeding, gametogenesis and environmental temperature (Cowey and Sargent, 1972). However, they present the problem of a seasonally (as well as other factors) changing nutritional value and thus, probably changing ability to induce optimal broodstock fecundity, in contrast to the farm-raised sandworms.

Phospholipid: The major component of the lipid of both groups of sandworms was phospholipids, which is consistent with that reported for the polychaete, *Neanthes arenaceodentata*, where the lipid component was principally composed of phospholipids (92%), followed by triacylglycerol (3%) and cholesterol (3%) (Lee *et al*., 2005). Wold and co-worker reported that the effect of dietary phospholipid fraction on growth and gut maturation in cod larvae compared more efficiently to those in the neutral lipid fraction (Wold *et al*., 2007). The major phospholipid class of both groups of sandworms in this study was found to be Phosphatidylcholine (PC), as has been reported for *P. cultrifera* oocytes where most of the associated phospholipids were PC (77%), followed by X (non-identified phospholipids, 13.3%) and LPC (lysophosphatidylcholine, 4.2%) (Taki *et al*., 1989). However, the most abundant PC species in wild-caught sandworms consist of C20:4 (ARA) reported to be the important PUFA derived from prostaglandins and known to play an important role in the reproductive cycle of numerous species (D'Croz *et al*., 1988). This result gave the significantly difference of the valuable nutrient of farm-raised and wild-caught sandworms.

Sterol composition: The steroid composition of farm-raised and wild-caught sandworms, as analyzed by GC, was found to be chiefly comprised of cholesterol, at least in the steroid extraction where this was the only steroid isolated. This somewhat concurs with that reported for deep-sea polychaetes, where cholesterol was reported to comprise of some 89-98% of the total sterol level (Phleger *et al*., 2005). Cholesterol is an important membrane component in earthworms (Peterson and Holmstrup, 2000).

Although cholesterol levels did not significantly differ between the different age cohorts of farm-raised sandworms, offering consistency in shrimp diet formulations based upon sandworms,

wild caught shrimps had significantly higher cholesterol levels. In most marine animals, cholesterol is important in oocyte and sperm development (Palacios *et al.*, 2000; Benkendorff *et al.*, 2005; Lee *et al.*, 2005; Palacios *et al.*, 2007) and mature animals store cholesterol in tissues for these activities (Buckup *et al.*, 2008). Therefore, in this one aspect, in contrast to the others, farm-raised sandworms may be less optimal than wild-caught sandworms. It may be possible to raise cholesterol levels in farm-reared sandworms, or else include other cheap cholesterol rich components into the diet formulations.

The outline of the fat composition and levels in farmed *P. nuntia*, in comparison to wild caught sandworms, suggests the optimal use of eight-month old farm-reared *P. nuntia* as live feed for shrimps. However, this database should provide a useful working basis for further optimization by both monitoring these valuable nutrient levels whilst screening for more economical rearing methods, and by attempting to alter (optimize) these nutrient levels and composition by changing the developmental conditions and diet supplements. Of course, for such progress to occur, the optimal composition for each stage of shrimp development, including broodstock rearing for maximum fecundity, also needs to be better characterized at the same time.

CONCLUSIONS

Present finding indicated that the farm-reared sandworms were more stable in their lipid composition, presumably through their more uniform controlled environment and diet and thus potentially more amenable to controlled manipulation. That their fatty acid profiles could be used to improve the quality of sandworms for broodstock shrimps remains to be fully tested but would provide an added benefit in addition to those of a ready supply of pathogen controlled live food for shrimp broodstocks and helping to reduce the destruction of the environment from wild sandworm collecting activities.

ACKNOWLEDGMENTS

This research was funded by The National Research Council of Thailand (NRCT) and the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). The authors wish to thank the Institute of Biotechnology and Genetic Engineering; Department of Chemistry, Faculty of Science; The Halal Science Center and Aquatic Resources Research Institute, of Chulalongkorn University (Bangkok, Thailand) for supporting and resources; Dr. Robert Douglas John Butcher, Publication Counselling Unit, Faculty of Science, Chulalongkorn University for language editing; plus the owners and management of the respective sandworm farms in Thailand for sandworm samples.

REFERENCES

AOAC, 1995. Official Methods of the Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC, USA.

Benkendorff, K., A.R. Davis, C.N. Rogers and J.B. Bremner, 2005. Free fatty acids and sterols in the benthic spawn of aquatic molluscs and their associated antimicrobial properties. J. Exp. Marine Biol. Ecol., 316: 29-44.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Boolootian, R.A., 1963. Reproductive physiology of echinoderms. Proceedings of the 16th International Congress of Zoology, August 20-27, 1963, Washington, DC, USA.

Buckup, L., B.K. Dutra, F.P. Ribarcki, F.A. Fernandes, C.K. Noro, G.T. Oliveira and A.S. Vinagre, 2008. Seasonal variations in the biochemical composition of the crayfish *Parastacus defossus* (Crustacea, Decapoda) in its natural environment. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 149: 59-67.

Chunhabundit, S., 1991. Culturing of sand worm *Perinereis nuntia* var.brevicirris (Grube) utilize for mariculture diet. Proceedings of the 3rd Technical Conference on Living Aquatic Resources, January 17-18, 1991, Chulalongkorn University, Thailand.

Cowey, C.B. and J.R. Sargent, 1972. Fish Nutrition. Adv. Marine Biol., 10: 383-492.

D'Croz, L., L.V. Wong, G. Justine and M. Gupta, 1988. Prostaglandins and related compounds from the polychaete worm *Americonuphis reesei* Fauchald (Onuphidae) as possible inducers of gonad maturation in Penaeid shrimps. Comunicaciones Rev. Biol. Trop., 36: 331-332.

Farmanfarmaian, A., A.C. Giese, R.A. Boolootian and J. Bennett, 1958. Annual reproductive cycles in four species of West Coast starfishes. J. Exp. Zool., 138: 355-367.

Fish, J.D., 1967. The biology of *Cucumaria elongate* (Echinodermata: Holothuroidea). J. Mar. Biol. Assoc. U.K., 47: 129-143.

Giese, A.C., 1966. Lipids in the economy of marine invertebrates. Physiol. Rev., 46: 244-298.

IUPAC, 1979. Standard Methods for the Analysis of Oils, Fats and Derivatives. 6th Edn., Pergamon Press, Paris, pp: 126.

JIRCAS, 2006. Arachidonic acid can help the aquaculture industry to take off in developing countries hatcheries in the tropics require arachidonic acid for stable fry production. Japan International Research Center for Agricultural Sciences, 2005 Annual Report (April 2005-March 2006), Japan, pp: 58-59.

Koivusalo, M., P. Haimi, L. Heikinheimo, R. Kostiainen and P. Somerharju, 2001. Quantitative determination of phospholipid compositions by ESI-MS: Effects of acyl chain length, unsaturation and lipid concentration on instrument response. J. Lipid Res., 42: 663-672.

Lee, R.F., A. Walker and D.J. Reish, 2005. Characterization of lipovitellin in eggs of the polychaete *Neanthes arenaceodentata*. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 140: 381-386.

Li, D., M. Dong, W.J. Shim and N. Kannan, 2007. Application of pressurized fluid extraction technique in the gas chromatography-mass spectrometry determination of sterols from marine sediment samples. J. Chromatogr. A, 1160: 64-70.

Luis, O.J. and A.M. Passos, 1995. Seasonal changes in lipid content and composition of the polychaete Nereis (Hediste) diversicolor. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 111: 579-586.

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

Middleditch, B.S., S.R. Missler, D.G. Ward, J.B. Mcvey, A. Brown and A.L. Lawrence, 1979. Maturation of penaeid shrimp: Dietary fatty acids. Proc. World Maricult. Soc., 10: 472-476.

Meunpol, O., P. Meejing and S. Piyatiratitivorakul, 2005. Maturation diet based on fatty acid content for male *Penaeus monodon* (Fabricius) broodstock. Aquacult. Res., 36: 1216-1225.

Millamena, O.M. and F.P. Pascual, 1990. Tissue lipid content and fatty acid composition of *Penaeus monodon* fabricius broodstock from the wild. J. World Aquacult. Soc., 21: 116-121.

Mnari, A., I. Bouhlel, I. Chraief, M. Hammami, M.S. Romdhane, M. El Cafsi and A. Chaouch, 2007. Fatty acids in muscles and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*. Food Chem., 100: 1393-1397.

Olsson, G.B., R.L. Olsen, M. Carlehog and R. Ofstad, 2003. Seasonal variation in chemical and sensory characteristics of farmed and wild Atlantic halibut (*Hippoglossus hippoglossus*). Aquacult., 217: 191-205.

Palacios, E., A.M. Ibarra and I.S. Racotta, 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannemei* broodstock. Aquaculture, 185: 353-371.

Palacios, E., I.S. Racotta, O. Arjona, Y. Marty, J.R. Le Coz, J. Moal and J.F. Samain, 2007. Lipid composition of the pacific lion-paw scallop, *Nodipecten subnodosus*, in relation to gametogenesis 2. Lipid classes and sterols. Aquaculture, 266: 266-273.

Peterson, S.O. and M. Holmstrup, 2000. Temperature effects on lipid composition of the earthworms *Lumbricus rubellus* and *Eisenia nordenskioeldi*. Soil Biol. Biochem., 32: 1787-1791.

Phleger, C.F., M.M. Nelson, A.K. Groce, S.C. Cary, K. Coyne, J.A.E. Gibson and P.D. Nichols, 2005. Lipid biomarkers of deep-sea hydrothermal vent polychaetes- *Alvinella pompejana, A. caudate, Paralvinella grasslei* and *Hesiolyra bergii*. Deep-Sea Res. I, 52: 2333-2352.

Sargent, J.R., L.A. McEvoy and J.G. Bell, 1997. Requirements, presentation polyunsaturated fatty acids larval feeds and sources of in marine fish. Aquaculture, 155: 117-127.

Sriket, P., S. Benjakul, W. Visessanguan and K. Kijroongrojana, 2007. Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. Food Chem., 103: 1199-1207.

Taki, H., J.L. Baert and A. Dhainaut, 1989. Synthesis and release of vitellogenin-associated phospholipids by the coelomocytes of *Perinereis cultrifera* (Annelida, Polychaeta). Comp. Biochem. Physiol. B Comp. Biochem., 92: 167-173.

Vijayan, K.K., V.S. Raj, C.P. Balasubramanian, S.V. Alavandi, V.T. Sekhar and T.C. Santiago, 2005. Polychaete worms: A vector for white spot syndrome virus (WSSV). Dis. Aquat. Org., 63: 107-111.

Wang, C., S. Xie, J. Yang, Q. Yang and G. Xu, 2004. Structural identification of human blood phospholipids using liquid chromatography/quadrupole-linear ion trap mass spectrometry. Anal. Chim. Acta, 525: 1-10.

Wold, P.A., K. Hoehne-Reitan, C.L. Cahu, J.Z. Infante, J. Rodriguez and E. Kjϕrsvik, 2007. *Phospholipids* vs. neutral lipids: Effects on digestive enzymes in Atlantic cod (*gadus morhua*) larvae. Aquaculture, 272: 502-513.

Wouters, R., P. Lavens, J. Nieto and P. Sorgeloos, 2001. Penaeid shrimp broodstock nutrition: An updated review on research and development. Aquaculture, 202: 1-21.