Effects of live feed *Perinereis nuntia* on prostaglandin biosynthesis and fatty acid regulatory pathways in the black tiger shrimp *Penaeus monodon*

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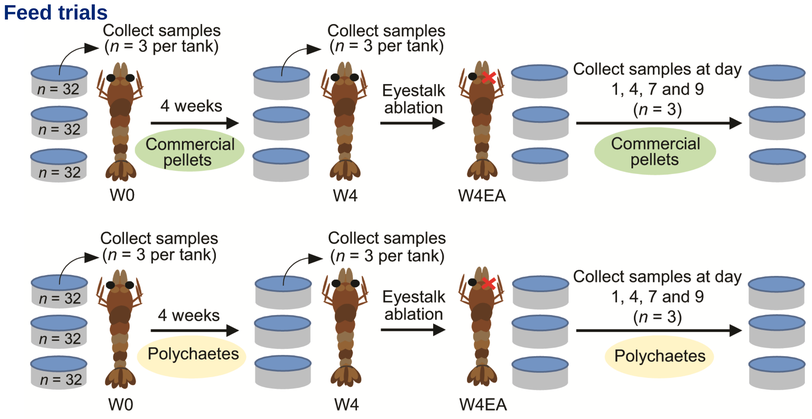
Introduction

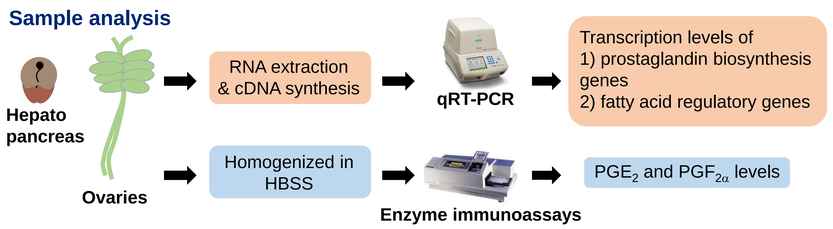
Domestication of the black tiger shrimp *Penaeus monodon* has always been hindered by low ovarian maturation rates in female broodstock. Levels of prostaglandins in ovaries have previously been correlated with ovarian maturation in several crustacean species, including the Florida crayfish *Procambarus paeninsulanus*, the freshwater field crab *Oziotelphusa senex senex* and *P. monodon* (1-4). In hatcheries, shrimp acquired dietary prostaglandins and its precursor, arachidonic acid (ARA), through consumption of polychaetes. This enhanced ovarian maturation rates, increased levels of ARA in hepatopancreases, and altered transcription levels of genes in the crustacean fatty acid regulatory pathway during ovarian development (3, 5-8). Nevertheless, comparative analysis on the effects of feed pellets and polychaetes on shrimp prostaglandin biosynthesis and fatty acid regulatory pathways have yet to be investigated.

Materials and Methods

Feed trials



Sample analysis



Results

To determine the feed effects on shrimp prostaglandin biosynthesis and fatty acid regulatory pathways, levels of nutrients in shrimp feed were determined. GC-FID and enzyme immunoassay analyses revealed that feed pellets had comparable levels of docosahexaenoic acid (DHA), but lower levels of ARA, eicosapentaenoic acid (EPA), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) than polychaetes (Fig 1A-B). After four weeks of feeding, shrimp were subjected to unilateral eyestalk ablation to induce ovarian maturation, revealing that polychaete-fed shrimp were able to reach stage 3 and 4 ovaries whereas pellet-fed shrimp only reached stage 2 ovaries.

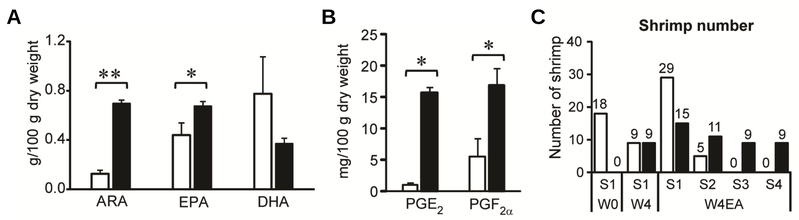


Figure 1 Nutritional assessment of shrimp feed and their effects on shrimp ovarian development. Feed pellets (white bars) and polychaetes (black bars) were subjected to analysis using (A) GC-FID and (B) enzyme immunoassays. (C) Shrimp were harvested at different time points and separated based on feed types and ovarian maturation stages. S1 to S4 indicates ovarian maturation stages 1 to 4. Asterisks indicate significant differences between the two feed groups at $P < 0.05$ (*) and $P < 0.01$ (**).

To assess the effects of polychaete consumption on crustacean prostaglandin biosynthesis, enzyme immunoassays were performed to estimate the levels of PGE_2 and $PGF_{2\alpha}$ in shrimp ovaries and hepatopancreases (Fig. 2). In ovaries, levels of PGE_2 were comparable in shrimp between the two feed types while levels of $PGF_{2\alpha}$ were higher in ovaries of pellet-fed shrimp compared to those of polychaete-fed shrimp. On the other hand, levels of PGE_2 and $PGF_{2\alpha}$ were 15 and 12 times higher, respectively, in hepatopancreases of shrimp fed with polychaetes compared to those fed with feed pellets (Fig. 2C-D).

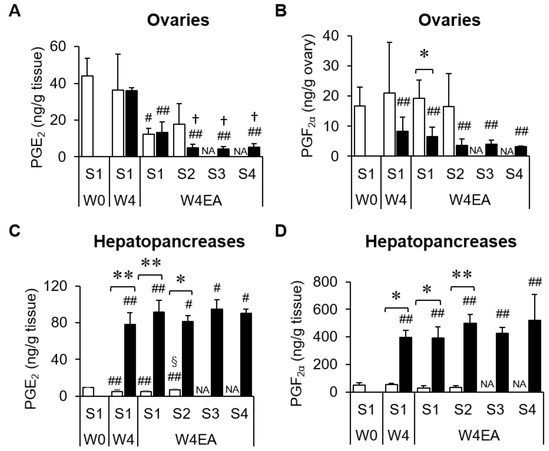


Figure 2 Levels of PGE_2 and $PGF_{2\alpha}$ in shrimp ovaries and hepatopancreases. Levels of PGE_2 (A and C) and $PGF_{2\alpha}$ (B and D) in shrimp fed with feed pellets (white bars) and polychaetes (black bars) were analyzed using enzyme immunoassays. Shrimp ovaries (A-B) and hepatopancreases (C-D) were harvested at different time points and separated based on ovarian maturation stages. NA indicates that there was no shrimp available at the designated stage.

Due to changes in prostaglandin levels in shrimp ovaries and hepatopancreases, quantitative real-time PCR (qRT-PCR) analysis were performed to monitor transcription levels of prostaglandin biosynthesis and fatty acid regulatory genes in these organs (Fig 3 and 4).

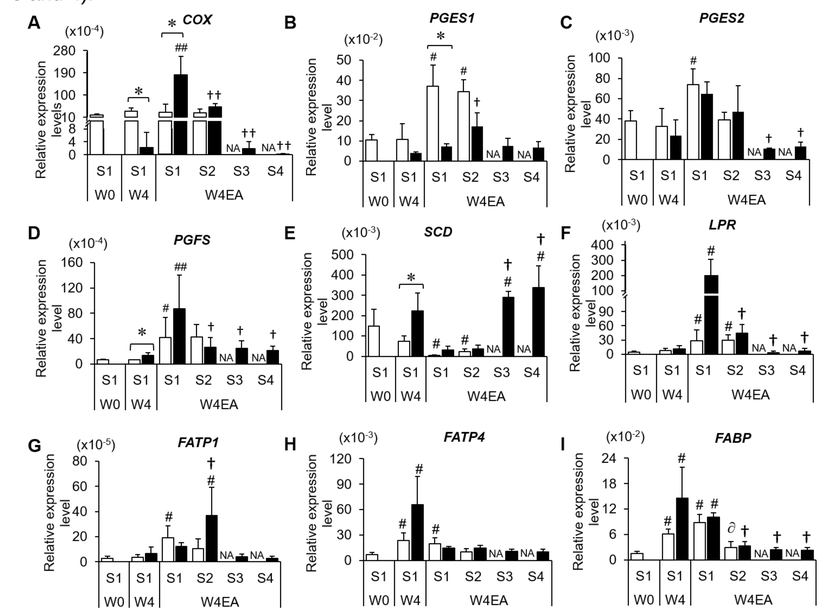


Figure 3 Transcription levels of prostaglandin biosynthesis and fatty acid regulatory genes in shrimp hepatopancreases. Hepatopancreas cDNA of pellet-fed (white bars) and polychaete-fed shrimp (black bars) were subjected to qRT-PCR analysis to assess the transcription levels of genes in the prostaglandin biosynthesis pathway, including (A) *cyclooxygenase*, (B) *prostaglandin E synthase 1*, (C) *prostaglandin E synthase 2* and (D) *prostaglandin F synthase*. Genes in the fatty acid regulatory pathway include (E) *stearoyl-CoA desaturase*, (F) *lipophorin receptor*, (G) *long-chain fatty acid transport protein 1*, (H) *long-chain fatty acid transport protein 4* and (I) fatty acid-binding protein. * indicates a significant difference between pellet-fed and polychaete-fed shrimp. # indicates a significant difference between the designated condition and the control at W0. ∂ and † indicates a significant difference between the designated condition and pellet-fed and polychaete-fed shrimp at W4EAS1, respectively. *, #, ∂ and † represent $P < 0.05$ while **, ##, ∂ ∂ and †† represent $P < 0.01$.

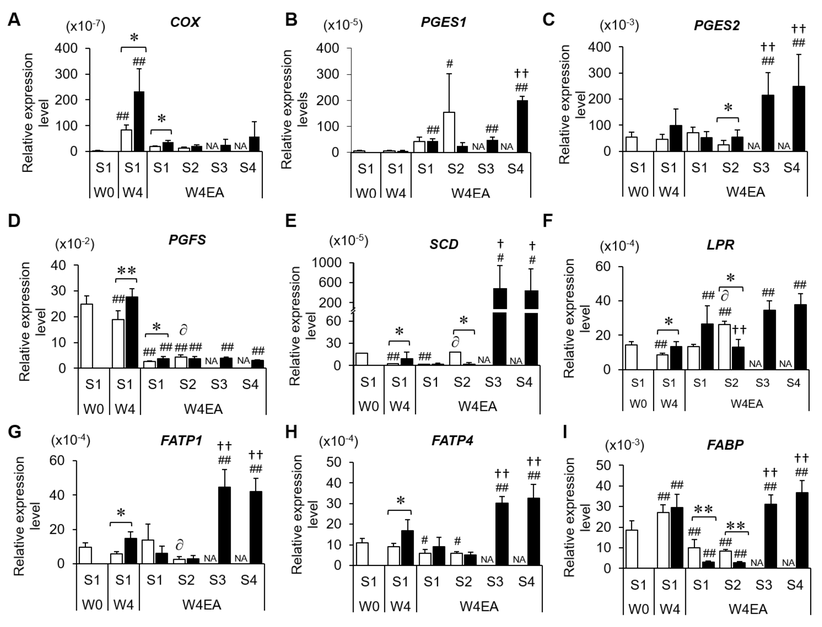


Figure 4 Transcription levels of prostaglandin biosynthesis and fatty acid regulatory genes in shrimp ovaries. Ovary cDNA of pellet-fed (white bars) and polychaete-fed shrimp (black bars) were subjected to qRT-PCR analysis to monitor the transcription levels of genes in the prostaglandin biosynthesis pathway, including (A) *cyclooxygenase*, (B) *prostaglandin E synthase 1*, (C) *prostaglandin E synthase 2* and (D) *prostaglandin F synthase*. Genes in the fatty acid regulatory pathway include (E) *stearoyl-CoA desaturase*, (F) *lipophorin receptor*, (G) *long-chain fatty acid transport protein 1*, (H) *long-chain fatty acid transport protein 4* and (I) fatty acid-binding protein. Description regarding symbols used in this figure can be found in the legend of figure 3.

Discussion

Changes in ovaries and hepatopancreases of shrimp fed with feed pellets and polychaetes can be summarized in Fig 5.

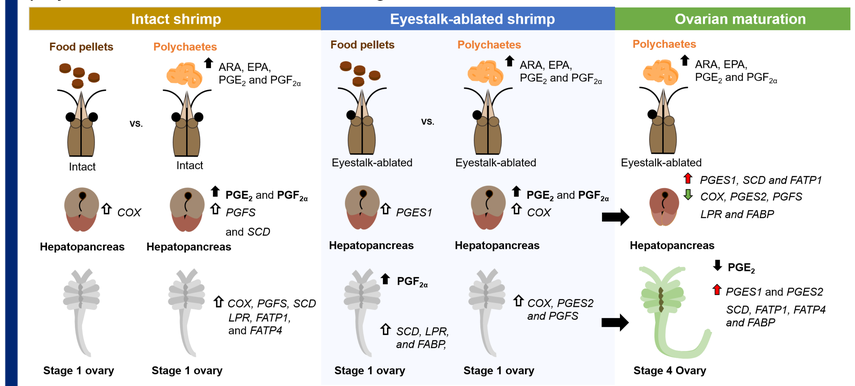


Figure 5 Summary of the effects of shrimp feed on prostaglandin biosynthesis and fatty acid regulatory pathways in *P. monodon*.

References

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Acknowledgement: This work was supported by the National Science and Technology Development Agency (P-11-01054 and P-13-50373); the Thailand Research Fund under the TRF mid-career research grant (RSA6180007) and partially supported by the European Union's Horizon 2020 research and 523 innovation programme under the Marie Skłodowska-Curie (grant number 734486).