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In situ fatty acid production supports shrimp yields in diets lacking fish oil and fishmeal

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Abstract

Using capture fishery-derived fish oil and fishmeal in aquafeeds is unsustainable. This study mimicked semi-intensive shrimp ponds, including primary producers, in mesocosm tanks. Fatty acid mass balances were computed to distinguish between diet-based and primary production-based LC-PUFA contributions to shrimp (*Litopenaeus vannamei*) production and meat quality. Performance and body fatty acid composition were compared of shrimp fed a commercial diet containing fish oil and fishmeal (control), with a fishmeal- and fish oil-free diet (low LC-PUFA diet: LOW). Six mesocosms were each stocked with 60 juvenile shrimp and randomly assigned to the two diets. After an 8-week grow-out period, biomass production, survival and proximate body composition were similar between diets. Control shrimp contained twice as much LC-PUFA and omega-3 fatty acids than LOW shrimp. Large quantitative losses (85%) were found in both treatments of the LC-PUFA-precursors alpha-linolenic acid (ALA) and linoleic acid (LA) that were being used as energy source by the shrimp instead for LC-PUFA synthesis. Whereas losses were also observed for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the control group, there was a gain for these components in the LOW tanks. LOW shrimp sourced at least 32% of their total EPA gain and 15% of their total DHA gain from the algal-based food web. This quantitative analysis of the fate of major dietary fatty acids strongly suggests that the pond's primary production can provide shrimp additional LC-PUFA. Finding a balance between LC-PUFA contribution through formulated feed and natural production seems possible and deserves further research.

KEYWORDS
DHA, EPA, fish oil, fishmeal, Litopenaeus vannamei, mesocosm, omega-3 fatty acids

1 | INTRODUCTION

1.1 | Dependency on fisheries hinders sustainable aquaculture

Aquaculture production needs to reach 80 million MT by 2030 to fulfil the growing global demand for animal protein (Kobayashi et al., 2015). In aquaculture, more than 98% of shrimp are produced in brackish water ponds. In semi-intensive and intensive ponds, the feed is the most expensive input, accounting for half of the production costs (NRC et al., 2011). Unfortunately, some raw feed ingredients such as fishmeal and fish oil—major sources of long-chain polyunsaturated fatty acids (LC-PUFA) for shrimp and fish—are becoming scarce and this may inhibit further aquaculture expansion (Boyd et al., 2007, FAO, 2018). Estimates for 2006 indicate that the aquaculture sector used an equivalent of 16.6 million MT small pelagic forage fish with an overall fish-in–fish-out ratio of 0.7 (Tacon & Metian, 2008). This highlights our inefficient and unsustainable use of natural resources, adding substantial pressure to natural ecosystems. Marine fisheries expanded rapidly since the 80s, and global fishing effort together with the related environmental impact continues to increase. Capture fisheries result in the decline of fish standing stocks and the alteration of life history traits. Effects are not limited to fish but extend often to the entire aquatic food web, including groups such as mammals, turtles, seabirds and the benthic community (Clark & Tilman, 2017; Dayton et al., 1995; Ortuño Crespo & Dunn, 2017). As a result, the overall biodiversity and resilience of natural systems is reducing. Avoiding the use of capture fishery-derived products in animal feeds is thus desired. This leads to an urgent need for alternative lipid sources other than fish oil in aquaculture diets, that can meet the dietary requirements for omega-3 (n-3) fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (NRC, 2011).

1.2 | Alternative fatty acid sources

Lots of research has been done to find alternative ingredients to substitute fishmeal or fish oil in aquaculture diets without compromising on current production rates. Studies on replacing both fishmeal and fish oil without EPA or DHA supplementation are rare. Outcomes suggest that nutritionally balanced diets can partially replace fishmeal or fish oil without negatively affecting shrimp survival and growth. These diets contain soybean meal, animal by-product meal, vegetable oils or insect-derived ingredients (Cummins et al., 2017; Turchini et al., 2009; Xu et al., 2016). Furthermore, research in biotechnology has made great progress in producing EPA and DHA from algae, fungi, bacteria or thraustochytrids (Amiri-Jami et al., 2014; Boelen et al., 2013; Wang et al., 2017), which are frequently used in human diet supplements or baby milk powder. Unfortunately, these ingredients are still too expensive to be used in aquafeeds.

A potential alternative to lipids from fishmeal and fish oil is plant oils, although also expensive and often containing higher amounts of n-6 oils instead of n-3 oils. Within the n-3 oils, plants mainly contain short-chain polyunsaturated fatty acids (short-chain PUFA) with up to three double bonds, such as alpha-linolenic acid (αLNA), compared to long-chain polyunsaturated fatty acids (LC-PUFA) such as EPA and DHA, containing 5 and 6 double bonds, respectively. In the search for fishmeal and fish oil replacements, the emphasis has been predominantly on n-3 fatty acids due to the important physiological functions of n-3 LC-PUFA and its limited availability. The importance of n-6 fatty acids, for instance arachidonic acid (ARA) and its precursor linoleic acid (LOA), has been largely overlooked but is now gaining more attention due to their role in fish and shrimp health performance (Bell & Sargent, 2003).

1.3 | Enzymatic conversion

Animals can enzymatically convert αLNA into EPA and DHA (n-3 pathway), and LOA into ARA (n-6 pathway), though efficiencies are low, ranging between 1% and 5% (Kanazawa et al., 1979). Therefore, EPA, DHA and ARA are considered conditionally essential for animals since enzymatic conversion can hardly provide sufficient EPA and DHA levels from αLNA (Stark 2008, Wall 2010, Davis 2003) or ARA from LA. Direct access to EPA, DHA and ARA through the diet is beneficial, and required for optimal animal health and performance.

Determining requirements for αLNA, LOA, EPA and DHA can be challenging in experimental set-up, as these components interact with each other. Nevertheless, in shrimp feed formulations, the growth promoting effect of dietary short-chain PUFA and LC-PUFA is acknowledged and can be ranked. The combination of EPA and DHA enhances growth best, followed by αLNA and LOA (Glencross & Smith, 1999, 2001a, 2001b; Glencross et al., 2002a, 2002b). The desaturase enzymes involved in biosynthesis of LC-PUFA from short-chain PUFA are driven by competitive substrate inhibition showing a preference for longer and more saturated molecules, leading to a hierarchy with DHA as most preferred substrate, followed by, in this order, EPA, ARA, αLNA and LOA (Glencross, 2009; Sargent et al., 1993). Both n-6 and n-3 are desaturated by these enzymes. Consequently, when the balance between n-6 and n-3 fatty acids is altered, for example by replacing n-3 LC-PUFA rich fish oil by n-6 rich plant oils, thus replacing DHA and EPA by ARA and LA, this may negatively affect the animal's capacity to desaturate n-3 LC-PUFA from their precursor αLNA since n-6 oils will occupy the majority of the enzymes.

1.4 | Fatty acid requirements versus meat quality

In feed formulation for *L. vannamei* diets, a minimum LC-PUFA requirement of 0.3%–0.5% (diet weight basis) is commonly used, including 0.2% EPA and 0.1% – 0.3% DHA (González-Félix et al., 2003).

Nowadays partial fishmeal and fish oil replacement by soybean meal and vegetable oils has become customary practice. Although replacement of fishmeal and fish oil by vegetable products in shrimp diets has no effect on growth or survival, it produces shrimp low in LC-PUFA content. Indeed, in the period 2006–2015 the n-3 LC-PUFA content of aquaculture seafood decreased drastically, for example 50% in Atlantic salmon and 52% – 68% in shrimp (Sprague et al., 2016, Izquierdo et al., 2006; NRC, 2011). Thus, although it is possible to make aquaculture less dependent on capture fisheries, it concurs with a decrease in nutritional quality. Such a reduction in quality can have far reaching consequences for human health, since seafood products are a major source of EPA and DHA for humans (Yashodhara et al., 2009).

1.5 | Pond's natural food as additional fatty acid source

Studies evaluating alternative lipid ingredients are often conducted in clear water systems, where growth of natural food is prevented and food supply is fully controlled by external inputs. This approach however neglects the potential contribution of natural food present to shrimp production in fed outdoor production ponds. Shrimp kept in mesocosms fed commercial diets including both fishmeal and fish oil show better performance than control shrimp kept in clear water systems due to additional available nutrients in the mesocosm's ecosystem (Tacon et al., 2002). Ignoring these additional available nutrients in the pond may lead to the overestimation of the utilization efficiency of supplemented feed. For example, shrimp reared in outdoor mesocosm systems incorporated higher levels of EPA and DHA when fed fish oil-poor diets than shrimp reared in clear water systems (Izquierdo et al., 2006). High-quality natural foods, such as copepods or diatoms, contain significant amounts of EPA and DHA, and are known to stimulate shrimp production (Delong et al., 1993; Johnson & Wiederholm, 1992; Napolitano et al., 1996). Numerous studies have shown that natural food production can contribute to shrimp nutrition in production ponds, ranging from extensive to hyper-intensive production systems (Jory, 1995, Anderson et al., 1987, Sangha et al., 2000, Lavens & Sorgeloos, 2000, McIntosh et al., 2000, Bojórquez-Mascareño & Soto-Jiménez, 2013, Martinez-Cordova et al., 2003, Soares et al., 2004, Decamp et al., 2002, Browdy & Moss, 2005, Wasielesky et al., 2006). Stable isotope measurements suggest that in shrimp ponds, the contribution of natural foods can reach up to 50% of the total diet selection (Burford & Williams, 2001). Unfortunately, most studies on fishmeal- and/or fish oil-free diets focus on protein instead of fatty acids and add extra marine fatty acids to the experimental diets (such as squid or menhaden oil, or microbial or algal products) to compensate LC-PUFA levels (Amaya et al., 2007; Davis & Arnold, 2000; Patnaik et al., 2006). This hampers the assessment of the LC-PUFA contribution from natural food through the comparison of clear water systems and pond systems.

1.6 | Study aim

In semi-intensive coastal brackish water ponds, primary production often exceeds 4 g C m^{-2} d^{-1}. The dry mass of algae produced in these ponds is similar to the amount of feed administrated. Some marine or brackish water algae are good sources of LC-PUFA and might contribute to the shrimp diet. Yet, the actual contribution of primary production-derived fatty acids to the shrimp diet is poorly understood nor quantified (Bojórquez-Mascareño & Soto-Jiménez, 2013; Izquierdo et al., 2006; Neori, 2011). The first aim of this study was to assess the LC-PUFA contribution by dietary fish oil and fishmeal on whiteleg shrimp (*Litopenaeus vannamei*) production and meat quality. Mesocosms were used to mimic a semi-intensive outdoor pond production system, including primary producers. The second aim was to compute PUFA mass balances considering formulated feed input and shrimp production. The goal was to distinguish between formulated diet-based and primary production-based contributions to shrimp production. Finally, the feasibility and sustainability to rely in semi-intensive production systems on in situ naturally produced short-chain PUFA and LC-PUFA for shrimp production was evaluated.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up

The experiment was conducted indoor under controlled temperature conditions at the aquaculture research institute 'Carus' of Wageningen University in The Netherlands. Six experimental mesocosm tanks with a working volume of 700 L (1.25 m diameter, 90 cm depth) were used as a model for outdoor commercial shrimp ponds. Seven agricultural lights (Gavita; three LEP 270–01 SUP EU, and four Digistar 400W e-series) were suspended above the tanks. Each individual tank received an incident irradiance of 300 µmol photons m^{-2} s^{-1} under a 12-hr/12-hr day/night regime to enable autotrophic natural food production in the tanks. The light system (Gavita; Master Controller EL1) controlled sunrise and sunset time and room temperature was maintained at 27–29°C. Tank water was continuously mixed and aerated by a looped aeration pipe, 7 cm above the sediment and perforated at 10-cm intervals. Water temperature was 25–27°C. All mesocosm tanks were filled with artificial seawater with a salinity of 25 ppt (Reef Crystals) and a 7 cm sediment layer consisting of homogenously sterilized pure sand. To inoculate the mesocosm ecosystem, 500 g of 'live rock' (NMFS, 1995) was added to the sediment of each tank (collected from shrimp mangrove tanks at tropical sea aquarium Burger's Zoo Arnhem, The Netherlands). The mesocosms were left to maturate for 1 year. Three days prior to the start of the experiment, all tank walls were scrubbed clean, and sediment and water were collected in a large basin and thoroughly mixed and redistributed to ensure a similar start situation for the experiment. One day before the start of the experiment (day 0), 60 1.5-g juvenile shrimp were stocked in each mesocosm (circa 50 ind

m^{-2}) (Florida Shrimp International Shrimp Harvesters USA, SPF-line, imported by Crevetec Belgium), intending to mimic a farming system of intensive shrimp farmers in the Vietnamese Mekong Delta with a potential shrimp production of 2000 – 3,000 kg/ha (Joffre, 2010).

2.2 | Dietary treatments and feeding regime

Treatments were a control diet and a diet low in n-3 LC-PUFA, randomly distributed over 6 mesocosms (3 replicates per treatment). The control diet was formulated according to common commercial practice containing 1% fish oil, 16% fishmeal and 10% soybean meal (standard LC-PUFA dietary group: control). In the low LC-PUFA treatment diet, fishmeal and fish oil were fully substituted by casein and coconut oil, respectively (low LC-PUFA treatment group: LOW). Both diets contained the same amount of crude protein, essential amino acids and vitamins, crude fat and energy (Table 1). Feeding regime was set initially to 4.9% body weight per day and gradually decreased reaching 3.4% body weight per day at the end of the experiment. Each tank received 433.5 g feed during the entire experiment. Feed was continuously and uniformly added during day and night with an automatic 24-hr belt feeder. The shrimp were not fed 24 hr before and after stocking, and 12 hr before and after sampling. The fatty acid composition of the experimental diets is presented in Table 2. The control diet contained sufficient amounts of LC-PUFA, EPA and DHA, while the LOW diet was deficient. In general, the control diet contained 9.7 times more LC-PUFA than the LOW diet, particularly EPA and DHA. αLNA content was comparable between both diets, while ARA content was 7.5 times higher in the control diet. Both diets contained deficient ARA levels. The n-6/n-3 ratio was 4.2 times higher in the LOW diet.

TABLE 1 Ingredient composition, proximate content and estimated digestibility of the experimental diets containing standard LC-PUFA levels (control) and low LC-PUFA levels (LOW)

	Control diet	LOW diet
Ingredient (in %):		
Fishmeal	16.00	---
Fish oil	1.00	---
Coconut oil	---	2.40
Casein	---	13.20
Wheat gluten	10.00	10.00
Soybean meal	10.00	10.00
Krill protein hydrolysate	1.00	1.00
Wheat flour	27.60	27.00
Wheat	20.00	20.00
Wheat bran	10.00	10.00
Cholesterol	0.20	0.20
Soy lecithin	0.50	0.50
Monocalcium phosphate ($Ca(H_2PO_4)_2$)	1.60	2.75
Calcium carbonate ($CaCO_3$)	0.40	0.95
Mineral and vitamin premix	1.00	1.00
Lysine hydrochloride	0.30	0.30
DL-methionine	0.20	0.20
L-Threonine	0.20	0.20
L-Arginine	---	0.30
Total	100.00	100.00
Proximate composition (g kg^{-1} dry matter):		
Crude protein	354.9	371.9
Crude fat	56.2	57.4
Crude ash	69.7	49.8
Carbohydrates	519.2	520.9
Energy (kJ/g DM)	19.8	20.4
Estimated digestibility:		
Digestible energy content (g kg^{-1} dry matter)	15.36	15.31
Digestible protein/Digestible energy	22.30	22.52

2.3 | Sampling and system control

During the 57 days of the experiment, shrimp were sampled on days 0 (= stocking day), 22, 43 and 57. On day 0, 20 shrimp were randomly selected as representatives of the initial population, euthanized using ice water and stored at −20°C prior to further analysis. At days 22 and 43, 20 shrimp were harvested, weighed, euthanized and stored at −20°C. At day 57, all remaining shrimp were harvested, counted, weighed, euthanized and stored at −20°C. Each week a grab sample was taken from the feed and added to an airtight container kept at 4°C. At the end of the experiment, the feed in the container was uniformly mixed to obtain a representative sample of the feed administrated during the experiment. Water quality parameters were weekly checked using a multi-parameter portable meter (WTW Multi 3,430) at 10:00a.m. for pH and oxidation reduction potential (ORP) (Sentix 940) and salinity (Tetracon 925). The dissolved oxygen (DO) concentration was measured continuously during 24 hr and recorded every 10 min (FDO 925). Orthophosphate, NO_2^-, NO_3^- and total ammonia nitrogen (TAN) were measured according to protocol NEN-ISO6777 and NEN-ISO7150-1 using a Smartchem (Smartchem 200, Alliance Instruments, AMS Systea, Frepillon, France). Nutrient concentrations and oxygen levels were managed to remain favourable for growth at < 2 mg NO_2^- L^{-1}, <50 mg NO_3^- L^{-1}, <3 mg TAN L^{-1}, 7.0–8.8 pH and > 6 mg DO L^{-1}. Salinity was kept constant by adding fresh tap water of 22°C twice weekly to compensate for evaporation losses. When multiple samples for measuring a parameter were taken, they were pooled within day and within mesocosm.

2.4 | Chemical analyses

First, the gastrointestinal tract of sampled shrimp was removed, and shrimp were subsequently freeze-dried (ZIRBUS technology, Sublimator 3X4X5, Zirbus technology GmBH, Bad Grund, Germany). Shrimp and feed samples were ground using a centrifugal grinding

mill operated at 60% amplitude for 3 min at 12,000 RPM (Retsch 200 ZM 1mm sieve). Chemical analysis of shrimp and feed included determination of dry matter (DM) (protocol ISO6496), ash (ISO5985), crude protein (CP) (ISO5983), crude fat (CF) (ISO6492) and gross energy (E) (ISO9831). Organic matter (OM) and carbohydrate (CH) content were calculated based on dry matter content minus ash content, and organic matter content minus crude protein and fat content, respectively. Productive protein value was calculated as protein gain divided by dietary protein intake. Feed conversion ratio was calculated as feed input divided by shrimp biomass gain. Fatty acid profiles of shrimp and feed were analysed following direct transesterification of fatty acid methyl esters (Lepage & Roy, 1984).

TABLE 2 Fatty acid composition of experimental diets and dietary LC-PUFA requirements for L. vannamei (mg/g DM feed). Control diet: diet with standard LC-PUFA content. LOW diet: diet with low LC-PUFA content. (αLNA—alpha-linolenic acid; EPA—eicosapentaenoic acid; DHA—docosahexaenoic acid; LOA—linoleic acid; and ARA—arachidonic acid.)

	Control diet	LOW diet	LC-PUFA Requirements[Δ]
∑ omega−3*	6.28	1.86	
∑ omega−6**	12.87	16.10	
omega−6/omega−3	2.05	8.63	
∑ saturates†	9.31	15.70	
∑ monounsaturates‡	9.99	6.55	
∑ short-chain PUFA§	13.9	17.43	
∑ LC-PUFA◊	5.25	0.54	3.0 – 5.0
αLNA 18:3n−3	1.19	1.35	
EPA 20:5n−3	2.07	0.17	2.0
DHA 22:6n−3	2.23	0.12	1.0 – 3.0
LOA 18:2n−6	12.67	16.08	
ARA 20:4n−6	0.15	0.02	5.0

*∑ includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:3n-3, 22:4n-3, 22:5n-3, 22:6n-3.

**∑ includes 18:2n-6, 18:3n-6, 19:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.

†∑ includes 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 21:0, 22:0, 23:0, 24:0.

‡∑ includes 14:1n-5, 15:1n-5, 16:1n-7, 17:1n-7, 18:1n-9, 18:1n-7, 19:1n-9, 20:1n-9, 20:1n-7, 22:1n-9, 22:1n-7, 23:1n-9, 24:1n-9.

§∑ includes 18:2, 18:3, 19:2, 20:3, 22:3.

◊∑ includes 18:4, 20:4, 20:5, 21:5, 22:4, 22:5, 22:6.

ΔFor L. *vannamei*, (González-Félix et al., 2003, González-Félix et al., 2002a, González-Félix et al., 2002b).

2.5 | Data analysis

The data analysis was carried out using IBM SPSS software package version 23 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Mesocosm tanks were the experimental units. Comparison of means was performed by independent *t*-tests. Outcomes are presented as treatment means (± standard deviation, $n = 3$).

3 | RESULTS

3.1 | Shrimp general performance

Shrimp growth, total biomass production and survival at the end of the experiment were similar between both diets. Final individual body weight and total produced biomass were not different between treatments, but the means of control shrimp were higher (Table 3). The intended production performance was reached with an equivalent of 3,047 kg/ha and 2,244 kg/ha (control and LOW groups, respectively) produced in 8 weeks. Survival of 96 ± 1.9% ($n = 6$) was high in all tanks, and mortality was mainly caused by shrimp jumping out of the tanks. Moulting seemed to occur simultaneously, and exoskeletons were left in the mesocosm to be re-eaten by the animals.

3.2 | Water quality in mesocosms

No significant differences between treatments were observed for water temperature, dissolved oxygen, pH, alkalinity, total

TABLE 3 Performance parameters. Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content

	Control shrimp	LOW shrimp	*Level of significance*
Total feed fed per tank	433.5 g	433.5 g	
Feed conversion ratio	1.1 ± 0.2	1.5 ± 0.2	$p = .112$
Survival (%)	98.8 ± 1.0	95.0 ± 1.7	$p = .067$
Initial shrimp biomass (g)	1.4 ± 0.1	1.6 ± 0.1	$p = .115$
Final shrimp biomass (g)	11.4 ± 1.9	9.4 ± 0.7	$p = .779$
Total produced biomass (g)	373.9 ± 68.4	275.4 ± 45.8	$p = .109$
Productive protein value (%)	58.5 ± 10.7	38.9 ± 14.7	$p = .135$
Individual final body weight (g)	11.4 ± 1.9	9.4 ± 0.7	$p = .165$
Individual initial body weight (g)	1.4 ± 0.1	1.6 ± 0.1	$p = .230$

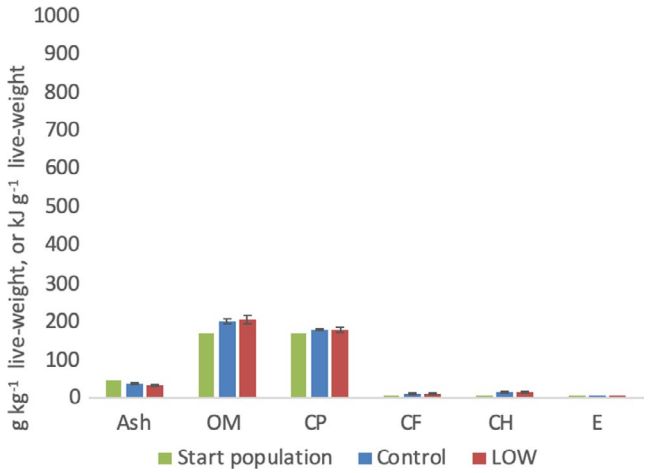

FIGURE 1 Shrimp biochemical body composition at the start (start population) and end of the experiment (control and LOW) expressed on live-weight basis and expressed on dry matter basis. Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. Abbreviations: organic matter (OM), crude protein (CP), crude fat (CF), carbohydrates (CH) and energy (E). No error bars of start population are shown; one sample was taken from base population (60 individuals pooled)

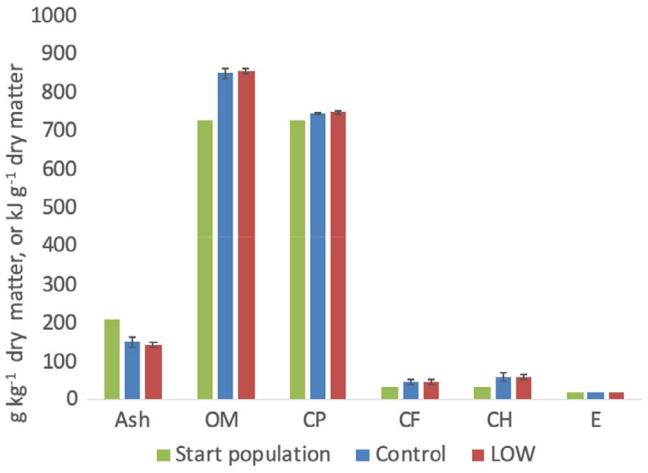

FIGURE 2 Shrimp biochemical body composition at the start (start population) and end of the experiment (control and LOW) expressed on live-weight basis and expressed on dry matter basis. Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. Abbreviations: organic matter (OM), crude protein (CP), crude fat (CF), carbohydrates (CH) and energy (E). No error bars of start population are shown; one sample was taken from base population (60 individuals pooled)

TABLE 4 Shrimp final fatty acid composition (mg/g shrimp DM) of dietary treatment groups. Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. *P*-values presented in bold highlight significant outcomes

	Control shrimp	LOW shrimp	*Level of significance*
∑ omega−3*	11.05 ± 0.52	5.45 ± 0.12	***p* < .001**
∑ omega−6**	11.61 ± 0.92	15.59 ± 0.66	***p* = .004**
omega−6/omega−3	1.05 ± 0.06	2.86 ± 0.15	***p* < .001**
∑ saturates†	15.18 ± 0.91	18.34 ± 1.38	***p* = .029**
∑ monounsaturates‡	11.92 ± 1.05	12.13 ± 1.23	*p = .833*
∑ short-chain PUFA§	10.84 ± 1.00	15.25 ± 0.77	***p* = .004**
∑ LC-PUFA◊	11.82 ± 0.54	5.79 ± 0.16	***p* < .001**
αLNA 18:3n−3	0.64 ± 0.13	1.13 ± 0.04	***p* = .003**
EPA 20:5n−3	5.43 ± 0.24	2.35 ± 0.08	***p* < .001**
DHA 22:6n−3	4.07 ± 0.32	1.40 ± 0.21	***p* < .001**
LOA 18:2n−6	10.02 ± 0.85	13.88 ± 0.80	***p* = .005**
ARA 20:4n−6	1.41 ± 0.04	1.48 ± 0.15	*p = .442*

*∑ includes 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:3n-3, 22:4n-3, 22:5n-3, 22:6n-3.

**∑ includes 18:2n-6, 18:3n-6, 19:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.

†∑ includes 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 21:0, 22:0, 23:0, 24:0.

‡∑ includes 14:1n-5, 15:1n-5, 16:1n-7, 17:1n-7, 18:1n-9, 18:1n-7, 19:1n-9, 20:1n-9, 20:1n-7, 22:1n-9, 22:1n-7, 23:1n-9, 24:1n-9.

§∑ includes 18:2, 18:3, 19:2, 20:3, 22:3.

◊∑ includes 18:4, 20:4, 20:5, 21:5, 22:4, 22:5, 22:6.

ammonia nitrogen, nitrite, nitrate, orthophosphate and oxidation reduction potential. Water temperature was on average 26.2°C ± 0.5 (*n* = 6) across all mesocosms, with a largely constant pH of 8.46 ± 0.06 (*n* = 6) for the duration of the experiment. All tanks showed low levels of TAN, NO_2^--N and NO_3^--N with maximal values recorded of 1.02 mg/L, 0.58 mg/L and 1.14 mg/L, respectively.

3.3 | Shrimp biochemical composition

Final body biochemical composition, expressed in g/kg live-weight and g/kg dry matter, did not show significant differences between treatment groups (Figures 1 and 2, respectively). Dry matter content on live-weight basis was similar between treatments and on average 23.7 ± 0.9%. Although no differences were observed in total

crude fat composition, fatty acid profiles showed clear differences between treatments (Table 4). Shrimp from the control diet contained twice as much LC-PUFA and n-3 fatty acids than LOW shrimp ($p < .001$). Shrimp fed the LOW diet contained significantly more n-6 fatty acids, short-chain PUFA and saturated fatty acids. When focussing on single essential fatty acids, shrimp ARA content was not affected by diet, while LOA and αLNA were higher and EPA and DHA lower in shrimp fed the LOW diet. The n-6/n-3 ratio was about 2.7 times higher in the LOW shrimp.

3.4 | Fatty acid retention

Total αLNA content in total shrimp biomass did not differ significantly between diets (control: 46.3 ± 12.2 mg per mesocosm tank, LOW: 67.2 ± 10.6 mg per tank, $p = .089$) (Figure 3a). The total shrimp αLNA content was 89% lower than the input, representing an overall ALA loss of 471 mg per tank, leading to a dietary αLNA retention of 10% after deducting fatty acid content of the start population. About twice as much EPA accumulated in total shrimp biomass fed the control diet than in shrimp fed the LOW diet (381 ± 49 mg versus 174 ± 17 mg per tank, respectively, $p = .002$) (Figure 3b). The total shrimp biomass fed the control diet contained only 55% of the EPA input, indicating a loss of 474 mg EPA per tank. In contrast, LOW shrimp contained 64.7 mg more EPA per tank than provided through initial biomass and feed. This concurs with a retention efficiency of 42% for control shrimp and an increase of 95% for LOW shrimp considering the EPA supplied with the feed. Control shrimp retained more DHA than LOW shrimp (285 ± 29 versus. 107 ± 2 mg per tank, $p < .001$) (Figure 3c). In the control treatment, similar as observed for EPA, 69% of the DHA fed, equalling 642 mg per tank, was not retained in shrimp biomass. With the LOW diet, 10.3 mg more DHA per tank was retained in shrimp biomass than the amount fed. This corresponds to a 73% loss of fed DHA with the control diet and a 22% gain with the LOW diet. For n-6 essential fatty acids, no differences were observed between treatments in total produced shrimp LOA content (control: 464 ± 52 mg per tank, LOW: 528 ± 75 mg per tank, $p = .290$) (Figure 3d) and ARA content (control: 113 ± 17 mg per tank, LOW: 108 ± 16 mg per tank, $p = .726$) (Figure 3e). Shrimp lost the majority of their LOA content in initial biomass and feed (5,210 mg per tank) giving an LOA retention of only 9%. Shrimp ARA content was overall 66.3 mg per tank higher than input through initial biomass and feed, highlighting an ARA increase of 51% considering ARA supplied through feed.

4 | DISCUSSION

4.1 | Performance

Individual shrimp growth, total biomass production and survival were similar between diets. Therefore, the absence of fish oil and fishmeal in the formulated diet did not reduce growth performance in the mesocosms. This is in line with similar outcomes of other studies as described in the introduction. Nevertheless, the lack of such difference should be considered with care. Outcomes of comparable studies using the same mesocosm tanks, feeding similar diets under the same environmental conditions (e.g. temperature, aeration, light intensity), demonstrate that the variability between replicates (standard deviation) was sufficiently small to detect a 25% difference for total biomass production between treatments (a = 0.05, b = 0.2, power 0.80) with three replicates per treatment (Tinh et al., 2020). Therefore, three replicates should be considered sufficient for such mesocosm study. However, when considering the variability amongst replicates for total biomass production in the present study, eight replicate tanks per treatment would be required for the LOW diet to be significantly different from the control diet (a = 0.05, b = 0.2, power 0.80) (Kabacoff & Action, 2011). More mesocosm experiments are needed to determine the expected variation between treatment in mesocosms. In literature, for pond experiments, the number of replicates per treatment commonly varies between three (Asaduzzaman et al., 2010; Hari et al., 2006) and six (Kabir et al., 2020).

Although water temperature was found to be on the low side in this current experiment compared to reported growth optima (i.e. 27 –30 °C; (Wyban et al., 1995)), shrimp showed normal growth. Given a production of 3,047 kg/ha and 2,244 kg/ha (control and LOW groups, respectively) over an 8-week period, our experimental mesocosms mimicked a farming system of semi-intensive shrimp farmers in the Vietnamese Mekong Delta well (Joffre, 2010). The mesocosms maintained low TAN, nitrite and nitrate concentrations during the entire experiment. This concurs with results found in literature where stocking density up to 50 shrimp m^{-2} in closed systems had no negative effect on water quality and shrimp performance during 90 days (Thakur & Lin, 2003). In this current study, survival rates were high (96 ± 1.0%, $n = 6$) and feed conversion ratio (1.3 ± 0.3, $n = 6$) was on the low side in the range 1.2 – 2.5 as observed in greenhouse-enclosed intensive shrimp production systems fed commercial diets (Venero et al., 2009). Shrimp performance was similar as reported in literature when feeding shrimp a fish oil-free diet in mesocosms (96% survival and a feed conversion ratio of 1.3) (Izquierdo et al., 2006).

4.2 | Shrimp biochemical composition

Although the composition of shrimp of both treatments was similar in terms of fat, carbohydrate, ash and organic matter, there were pronounced differences in fatty acid composition. Shrimp fed the fish oil- and fishmeal-free diet had significantly lower LC-PUFA content, mainly due to a lower EPA and DHA content, and a higher n-6/n-3 ratio. A comparison between fatty acid contents in this current study (presented as % of total fatty acid content) to cultured shrimp and wild caught shrimp is presented in Table 5. Captured wild shrimp stand out to cultured shrimp in higher n-3 fatty acid content, especially EPA, and consequently a low n-6/n-3 ratio. Compared to

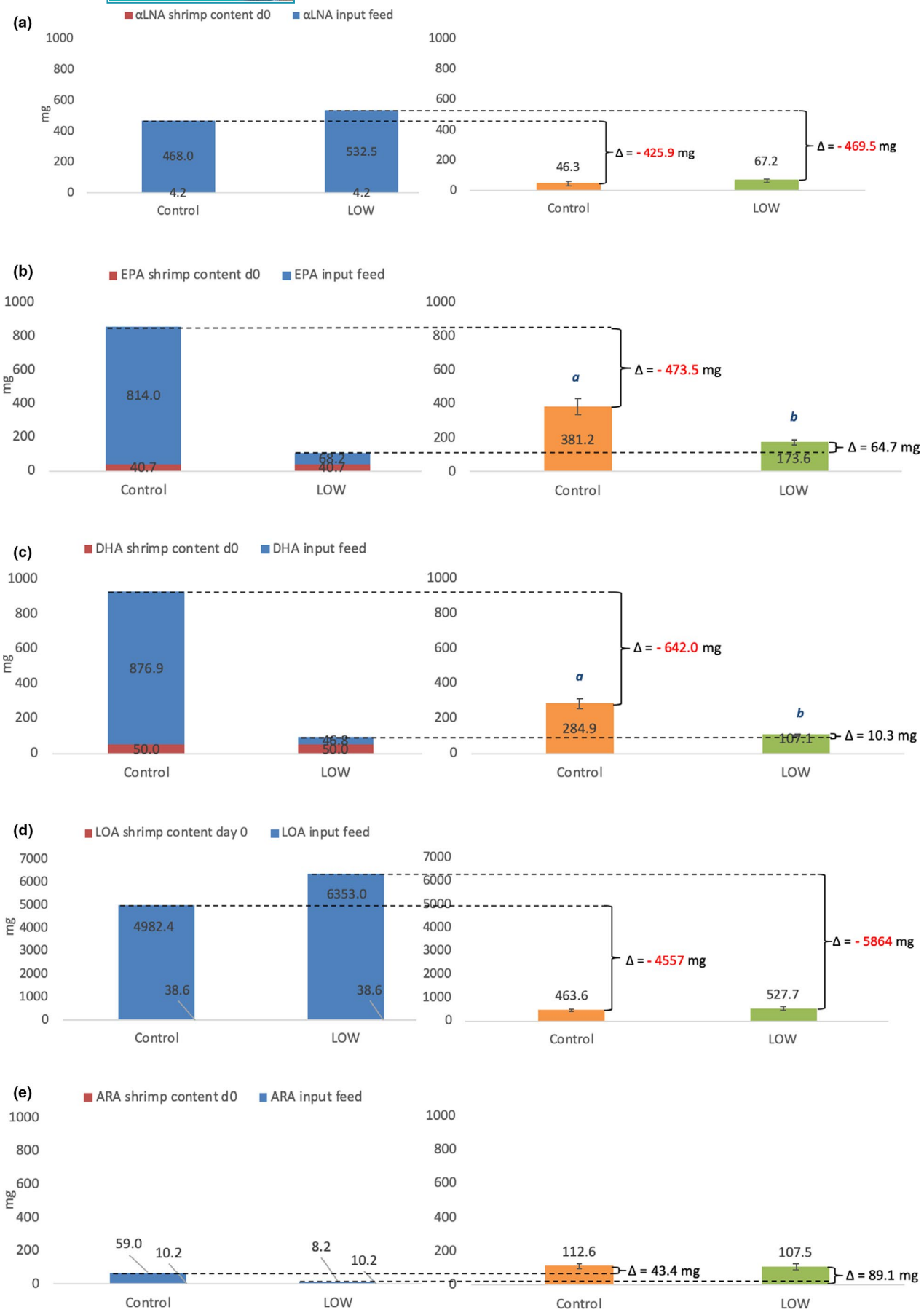
(a)
αLNA shrimp content d0
αLNA input feed
mg
468.0
4.2
532.5
4.2
Control
LOW
46.3
67.2
Δ = - 425.9 mg
Δ = - 469.5 mg
Control
LOW
(b)
EPA shrimp content d0
EPA input feed
814.0
40.7
68.2
40.7
381.2
173.6
a
b
Δ = - 473.5 mg
Δ = 64.7 mg
(c)
DHA shrimp content d0
DHA input feed
876.9
50.0
46.8
50.0
284.9
107.1
Δ = - 642.0 mg
Δ = 10.3 mg
(d)
LOA shrimp content day 0
LOA input feed
4982.4
38.6
6353.0
38.6
463.6
527.7
Δ = - 4557 mg
Δ = - 5864 mg
(e)
ARA shrimp content d0
ARA input feed
59.0
10.2
8.2
10.2
112.6
107.5
Δ = 43.4 mg
Δ = 89.1 mg

FIGURE 3 **A-E** Shrimp and feed essential fatty acid content presented as absolute amounts. Fatty acid input (amount in the shrimp start population plus amount fed) and retention (amount accumulated in shrimp) of n-3 fatty acids αLNA, EPA, DHA and n-6 essential fatty acids LOA and ARA. The horizontal lines in the figures indicate the expected final level of essential fatty acid content of the total produced shrimp biomass based on input and disregarding fatty acid synthesis by the shrimp. **A)** Alpha-linolenic acid (18:3n-3 αLNA) balance per tank. **Left**: αLNA shrimp start content plus external αLNA input through feed (mg); **right**: shrimp final total αLNA content (mg). Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. **B)** Eicosapentaenoic acid (20:5n-3 EPA) balance per tank. **Left**: EPA shrimp start content plus external EPA input through feed (mg); **right**: shrimp final total EPA content (mg). Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. **C)** Docosahexaenoic acid (22:5n-3 DHA) balance per tank. **Left**: DHA shrimp start content plus external DHA input through feed (mg); **right**: shrimp final total DHA content (mg). Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. **D)** Linoleic acid (18:2n-6 LA) balance per tank. **Left**: LOA shrimp start content plus external LOA input through feed (mg); **right**: shrimp final total LOA content (mg). Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content. **E)** Arachidonic acid (20:4n-6 ARA) balance per tank. **Left**: ARA shrimp start content plus external ARA input through feed (mg); **right**: shrimp final total ARA content (mg). Control: dietary group with standard LC-PUFA content. LOW: dietary group with low LC-PUFA content

cultured shrimp fed other plant-based diets (Browdy et al., 2006; Ramezani-Fard et al., 2014), control shrimp in this experiment show comparable n-6/n-3 ratios and similar essential fatty acid composition. LOW shrimp contained far less LC-PUFA and n-3 fatty acids than cultured and wild shrimp.

4.3 | Shrimp meat quality

While leaving out fishmeal and fish oil from formulated shrimp feed has no effect on protein production, meat quality is deteriorated due to decreased n-3 LC-PUFA levels and increased n-6/n-3 ratios. Unfortunately, one cannot escape the consequence of increasing n-6/n-3 ratios when replacing fish oil and fishmeal by plant products without making use of n-3 supplements. On top of low n-3 LC-PUFA dietary input, the high n-6/n-3 ratio might have further reduced the n-3 synthesis pathway inside the shrimp body due to enzyme substrate competition. As seafood is the main source of LC-PUFA to humans and is therefore essential for health, fully leaving out fish oil and fishmeal from shrimp diet formulations may therefore be undesired. However, the total lipid content of shrimp is low compared to fish. Therefore, if one is aiming for seafood high in n-3 LC-PUFA content, the choice for fish is easily made over shrimp regardless of shrimp diet, even though also the fish n-3 LC-PUFA contents depend on diet formulation. Further, lipid and EPA and DHA composition of shrimp fed plant-based diets is still of better quality compared to beef, pork and chicken meat. In addition, meat products contain higher fat and lower EPA and DHA levels (Browdy et al., 2006). Therefore, shrimp fed vegetable diets remain a healthy diet choice for human consumption regarding protein and lipid composition.

4.4 | Fatty acid quantitative losses and gains

In both treatments, there were large quantitative losses in total amounts of the precursors αLNA and LA. Whereas this was also observed for EPA and DHA in the control group, there was a gain for these components in the tanks fed a diet without fish oil and fishmeal. The observed balance losses can be partially explained by fatty acid synthesis from precursors into LC-PUFA, and by a poor lipid and fatty acid digestive capacity in crustaceans due to a lack of gastric fat emulsifiers such as bile salt (Brockerhoff & Hoyle, 1967; Glencross et al., 1998). Although selective retention and bioaccumulation of essential fatty acids are observed in a wide variety of animals at different trophic levels (Gladyshev et al., 2013), this capacity is species-dependent and influenced by diet composition and the nutritive status of the animal. Starvation and malnutrition in different fish species showed that fish have a retention preference of n-3 LC-PUFA over n-6 LC-PUFA and DHA over EPA. Nevertheless, high catabolism of n-3 LC-PUFA can also be observed in fish, and this increases further during malnutrition (Glencross, 2009; Glencross et al., 2003b; Oxley et al., 2005; Stubhaug et al., 2007). Shrimp have been reported to catabolize over a third of their dietary EPA by ß-oxidation for ATP production (Dall et al., 1993). Similar large losses of n-3 LC-PUFA are also observed in this current study in the control group.

In contrast to the quantitative n-3 LC-PUFA losses in the control group, shrimp without dietary fish oil and fishmeal showed a remarkable gain in EPA and DHA. These gains cannot be fully explained by enzymatic conversion of αLNA into EPA and DHA. Shrimp are poor fatty acid synthesizers due to low enzyme substrate affinity with a conversion rate of between 1% and 5% (Kanazawa et al., 1979). But even when calculating with a high 5% αLNA to EPA conversion and subtracting standard deviation of total biomass EPA content, LOW shrimp acquired at least 20.9 mg EPA de novo (calculated: 64.7 mg EPA gain minus 5% of 536.7 mg αLNA balance input, minus standard deviation of 17 mg). Since it is unlikely shrimp converted body and dietary DHA to EPA under suboptimal nutritional condition caused by the absence of dietary fishmeal and fish oil, it is most likely this additional EPA gain originates from primary production in the mesocosm, suggesting that the shrimp were able to exploit these alternative sources. This means that LOW shrimp sourced at least 32% of their total EPA gain from the algal-based food web. Similarly, 1.5 mg de novo DHA must have been sourced from primary producers directly (calculated: 10.3 mg DHA gain minus 5% of 5% of αLNA balance input since it requires two elongation steps from αLNA to DHA, minus 5% of EPA balance input, minus standard deviation of 2 mg), or indirectly via EPA derived from the primary production in the mesocosm. This means that LOW shrimp sourced at least 15% of their total DHA gain from the algal-based food web. Due to the

TABLE 5 Comparison of fatty acid compositions of *L. vannamei* (unless otherwise stated) between this experiment, cultured (fed standard diets containing fishmeal and fish oil unless otherwise stated) and wild caught shrimp (Browdy et al., 2006; Li et al., 2011; Lim et al., 1997; Ramezani-Fard et al., 2014)

	Present study		Brody et al. 2006		
Fatty acid: % of total fatty acids Lipid content: % of dry matter	**Control shrimp**	**LOW shrimp**	**Cultured**	**Cultured; plant-based diet plus DHA and ARA additives**	**Wild caught—Mexico**
L n-	1.29	2.19	0.98	4.63	0.24
EPA 20:5n-3	10.9	4.56	15.8	10.8	17.2
DHA 22:6n-3	8.18	2.72	11.8	8.75	14.2
ARA 20:4n-6	2.82	2.88	3.46	3.00	5.05
Total n-3	22.2	10.6	30.3	25.4	34.2
Total n-6	23.3	30.3	17.6	28.7	8.45
n-6/n-3	1.05	2.86	0.58	1.13	0.49
Lipid content	3.57	3.74	1.86[§]	1.79[§]	n/a

[§]Adapted from Browdy et al., 2006 converting values given in % of shrimp wet weight into % of shrimp dry matter, assuming a 25 % dry matter shrimp composition.

large balance losses in control shrimp for EPA and DHA, it cannot be calculated if and to what extend control shrimp sourced EPA and DHA from the mesocosms, but it is clear that they were much less efficient in their use of these valuable fatty acids compared to shrimp with a diet deficient in these components (control shrimp: 42% EPA and 27% DHA retention from feed, versus LOW shrimp: 195% EPA and 122% DHA retention from feed). The n-6 fatty acid ARA showed gains in both treatments, but these observed gains can entirely be explained by enzymatic synthesis from the precursor LA. LOA is usually widely abundant in plant-based diets, as well as in both experimental diets in this current experiment. Calculating with 5% enzyme efficiency converting LOA into ARA, the LOA content of the initial biomass plus input through the feed of total 5,706 mg can potentially have led to 285 mg ARA, covering the observed shrimp ARA gain of 99.9 mg.

4.5 | Mesocosm contribution allows changes in diet formulation

Our quantitative analysis of the fate of major dietary fatty acids strongly suggests that the pond's primary production can provide shrimp additional dietary EPA and DHA. Nevertheless, when fully excluding fishmeal and fish oil from formulated feed, the LC-PUFA content is lower than normally observed in cultured or wild caught shrimp (Table 5). Overall, the EPA and DHA contents were 2.4 to 3.0 times lower in LOW shrimp compared to the control. Evaluation of a comparable study found in literature, shows similar results. Shrimp produced in mesocosms fed diets free of both fishmeal and fish oil resulted in unaffected general shrimp performance, but significantly lower levels of n-3 LC-PUFA (EPA and DHA) in shrimp. This latter study, however, started the experiment with an unmatured mesocosm consisting of clear chlorinated water (González-Félix et al., 2010). The contribution of primary production was therefore assumed to be suboptimal and much lower than in our study. Since EPA and DHA production by primary producers is surface area-dependent, based on the current set-up in our study, it is expected that when feeding a fishmeal- and fish oil-free diet, the pond might be able to fulfil the LC-PUFA demand at a shrimp biomass production 2.4 to 3.0 times smaller than in this experiment. The latter statement is highly speculative, but supported by a similar study (Glencross et al., 2014). In that study, shrimp were fed fishmeal- and fish oil-free diets during approximately similar experimental length and comparable mesocosm tanks. The produced shrimp showed, besides similar general performance, only a minor decrease in LC-PUFA levels (EPA was unaffected, DHA only slightly lower) compared to a control diet containing fishmeal and fish oil. In that study however, stocking density was 2.3 times lower than our study, and it can thus be assumed that primary production had a relatively higher contribution as result. Quantifying the LC-PUFA accumulation in the whole mesocosm will be needed for confirmation, because it could be possible that less LC-PUFA will be produced at a lower culture intensity in the mesocosm.

At the same time, an inclusion level of 16% fishmeal and 1% fish oil as used in the standard diet treatment of this experiment seems too high regarding the relatively large ALA, EPA, DHA, LOA and ARA balance losses. From a diet formulating perspective, the large balance losses of αLNA in both dietary groups suggest that it might be possible to replace a part of the ALA-containing diet ingredients, such as plant oils, by cheaper fat sources since the major part of αLNA seems to have been used as energy source instead of acting as EPA and DHA precursor. However, this is only possible when the overall dietary n-6/n-3 ratio will not further increase to prevent stronger preference of the desaturase enzyme towards n-6 LC-PUFA synthesis leading to reduced activity in the n-3 LC-PUFA synthesis pathway. Therefore, when replacing αLNA with alternative energy sources, dietary n-6 fatty acid containing ingredients should be lowered in same or higher amounts. This is possible since LOA balance loss was found to be of relatively similar level as αLNA

			Ramezani-Fard et al. 2004	Lim et al. 1997		Li et al. 2011
Wild caught *L. setiferus*—Southeast Atlantic	Cultured	Cultured	Cultured	Cultured; fishmeal and coconut oil diet	Cultured	Cultured
0.55	0.46	0.70	1.56	1.60	1.30	1.60
14.8	11.7	16.1	10.1	12.8	20.7	12.3
8.61	9.76	10.7	7.19	9.20	13.1	9.10
6.02	2.57	4.12	4.10	3.60	2.60	4.20
25.9	22.6	28.5	19.7	n/a	n/a	23.7
10.3	19.4	16.8	23.2	n/a	n/a	15.1
0.4	0.86	0.59	1.18	n/a	n/a	0.64
n/a	n/a	n/a	n/a	3.13	4.45	1.32

balance loss, both around 90%. If 5% of the αLNA and LOA input would be used in the LC-PUFA synthesis pathway, this suggests 85% is being used as energy source.

Considering diet formulation, finding a balance between LC-PUFA contribution through formulated feed and natural production seems possible but deserves attention for further research. Flows of energy, nutrients and LC-PUFA through food webs in aquaculture production ponds are very unpredictable and presently not well understood. While the results show that algae provide LC-PUFA, it is not known how and where LC-PUFA accumulates in the system. This should be explored first before speculating on how to incorporate possible contributions through the food web into a feeding strategy for semi-intensive shrimp ponds. There is need of a better understanding of the flow and fate of energy and essential fatty acids from primary producers and external feed into consumer biomass. In this study, the focus was on feed and shrimp, whereas no assessment was made of the biochemical composition of the other food web components in the mesocosm. Therefore, the next step will be a follow-up research with focus on specific LC-PUFA content and quantified contribution of different food web compartments of the mesocosms to shrimp production. Understanding underlying metabolic processes in the natural food web of shrimp ponds may aid in moving towards more sustainable aquaculture.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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