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Determination of poly-β-hydroxybutyrate assimilation by postlarval whiteleg shrimp, *Litopenaeus vannamei* using stable ^{13}C isotope tracing

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Abstract

A preliminary study was conducted to demonstrate fate of the bacterial storage compound poly-β-hydroxybutyrate (PHB) once ingested and degraded in vivo in crustaceans. The 2% supplementation of ^{13}C-labeled *Ralstonia eutropha* DSM545 containing 75% PHB on dry weight in postlarval whiteleg shrimp, *Litopenaeus vannamei* resulted in consistent enrichment of two fatty acids (containing 14–24 carbons [C] in length) pentadecanoic acid (15:0) and palmitic acid (16:0) in all lipid fractions. This could indicate that ^{13}C signal

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may not only originate from the PHB but partly from the structural components of the bacterial cell. There was a trend of lower ^{13}C enrichment in various lipid fractions of the postlarvae as compared to the diet. These findings may have important implications as to potential of PHB as energy source rather than as building block. There was a rapid transfer of ^{13}C in the postlarvae as seen by the significant ^{13}C enrichment in the tissues 4 hr after feeding with labeled bacterial cells. Overall, the assimilation of PHB in crustacean tissues is demonstrated for the first time. Our results indicate that PHB once ingested is rapidly assimilated in the tissues and could probably act as an energy source rather than as a building block.

KEYWORDS
assimilation, fatty acids, poly-β-hydroxybutyrate (PHB), stable carbon isotope, whiteleg shrimp

1 | INTRODUCTION

The application of the bacterial storage compound poly-β-hydroxybutyrate (PHB) in aquatic farmed species has been reported to result in the promotion of growth (Nhan et al., 2010; Sui, Cai, Sun, Wille, & Bossier, 2012), improved robustness against adverse environmental conditions (Laranja et al., 2014), and enhanced resistance against pathogens (Defoirdt et al., 2007; Halet et al., 2007; Laranja et al., 2014; Suguna et al., 2014; Sui et al., 2012; Thai et al., 2014). PHB is also hypothesized to be degraded in the gut of the *Artemia* (Defoirdt, Halet, et al., 2007) where it functions as an energy source (De Schryver, 2010; Defoirdt, Boon, Sorgeloos, Verstraete, & Bossier, 2007). In more recent findings, PHB has been shown to affect lipid metabolism in selected fish species (Najdegerami et al., 2015; Situmorang, 2015). Despite the growing evidence on the beneficial effects of PHB in many aquatic farmed species, its assimilation in the tissues once ingested and degraded in vivo remained largely unexplored. A recent study employing the use of ^{13}C stable isotope analysis has demonstrated that PHB is assimilated and distributed in different organs of Nile tilapia, *Oreochromis niloticus* fingerlings (Situmorang, 2015). However, there have been no reports described for crustaceans. The aim of the present study is to determine the assimilation of PHB offered in its natural matrix to the crustacean species, postlarval whiteleg shrimp, *Litopenaeus vannamei* by means of ^{13}C stable isotope tracing. The ^{13}C signal of different fatty acid groups in postlarval *L. vannamei* fed a diet supplemented with ^{13}C-labeled *Ralstonia eutropha* DSM545 is also examined to determine whether PHB is assimilated in the structural components of crustaceans.

2 | MATERIALS AND METHODS

2.1 | Whiteleg *L. vannamei* postlarvae

Postlarval whiteleg shrimp, *L. vannamei* (PL10, 0.11 ± 0.01 g) were obtained from Shrimp Improvement Systems (Islamorada, Florida) and maintained in a recirculating system belonging to the Shrimp Facility of the Laboratory of

Aquaculture and *Artemia* Reference Center (ARC), Ghent University Belgium. Salinity and temperature in the recirculating system averaged to 35 g/L and 28°C, respectively. The photoperiod was maintained at 12 hr light:12 hr dark cycle. During the rearing period, postlarvae were fed commercial diets (CreveTec bvba, Ternat, Belgium) containing 54% crude protein and 12% crude fat at a level of 10% of wet body weight (BW). The daily ration was divided into equal amounts and offered two times a day (9:00, 16:00 hr).

2.2 | ^{13}C-labeled *R. eutropha* DSM545

The lyophilized cells of ^{13}C-labeled *R. eutro*pha DSM545 containing 75% PHB on dry weight (d.w.) were produced by Vito NV, Belgium, as described by Situmorang (2015). Before utilization, the lyophilized cells of *R. eutropha* were homogenized using a Polytron PT 10/35 homogenizer (Kinematica, Luzern Switzerland) for 30 s.

2.3 | Experiment 1: Assimilation of ^{13}C-labeled *R. eutropha* DSM545 through time

The assimilation of ^{13}C-labeled *R. eutropha* DSM545 containing 75% PHB on d.w. by postlarval whiteleg shrimp, *L. vannamei* was determined through time. Postlarvae were stocked at 1 individual/L in two acclimation tanks consisting of 50-L plastic tank. Temperature and level of dissolved oxygen in the acclimation tanks were maintained at 28°C and ≥5 mg/L, respectively. During this period, postlarvae were acclimated to a diet containing 2% non-^{13}C-labeled *R. eutropha* DSM545. The diet, which was given at a level of 10% on wet BW, was divided into two feeding rations daily (9:00 and 16:00 hr). The composition of the diet used during the acclimation period is shown in Table 1. The uneaten feed and fecal matter were removed daily using a siphon and approximately 50% of the rearing water was replaced daily with preheated (28°C) seawater in order to maintain the levels of total ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen below 0.2, 0.1, and 10.0 mg/L,

TABLE 1 Dietary feed composition

Feed ingredients	Composition (g/kg)
Soy flour	302.0
Wheat flour	282.0
Fish meal 82	132.0
Poultry by product meal	74.0
Sepiolite 5	23.0
Vitamins and minerals premix	39.0
Gelatin 3	29.0
Tuna oil	18.0
Calcium carbonate	16.0
Monosodium phosphate	13.0
Soybean oil	37.0
Lecithin powder 95	8.0
Salt	5.0
Cholesterol 80% XG	2.0
PHB amorphous	20.0

Abbreviation: PHB, poly-β-hydroxybutyrate.

respectively. At the end of the 14-day acclimation period, postlarvae were transferred to the experimental setup.

The experimental units consisted of a 1,500-ml rectangular plastic reservoir filled with 1 L preheated (28°C) seawater with an individual cover and contained a single postlarva that was fed non-PHB supplemented diet for a period of 7 days to acclimate them to the new environment. Diets were given as mentioned previously. Air was continuously provided in each unit through the cover. These units were placed in a larger tank containing seawater and provided with a heater (100 W, JBL Neuhofen Germany) serving as a water bath. The temperature in the water bath system was maintained at 28°C. Photoperiod was kept at 12 hr light: 12 hr dark cycle and water change was administered daily at 50%. During the actual experiment, the postlarvae were fed a single dose of diet containing 2% ^{13}C-labeled *R. eutropha* DSM545 during the first feeding ration. Feeding with nonlabeled diets resumed in the second feeding ration. Postlarvae (n = 4 biological replicates) were sampled at 0, 4, 8, 16, 32, 48, 72, and 120 hr. These postlarvae were degutted and transferred to 1.5-ml tubes, flash-frozen in liquid nitrogen, and stored at − 80°C until further analysis. Frozen samples of postlarvae were oven-dried at 60°C until constant weight. Subsequently, dried samples of postlarvae were homogenized using a mortar and pestle and 1 ± 0.1 mg of the homogenized sample was separated and carefully transferred to tin capsules (8 × 5 mm; Sercon, UK) and pinched closed for analysis of δ^{13}C (‰).

2.4 | Experiment 2: Fatty acid-specific stable isotope analysis in whiteleg shrimp, *L. vannamei* postlarvae

The postlarval whiteleg shrimp were subjected to a 14-day acclimation period as previously described. At the end of the acclimation period, postlarvae were transferred to the experimental setup.

The experimental setup and conditions applied in this experiment were similar to those of Experiment 1. This time, however, the postlarvae (n = 4) were sampled only at the start (0 hr) and after 24 hr for fatty acid-specific stable isotope analysis. Sampling was carried out as described in Experiment 1.

2.5 | Lipid extraction and fractionation

The freeze-dried shrimp samples were extracted using a modified Bligh–Dyer method (Bligh & Dyer, 1959; Rütters, Sass, Cypionka, & Rullkotter, 2002). Briefly, 100 mg of homogenized whole shrimp sample was extracted with methanol–chloroform–water (3:1.5:2 vol/vol/vol) by shaking (Innova 2000) at 130 rpm for 2 hr. Next, the supernatants were collected, and chloroform (1.5 ml) and MilliQ water (1.5 ml) were added to enhance phase separation. The chloroform fraction was transferred to a 10-ml round-bottom tube, dried under a nitrogen flow, and stored dry at −20°C. Separation of total lipids into neutral lipids, glycolipids, and phospholipids was achieved on a chloroform-prerinsed silica column (0.5 g; activated for 3 hr at 150°C) by eluting with 2.5 ml chloroform, 2.5 ml acetone, and 5 ml methanol, respectively (Boschker, 2004). The resulting fractions were dried under nitrogen and stored at −20°C for further analysis (Heinzelmann et al., 2014).

2.6 | Fatty acid-specific stable isotope analysis

The FA composition is determined as the methyl esters of FAs (FAMEs) and its isotopic composition was determined using gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS; constituted of a Trace GC Ultra interfaced by GC/C III interface to Delta^{Plus}XP IRMS, all Thermo Scientific, Bremen, Germany) equipped with a BPX5 column (30 m, 0.25 mm i.d., 0.5 μm film thickness; SGE). The identity of individual FAME was based on retention

times and ion composition (mass spectrometry) compared with multiple reference materials (S. Bode, personal communication, April 12, 2017; Denef, Roobroeck, Wadu, Lootens, & Boeckx, 2009).

2.7 | $\delta^{13}C$ analysis

All $\delta^{13}C$ values were defined in delta notation (δ) and reported in parts per thousand (‰ or per mil). The values were calculated according to the formula used by Middelburg et al. (2000):

$$\delta^{13}C = \left[\left(\frac{\text{Rsample}}{\text{Rstandard}}\right)\right] - 1] \times 1000,$$

where $\delta^{13}C$ is the stable isotope ratio while R_{sample} and $R_{standard}$ are the molar ratios of the heavy/light isotope ($^{13}C/^{12}C$) of the sample and the reference, respectively. Samples were referenced against the International Standard Vienna Pee Dee Belemnite (VPDB; 0.0112372). Routine measurements were precise to within ±0.2‰ for natural samples while ±0.4‰ for enriched materials (Situmorang, 2015).

2.8 | Statistical analysis

Descriptive statistics were used to assess normality of the data before and after various transformations. An independent samples *t* test was used to analyze data in Experiments 1 and 2 to compare data to the control or time 0. All statistical analyses were performed using the SPSS Statistical software v.11.5 with an alpha level set at ≤0.5. Graphic presentations of results were carried with Sigmaplot 13.0 (Systat Software, Inc., Chicago, IL).

3 | RESULTS

3.1 | Experiment 1: Assimilation of ^{13}C-labeled *R. eutropha* DSM545 through time

Table 2 shows the mean $\delta^{13}C$ of postlarval whiteleg shrimp fed ^{13}C-labeled *R. eutropha* DSM545 at various time points. Statistical analysis performed 4 hr after feeding showed a significant increase in $\delta^{13}C$ (p = .000). Mean $\delta^{13}C$ values were −22.95 ± 0.23 at time 0 while this was −8.00 ± 1.49 4 hr after feeding with the test diet containing 2% ^{13}C-labeled *R. eutropha* DSM545.

TABLE 2 Mean $\delta^{13}C$ of postlarval whiteleg shrimp fed diet supplemented with ^{13}C-labeled *R. eutropha* DSM545 at various time points

Time (hr)	$\delta^{13}C$
0	−22.95 ± 0.23
4	−8.00 ± 1.49
8	−3.88 ± 1.21
16	3.73 ± 4.26
32	32.40 ± 24.22
48	4.90 ± 7.02
72	−6.88 ± 2.14
120	−4.48 ± 1.35

Note: Values represent mean ± *SEM* (n = 4). Statistical analysis performed at 4 hr after feeding showed a significant increase in $\delta^{13}C$ (p = .000).

3.2 | Experiment 2: Fatty acid-specific stable isotope analysis in whiteleg shrimp, *L. vannamei* postlarvae

Table 3 shows the $\delta^{13}C$ values in diets supplemented with either nonlabeled *R. eutropha* DSM545 or ^{13}C-labeled *R. eutropha* DSM545. There was a major ^{13}C enrichment in most long-chain fatty acids (LCFAs) belonging to the saturated and monounsaturated FA groups in diets supplemented with 2% ^{13}C-labeled *R. eutropha* DSM545. Furthermore, it also resulted in ^{13}C enrichment of two LCFAs considered essential to penaeid shrimp: linoleic acid (LOA; 18:2[n-6]) and eicosapentanoic acid (EPA; 20:5[n-3]). The two other LCFAs considered essential to penaeid shrimp, namely docosahexaenoic acid (DHA; 22:6[n-3]) and linolenic acid (LNA; 18:3[n-3]) were either nonenriched with ^{13}C or not detected in both diets.

TABLE 3 $\delta^{13}C$ values in diet either supplemented with non-labeled *R. eutropha* DSM545 or ^{13}C-labeled *R. eutropha* DSM545

LCFAs	Carbon:double bond (C:D)	Non-^{13}C supplemented diet	^{13}C supplemented diet
Saturated LCFAs			
Myristic acid	14:0	−23.18; −23.24	428.37; 422.11
Pentadecanoic acid	15:0	−14.22; −3.60	2,473.53; 2,143.55
Palmitic acid	16:0	−26.95; −27.50	308.55; 291.74
Heptadecanoic acid	17:0	−27.98; −28.10	338.17; 328.18
Stearic acid	18:0	−28.51; −29.32	−6.26; −7.74
Arachidic acid	20:0	−21.78; −23.73	−210.19; −149.20
Docosanoic acid	22:0	−35.65[a]	−32.90; −32.04
Tetracosanoic acid	24:0	−32.00; −37.21	−65.80; −56.11
Monounsaturated LCFAs			
Myristoleic acid	14:1(n-5)	−16.62[a]	5,414.96; 5,644.25
Pentadecenoic acid	15:1(n-5)	—	97,283.60; 96,017.66
Palmitoleic acid	16:1(n-7)	−23.88; −24.11	506.76; 496.30
Heptadecenoic acid	17:1(n-7)	17.51; 6.17	29,626.50[a]
Oleic acid	18:1(n-9)	−28.44; −29.55	−22.08; −20.11
Vaccenic acid	18:1(n-7)	−26.88; −33.82	204.28; 218.69
Nervonic acid	24:1(n-9)	−28.93; −29.54	142.87; 165.96
n-6 LCFAs			
Linoleic acid	18:2(n-6)-c	−29.78; −31.92	−20.00; −21.69
Dihomo-γ-linolenic acid	20:3(n-6)	−42.21[a]	32.17; 36.56
Arachidonic acid	20:4(n-6)	−29.77; −26.79	1,593.22; 1814.19
Ecosadienoic acid	20:2(n-6)	−30.72; −31.71	−33.50;34.10
Docosadienoic acid	22:2(n-6)	−32.21; −35.34	−34.87[a]
n-3 LCFAs			
Eicosapentenoic acid	20:5(n-3)	−31.02; −31.40	33.66; 33.39
Docosahexaenoic acid	22:6(n-3)	−28.95; −29.93	−31.60; −31.92

Note: Values are from two replicates (separated by semi-colon, no statistical support made due to insufficient number of replicates). —, no value obtained.
Abbreviation: LCFAs, long-chain fatty acid groups.
[a]Only one replicate.

The $\delta^{13}C$ values of the LCFAs in the neutral, glycolipid, and phospholipid fractions of the postlarval whiteleg shrimp after 24 hr are shown in Table 4. The saturated LCFA palmitic acid (16:0) was significantly enriched in both neutral and glycolipid fractions. Although this LCFA was also enriched in the phospholipid fraction, no statistical support was made due to insufficient number of replicates. The saturated LCFA stearic acid (18:0) in glycolipid and phospholipid fractions was significantly enriched. The monounsaturated LCFAs, namely palmitoleic acid (16:1[n-7]), heptadecenoic acid (17:1[n-7]), oleic acid (18:1[n-9]), vaccenic acid (18:1[n-7]), and erucic acid (22:1[n-9]), were significantly enriched in one or two lipid fractions. Significant enrichment in one or two lipid fractions was also observed for the n-6 LCFAs arachidonic acid (20:4[n-6]) and docosadienoic acid (22:2[n-6]). The n-3 LCFAs

TABLE 4 $\delta^{13}C$ values in various lipid fractions extracted from degutted postlarval whiteleg shrimp, *Litopenaeus vannamei* 24 hr after feeding with optimal diet containing 2% ^{13}C-labeled *R. eutropha* DSM545

LCFAs	Carbon:double bond (C:D)	Neutral lipids	Glycolipids	Phospholipids
Saturated LCFAs				
Myristic acid	C14:0	—	−8.31; −6.52[a]	−1.40; 5.68[a]
Pentadecanoic acid	C15:0	6.44[b]	16.21; 36.88[a]	39.37; 54.83[a]
Palmitic acid	C16:0	16.32 ± 1.73[E]	21.78 ± 1.15[E]	93.29; 110.46[a]
Heptadecanoic	C17:0	−18.14 ± 2.53[NSS]	−11.02 ± 5.15 [NSS]	—
Stearic acid	C18:0	−23.21 ± 1.99[NE]	−15.95 ± 3.04[E]	43.28 ± 21.50[E]
Arachidic acid	C20:0	—	−24.95 ± 3.38[NSS]	−16.44; −14.93[a]
Heneicosanoic acid	C21:0			−20.22[b]
Tricosylic acid	C23:0	—	−25.45 ± 0.19[NSS]	
Hexacosanoic acid	C26:0	−24.86 ± 0.45[NE]	−25.07 ± 0.43[NE]	
Monounsaturated LCFAs				
Pentadecenoic acid	C15:1(n-5)	−18.29[b]	17.90 ± 19.47[NSS]	2.19; 5.70[a]
Palmitoleic acid	C16:1(n-7)	80.96; 102.31[a]	83.32 ± 8.58[E]	39.02;37.78[a]
Heptadecenoic acid	C17:1(n-7)	38.46 ± 3.30[E]	243.70; 175.73[a]	12.56 ± 2.47[E]
Vaccenic acid	C18:1(n-7)	53.34 ± 7.90[E]	38.13 ± 4.38[E]	−19.60 ± 0.66[E]
Oleic acid	C18:1(n-9)	−22.01 ± 0.68[E]	−20.91 ± 0.80[E]	—
Eicosenoic acid	C20:1(n-9)	−20.17[b]	−18.96 ± 2.87[NE]	−23.77 ± 2.27[NE]
Erucic acid	C22:1(n-9)	—	−22.99 ± 0.19[E]	
n-6 LCFAs				
Linoleic acid	C18:2(n-6)	−27.70 ± 0.91[NE]	−29.56 ± 0.26[NE]	
Ecosadienoic acid	C20:2(n-6)	−25.77 ± 2.06[NSS]	−25.84 ± 2.23[NE]	—
Dihomo-γ-linolenic acid	C20:3(n-6)	—	−16.28[b]	−23.66 ± 0.41[NE]
Arachidonic acid	C20:4(n-6)	−21.71 ± 1.77[E]	−18.91 ± 4.32[NE]	−16.58 ± 1.80[E]
Docosadienoic acid	C22:2(n-6)		−22.99 ± 0.19[E]	−23.76 ± 0.26[NE]
n-3 LCFAs				
Eicosatrienoate	C20:3(n-3)		−20.81[b]	−17.97 ± 1.39[E]
Docosahexaenoic	C22:6(n-3)	−23.41 ± 0.43[NE]	−23.98 ± 0.36[E]	—
Eicosapentenoic acid	C20:5(n-3)	−23.64 ± 0.54[NE]	−23.60 ± 0.67[NE]	−22.03 ± 2.26[NE]

Note: Values are means (either having three or four replicates) ± *SEM*. Statistical analysis made for $\delta^{13}C$ values at time 0 and 24 hr ($\delta^{13}C$ values at time 0 not presented). —, no value obtained; [E], significantly enriched 24 hr after feeding.
[a]Only two replicates; [NSS], no statistical support made.
[b]Only one replicate; [NE], nonsignificantly enriched 24 hr after feeding.

eicosatrienoate (20:3[n-3]) and docosahexaenoic acid (22:6(n-3) were significantly enriched only in the phospholipid and glycolipid fractions, respectively.

Table 5 shows a comparison of the ^{13}C enrichment between diets supplemented with ^{13}C-labeled *R. eutropha* DSM545 and LCFAs in various lipid fractions of postlarval whiteleg shrimp. The majority of the ^{13}C-enriched FAs detected in the diet were also enriched in one or two lipid fractions of the postlarvae except for those FAs in the lipid fractions that were not statistically tested. There were, however, FAs that were detected in the diet but either absent or nonenriched in one of two lipid fractions of the postlarvae. Examples for this were the two saturated LCFAs docosanoic acid (22:0) and the tetracosanoic acid (24:0) and the monounsaturated LCFAs myristoleic acid (14:1[n-5]) and nervonic acid (24:1[n-9]). The n-6 LCFAs linoleic acid (18:2[n-6]) and the n-3 LCFAs eicosapentenoic acid (20:5(n-3) were also detected in the diet but either absent or nonenriched in the lipid fractions of the postlarvae. Surprisingly, the monosaturated LCFA erucic acid (22:1[n-9]) and the n-3 LCFA eicosatrienoate (20:3[n-3]) were enriched in glycolipids and phospholipids, respectively, but these FAs were not detected in the diet.

4 | DISCUSSION

In the present study, the measurement of the stable carbon isotope ratio ($\delta^{13}C$) was applied to demonstrate for the first time the fate of the bacterial storage compound PHB once ingested and degraded in vivo in crustacean tissues.

According to Situmorang (2015), the production of PHB in the bacterial strain *R. eutropha* DSM545 is triggered by nutrient limitation (particularly ammonium) combined with the provision of ^{13}C-labeled glucose to the cultivation broth. This procedure is intended to redirect the carbon flux from production of bacterial biomass to PHB synthesis, typically from 10 to 75% (Mozumder, De Wever, Volcke, & Garcia-Gonzalez, 2014). Based on this information, it is expected that there was a massive transfer of the ^{13}C signal originating from the labeled glucose to the PHB. The data also show that the ^{13}C may be partly transferred to the structural components (e.g., FAs) of the bacterial cell. This is based on the consistent enrichment of the two LCFAs pentadecanoic acid (15:0) and palmitic acid (16:0) in the ^{13}C-enriched shrimp diet and in the postlarval whiteleg shrimp. These LCFAs are reported to be two of the most dominant saturated FAs in *Ralstonia* spp. For instance, Behiry, Younes, Mohamed, and Mohamed (2015) reported the LCFAs pentadecanoic acid (15:0) and palmitic acid (16:0) to account for 13 and 48% and 18 and 22%, respectively, of the total FA contents in two European *Ralstonia solanacearum* isolates Scotland RsSc1 and NetherlandsRsNe1, respectively. In the present study, however, the exact ratio of the ^{13}C transferred in PHB and in the structural components of *R. eutropha* DSM545 is not known. This would warrant further investigation.

It could be observed that the LCFA composition of diet supplemented either nonlabeled or ^{13}C-labeled *R. eutropha* DSM545 closely reflect each other. The ^{13}C enrichment in LCFAs from various lipid fractions of the postlarval whiteleg shrimp was generally lower than that in the shrimp diet. These findings may have important implications as to the potential of PHB as an energy source as reported by De Schryver (2010) and Defoirdt, Halet, et al. (2007).

Interestingly, the LCFAs erucic acid (22:1[n-9]) and eicosatrienoate (20:3[n-3]) were enriched in one of the lipid fractions of the postlarval whiteleg shrimp, but these were not detected in the diet. The capacity of crustaceans to bioconvert FAs was demonstrated by De Troch et al. (2012) using harpacticoid copepods. These authors have measured arachidonic acid (20:4[n-6]) in the copepod while this was missing from the bacterial diet. In the present study, the presence of erucic acid (22:1[n-9]) in the glycolipid fraction of the postlarvae may have resulted as an elongation product of oleic acid (18:1[n-9]), which was present in the diet (Akoh & Min, 1998). On the other hand, eicosatrienoate (20:3[n-3]) is usually an elongation product of alpha-linolenic acid (18:3[n-3]) (British Nutrition Foundation, 1992); however, the eicosatrienoate (20:3[n-3]) in the phospholipid fraction of the postlarvae may have come from elongation or desaturation systems other than α-linolenic acid (18:3[n-3]) as this FA was not detected in the diet.

It is also noteworthy to mention that ^{13}C was rapidly assimilated in the tissues of postlarval whiteleg shrimp based on the higher $\delta^{13}C$ value 4 hr after feeding. These findings may indicate that PHB offered in natural matrix is

TABLE 5 Comparison between ^{13}C enrichment in diet supplemented with ^{13}C-labeled *R. eutropha* DSM545 and in various lipid fractions of degutted postlarval whiteleg shrimp

LCFAs	Carbon:double bond (C:D)	^{13}C-supplemented diet	Lipid fractions in postlarval *L. vannamei*		
			Neutral lipids	Glycolipids	Phospholipids
Saturated LCFAs					
Myristic acid	14:0	+++		**'+'**	**'+'**
Pentadecanoic acid	15:0	++++	**+**	**++**	**++**
Palmitic acid	16:0	+++	++	++	++
Heptadecanoic acid	17:0	+++	**"++"**	**'++'**	x
Stearic acid	18:0	'+'	–	"++"	++
Arachidic acid	20:0	"+++"	x	**'++'**	**'++'**
Heneicosanoic acid	21:0	x	x	x	'++'
Docosanoic acid	22:0	'++'	x	x	x
Tricosylic acid	23:0	x	x	**'++'**	x
Tetracosanoic acid	24:0	'++'	x	x	x
Hexacosanoic acid	26:0	x	'++'	'++'	x
Monounsaturated LCFAs					
Myristoleic acid	14:1(n-5)	++++	x	x	x
Pentadecenoic acid	15:1(n-5)	+++++	**'++'**	**++**	**+**
Palmitoleic acid	16:1(n-7)	+++	**++**	++	**++**
Heptadecenoic acid	17:1(n-7)	+++++	++	**+++**	++
Oleic acid	18:1(n-9)	'++'	'++'	'++'	x
Vaccenic acid	18:1(n-7)	+++	++	++	'++'
Eicosenoic acid	20:1(n-9)	x	**'++'**	–	–
Erucic acid	22:1(n-9)	x	x	'++'	x
Nervonic acid	24:1(n-9)	+++	x	x	x
n-6 LCFAs					
Linoleic acid	18:2(n-6)-c	'++'	–	–	x
Ecosadienoic acid	20:2(n-6)	x	**'++'**	–	x
Dihomo-γ-linolenic acid	20:3(n-6)	++	x	**'++'**	–
Arachidonic acid	20:4(n-6)	++++	'++'	–	'++'
Docosadienoic acid	22:2(n-6)	'++'	x	'++'	–
n-3 LCFAs					
Eicosatrienoate	20:3(n-3)	x	x	**'++'**	'++'
Eicosapentenoic acid	20:5(n-3)	++	–	–	–
Docosahexaenoic acid	22:6(n-3)	'++'	–	'++'	–

Note: **+**, $\delta^{13}C$ value presented as ones (no statistical support because of insufficient number of replicates); **'+'**, $\delta^{13}C$ in negative value presented as ones; '+', $\delta^{13}C$ in negative value presented as ones (no statistical support because of insufficient number of replicates); ++, $\delta^{13}C$ value presented as tens; **++**, $\delta^{13}C$ value presented as tens (no statistical support because of insufficient number of replicates); '++', $\delta^{13}C$ negative value presented as tens; **'++'**, $\delta^{13}C$ negative value presented as tens (no statistical support because of insufficient number of replicates); +++, $\delta^{13}C$ value presented as hundreds; **+++**, $\delta^{13}C$ value presented as hundreds (no statistical support because of insufficient number of replicates); '+++', $\delta^{13}C$ negative value presented as hundreds; ++++, $\delta^{13}C$ value presented as thousands; +++++, $\delta^{13}C$ value presented as tens of thousands; –, non-enriched FAs; x, no $\delta^{13}C$ value obtained.

Abbreviation: LCFAs, long-chain fatty acids.

rapidly assimilated in the tissues of crustaceans. Earlier results obtained by Situmorang (2015) on Nile tilapia, *O. niloticus* fed a diet supplemented with ^{13}C-labeled *R. eutropha* DSM545 containing 75% of PHB on d.w., which showed nonsignificantly different δ^{13}C values from 2 hr until 24 hr postfeeding. In the fecal matter, however, these authors have found significantly higher δ^{13}C value 2 hr postfeeding indicating that majority of the ^{13}C at this time point was not transferred to the tissues of the Nile tilapia but excreted as waste product. The difference in the results obtained in the present study and the earlier study may be species dependent.

5 | CONCLUSIONS

In conclusion, this study is the first to demonstrate the assimilation of PHB in crustacean tissues. There was a trend of lower ^{13}C enrichment in LCFAs from various lipid fractions of the postlarval whiteleg shrimp than in the bacterial lipids. Furthermore, PHB offered in natural matrix was rapidly assimilated in the tissues of the crustacean species. For future work, it is recommended to investigate the assimilation of PHB offered in natural matrix in short chain FAs (SCFAs) of crustacean species as the literature has mentioned that PHB is degraded into SCFAs (C2–C6) (Defoirdt, Boon, Sorgeloos, Verstraete, & Bossier, 2009).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

G.L.P. contributed to the crafting of the manuscript, experimental conduct, monitoring, collection of results, data analyses, and interpretation. J.L.L. contributed to the experimental conduct and data analyses. S.B. and P.B. contributed to the sample preparation and analyses. F.A. contributed to the experimental conduct, monitoring, and collection of data. E.A. and M.D.T. contributed to the experimental design and data analyses. P.B. contributed to the preparation of manuscript, experimental layout, data analyses, and review of the manuscript. P.D.S. contributed to the experimental layout, data analyses and interpretation, review of manuscript.

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