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The effect of dietary n-3 HUFA and 22:6n-3/20:5n-3 ratio on white shrimp larvae and postlarvae

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The influence of varying dietary n-3 HUFA concentrations and ratios of 22:6n-3/20:5n-3 (DHA/EPA) on culture performance and fatty acid composition of white shrimp (*Penaeus vannamei*) larvae and postlarvae was verified in two experiments. In a first experiment, shrimp (zoea 2 – postlarvae 11) were fed rotifers (*Brachionus plicatilis*) and *Artemia* spp. *nauplii* that were enriched with different self-emulsifying concentrates: one HUFA-free coconut oil (T1), and three iso-HUFA concentrates with DHA/EPA ratios of 0.6 (T2), 2 (T3) and 4 (T4), respectively. In a second experiment, postlarvae were fed for 25 days (starting from postlarvae 10) on iso-lipidic semi-purified diets: one HUFA-free control (TA), and four iso-HUFA diets with DHA/EPA ratios of 0.6 (TB), 0.9 (TC), 1.8 (TD) and 3.64 (TE), respectively. Experiment 1 revealed no significant differences in production characteristics, except that resistance to a counter-current stress in postlarvae 11 was significantly lower in T1 than in T2 and T3 ($p < 0.05$). In experiment 2, postlarvae of TA had a significantly lower dry weight than the other treatments at the end of the trial. The effect of the DHA/EPA ratio within the experimental limits could not be demonstrated. Analysis of the fatty acid composition of the shrimp showed notable changes of n-3 HUFA concentrations during the metamorphosis from mysis to postlarvae, and a different response to the dietary concentrations of DHA compared to EPA.

KEYWORDS: Essential fatty acids, Fatty acid composition, n-3 HUFA, Nutritional requirements, White shrimp (*Penaeus vannamei*)

INTRODUCTION

The nutritional value of lipids for marine fish and crustaceans depend mainly on the type and quantity of fatty acids (FA) they contain. In marine aquatic species FA of the n-3 family have a greater nutritional value than FA of the n-6 family, while long-chain FA prevail over shorter-chain FA of the same family (Kanazawa *et al.*, 1979b; Xu *et al.*, 1993). n-3 Highly unsaturated fatty acids (n-3 HUFA; long-chain FA) are required for optimal culture performance and production of quality fish fry and shrimp postlarvae (Watanabe *et al.*, 1983; Léger *et al.*, 1985; Sorgeloos and Léger, 1992; Rodriguez *et al.*, 1994). A dietary n-3 HUFA input is needed to compensate the

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performed according to the method described by Dhert *et al.* (1993). The zooplankton was distributed to the experimental tanks in four daily rations.

The treatments differed in the lipid composition of the enrichment products: one was based on HUFA-free coconut oil (T1) while the others were iso-HUFA preparations with DHA/EPA ratios of 0.6 (T2), 2 (T3) and 4 (T4) respectively. The experimental design was completely randomised with four replicates per treatment. Samples of enriched rotifers, enriched *Artemia*, larvae (Z2 and M3) and postlarvae (PL1 and PL10) were collected, washed with freshwater and stored at –85° C until analysis. FA composition was determined on duplicate samples. The lipid extraction followed the method described by Folch *et al.* (1957). FA were saponified with a methanolic sodium hydroxide solution (NaOH 0.5 N in methanol) and were esterified by adding 2 ml of a boron trifluoride methanol complex (14% BF_3MeOH). The fatty acid methyl esters (FAME) were analysed using a gas chromatograph SHIMADZU GC-14A instrument provided with a flame ionization detector. FAME were separated in a glass column (2.1 m × 3.2 mm ∅) packed with GP10% SP2330. The column was set to a programme temperature of 160 – 225° C (3° C min^{-1}). The temperatures of injector and detector were kept at 250° C. Nitrogen was used as gas carrier. FA were identified by comparison between different retention times of SUPELCO (PUFA-1 and PUFA-2; Sigma) qualitative standards. The quantitative analysis was done through GLC-10, RM-3 and rapeseed oil (SUPELCO, Sigma) standards which contain saturated and unsaturated fatty acid mixtures.

On day 8 of the experiment (larval stage M3/PL1) the fraction of the animals that had successfully metamorphosed into postlarvae was determined. At PL10 dry weight (60° C, 24 h) and pooled survival were determined. Also at PL10, the osmotic stress resistance was estimated by transferring postlarvae abruptly from 34 to 0 g l^{-1} and measuring the survival after 1 h. At PL11 the fraction of the animals that was able to maintain themselves during 6 min in a constant water current of 7 $cm.s^{-1}$ was determined per treatment pool according to the counter-current stress test described by Naessens *et al.* (1995). All data were analysed using one-way ANOVA and Scheffe's multiple range test ($p < 0.05$).

Experiment 2 (PL10-PL35)

PL9 of white shrimp were obtained from the Langolit S.A. hatchery in Punta Carnero, Ecuador, where they had been fed *C. gracilis* from nauplii 5 (N5) to M3 ($3.10^4 – 18.10^4$ cells ml^{-1}), *Artemia* instar I nauplii (GSL) from M3/PL1 onwards (1–5 ml^{-1} day^{-1}), commercial diets of the LANSY range (ZM, MPL and PL from INVE Aquaculture, Belgium) and supplements of Brine Shrimp Flakes (San Francisco Bay Brand, USA) from PL1 onwards according the user's instructions. They were acclimated for 1 day in a 200 l tank. Then, 250 PL10 were stocked in rectangular aquaria of 50 l provided with an air stone on the bottom. Initial individual dry weight was 0.53 mg. During 25 days, the postlarvae were reared in 34 g l^{-1} seawater which was treated as in experiment 1. The culture water was continuously exchanged at a daily rate of ~ 1000%. The temperature was maintained at 28(1)° C. Five semi-purified micro-bound diets were formulated and processed as described by Camara (1994). The formulations referred to as TB, TC, TD and TE had different concentrations of fish oils resulting in iso-lipidic and iso-HUFA diets with DHA/EPA ratios of 0.6, 1, 2 and 4 respectively. In a fifth formulation (TA) fish oils were replaced by an equal amount

very limited capacity of bio-conversion of dietary 18:3n-3 to n-3 HUFA such as 20:5n-3 (EPA) and 22:6n-3 (DHA) in marine fish and crustaceans, including white shrimp (*Penaeus vannamei*) (Kanazawa *et al.*, 1979a). For example, feeding n-3 HUFA-enriched *Artemia* or artificial diets containing these FA improved survival and/or growth of several penaeid shrimp (Kanazawa *et al.*, 1979b; Léger *et al.*, 1985; Bengtson *et al.*, 1991; Kanazawa, 1992; Xu *et al.*, 1993). Other authors reported a positive effect of dietary n-3 HUFA on the ability of shrimp to resist stress conditions, such as osmotic shocks (Tackaert *et al.*, 1989; Rees *et al.*, 1994). However, Rees *et al.* (1994) found that very high n-3 HUFA concentrations (31.2 mg g^{-1} DW) have no beneficial effect on growth in *P. monodon* postlarvae. Kanazawa (personal communication) recommended a minimal dietary provision of 1% n-3 HUFA for postlarval shrimp.

Recently, the importance of balancing these essential fatty acids (EFA) in the diets for marine fish larvae, and particularly of the superior value of DHA in comparison to EPA has been documented by Koven *et al.* (1989), Sargent *et al.* (1991), Kanazawa (1993), Mourente *et al.* (1993), and Watanabe (1993).

The present study evaluates the effect of various dietary n-3 HUFA levels and DHA/EPA ratios on white shrimp larvae and postlarvae. In a first experiment, representing the hatchery phase, animals were cultured from zoea 2 (Z2) to postlarvae 11 (PL11) stage on rotifers and *Artemia* nauplii, both enriched with experimental emulsified concentrates. A second experiment, representing the nursery phase, was performed to test experimental semi-purified diets in a culture from PL10 to PL35.

MATERIALS AND METHODS

Experiment 1 (Z2-PL11)

White shrimp larvae were obtained from the maturation department of Centro Nacional de Acuicultura e Investigaciones Marinas (CENAIM), Ecuador. They were kept in a 500 l tank and fed *Chaetoceros gracilis* until moulting to the second zoea stage (Z2). The experimental phase started when the Z2 larvae were stocked in cylindro-conical fibreglass tanks (40 l) at densities of ~ 100 Z2 l^{-1}. These tanks were provided with a central air stone. The larvae were cultured for 18 days up to PL11 (the number of days since metamorphosis from mysis to postlarvae is used here to indicate postlarval age) in filtered (1 μm) UV-treated seawater (34 g l^{-1} salinity) at 30(1)° C. Approximately 90% of the culture water was renewed using filters of varying mesh size (Z2-M1: 250 μm; M2-PL2: 300 μm; PL2-PL11: 500 μm). *C. gracilis* was fed through the mysis 3 (M3)/PL1 stage (densities decreasing from 15 10^4 to 10^4 cells ml^{-1}), and *Tetraselmis* sp. was fed from M3/PL1 through PL2 (10^4 cells ml^{-1}). Algae were added only 2 h prior to water exchange to minimize possible uptake by the live food and hitherto the algal HUFA input. Enriched rotifers (*B. plicatilis*) were fed from Z2 through M3/PL1 (20–40 ml^{-1} day^{-1}) and *Artemia* instar I nauplii (Great Salt Lake strain, GSL) were supplemented in small amounts from Z3/M1 through M3/PL1 (0.1–1.5 ml^{-1} day^{-1}). Enriched *Artemia* nauplii (GSL) were the only food as from the PL1 stage onwards (2.5–16.5 ml^{-1} day^{-1}). Enrichment of rotifers and *Artemia* was

of n-3 HUFA-free coconut oil. Each treatment consisted of three replicates. Shrimp were fed 30% of their biomass, in three daily rations. This amount was weekly adapted per treatment according to the wet weight of 10 subsampled postlarvae per aquarium and the estimated survival. Final evaluation of the culture performance was based on individual dry weight (60° C, 24 h), survival and stress resistance (osmotic stress according to Tackaert *et al.* (1989) during 30 min in 80 g l^{-1} salinity). Statistical analysis of these data was as in experiment 1. FA composition of food samples and pooled samples of postlarvae were analysed in duplicate as in experiment 1.

RESULTS

Experiment 1 (Z2-PL11)

The enrichment notably affected the FA composition and DHA/EPA ratio in the live food organisms (Table 1): it was possible to vary the DHA/EPA ratio in ranges from 0.70 to 2.25 and 0.45 to 1.77 in rotifers and *Artemia* respectively. After enrichment rotifers had an average lipid content of 18.6% on a dry weight basis (DWB), while *Artemia* had a much higher average of 37.1% on DWB.

Final individual dry weight, pooled survival, counter-current stress resistance, survival after osmotic stress and the fraction PL1 at day 8 are summarized in Table 2. No significant differences were obtained for production characteristics among the

TABLE 1. Fatty acid composition (area % of FAME), n-3/n-6 and DHA/EPA ratios in rotifers and *Artemia* nauplii

	Rotifers				*Artemia*			
	T1	T2	T3	T4	T1	T2	T3	T4
Fatty Acids								
12:0	nd	0.27	0.10	0.17	nd	0.36	0.85	0.36
16:0	18.25	13.58	15.35	15.57	18.60	9.84	10.50	10.00
18:0	7.14	4.44	4.94	5.26	6.23	3.75	3.80	3.69
18:1n-9	27.64	22.95	22.76	22.78	29.53	23.08	24.21	24.88
18:2n-6	13.48	9.72	8.49	8.74	11.62	7.42	7.21	7.04
18:3n-3	3.73	4.09	3.87	3.76	17.55	12.17	11.52	12.81
20:4n-6 + 22:1n-11	2.44	2.66	2.70	2.90	1.13	1.99	2.27	2.86
20:5n-3	2.75	9.13	6.03	4.55	2.85	14.62	9.43	7.61
22:6n-3	2.88	6.43	9.21	10.26	nd	6.57	10.23	13.52
n-3	10.95	25.05	24.61	24.05	24.33	37.93	36.42	37.57
n-6	21.82	14.71	13.71	14.35	13.77	10.26	10.71	11.10
n-3 HUFA	7.22	18.56	19.21	19.09	4.34	23.30	22.92	22.89
n-3/n-6	0.50	1.70	1.80	1.68	1.77	3.70	3.40	3.38
DHA/EPA	1.05	0.70	1.53	2.25	nd	0.45	1.08	1.77

TABLE 2. Individual dry weight, survival and osmotic stress resistance (OSR, survival after stress) at PL10, counter current resistance at PL11 (CCR), and percentage of PL1 on day 8 of the culture (experiment 1)

Treatment	Dry Weight (mg)	Survival (%)	OSR (%)	CCR (%)	PL1 (%)
T1	0.66[a](0.11)	64.2	17.8[a](5.6)	11[a](3)	77[a](26)
T2	0.73[a](0.18)	58.2	35.8[a](28.4)	36[b](7)	92[a](5)
T3	0.61[a](0.04)	78.3	23.2[a](5.3)	23[bc](6)	97[a](5)
T4	0.54[a](0.02)	71.8	21.7[a](20.7)	21[ac](7)	100[a](0)

[ab]Values within the same column not sharing a common superscript, are significant different ($p < 0.05$, Scheffe's multiple range test).

various groups ($p < 0.05$). Only the counter-current stress test indicated that postlarvae of T2 and T3 were significantly more resistant than those of T1 and T4, respectively T1; the osmotic stress test produced a similar ranking, but without significant differences. The metamorphosis fraction was smallest in T1, although not significant because of the high variation among replicates in this treatment.

Table 3 gives the results of the FA analysis of the larvae and postlarvae. Fig. 1 illustrates the n-3 HUFA composition of the animals as a function of the larval stage. At M3, PL1 and PL10 stages the DHA/EPA ratio in the animals reflected the one of the diets. However, larval DHA/EPA proportions never exceeded 1 whereas in the food organisms values as high as 2.25 were obtained. At the Z2 stage, up to which only *C. gracilis* was fed, the concentrations of DHA and EPA were high: 6.28 and 11.67% respectively. Under the experimental culture conditions these levels were never reached again with the exception of DHA content in M3 stages of T2, T3 and T4. The n-3/n-6 ratios remained relatively stable during the culture period and did not differ substantially among treatments; ratios from 1.13 to 2.06 were recorded.

During the Z2-M3 period, it was observed that when the low HUFA diet was offered (T1), the larval DHA and EPA concentrations both dropped although they remained higher than the live food level (rotifers T1). In all treatments the EPA concentration dropped considerably and ranged between 7.57 and 8.87% in M3. DHA increased along with the concentrations in the food, although never reaching concentrations higher than 7.42% even when rotifers with a DHA concentration of 10.26% were fed.

In all treatments the critical phase of metamorphosis from M3 to PL1 was characterized by an increase of EPA and a decrease of DHA concentrations, resulting in a drop of the DHA/EPA ratios.

Shortly upon completion of metamorphosis the postlarvae were fed enriched *Artemia* as their only diet. According to the treatment, EPA concentrations in this diet varied within a range of roughly 3 – 15%. Nevertheless, in all treatments larval concentrations exhibited a steady decrease compared to the levels at PL1 and converged to the narrow range of 7.39–8.02% (i.e. comparable values as observed in M3). DHA levels at the PL10 stage again tended to reflect the dietary values, although at a lower scale.

TABLE 3. Selected fatty acid composition (area % of FAME), n-3/n-6 and DHA/EPA ratios in larvae (Z2, M3) and postlarvae (PL1, PL10) of *P. vannamei* as a function of treatments (experiment 1)

		T1			T2			T3			T4		
	Z2	M3	PL1	PL10	M3	PL1	PL10	M3	PL1	PL10	M3	PL1	PL10
Fatty acids													
12:0	0.16	0.37	0.20	0.24	0.18	nd	0.12	0.2	0.17	0.13	0.16	0.21	0.18
16:0	15.83	12.76	12.69	16.83	13.41	16.42	15.89	14.6	14.59	15.66	14.96	15.98	15.67
18:0	7.78	8.37	8.01	9.72	7.43	7.75	8.81	8.87	8.12	9.62	7.58	8.29	9.04
18:1n-9	13.97	17.86	21.86	22.85	18.17	25.00	22.69	17.33	21.50	22.50	20.24	22.79	20.43
18:2n-6	4.13	5.92	6.11	4.93	5.98	4.19	5.45	5.86	4.51	6.23	5.71	4.05	3.88
18:3n-3	1.87	4.34	5.50	10.29	4.25	4.65	8.87	3.79	4.95	8.72	4.55	4.69	7.26
20:4n-6	3.43	2.77	4.00	3.91	4.00	3.51	3.84	3.81	3.87	4.09	3.41	3.93	4.23
+22:1n-11													
20:5n-3	11.67	8.42	9.28	7.81	8.87	8.56	8.02	7.77	8.56	7.39	7.57	9.00	7.71
22:6n-3	6.28	4.70	2.37	2.18	6.11	4.06	4.50	7.07	4.06	5.07	7.42	4.76	6.83
n-3	23.81	23.62	20.34	22.91	24.36	22.48	24.59	21.46	21.24	24.13	24.91	21.68	24.84
n-6	12.27	15.42	13.93	12.09	15.68	11.43	12.25	18.99	12.85	11.79	12.10	12.04	12.10
n-6 HUFA	20.49	18.07	13.53	11.51	18.61	17.13	14.71	16.08	15.11	14.49	19.04	16.05	16.58
n-3/n-6	1.94	1.53	1.46	1.89	1.55	1.97	2.01	1.13	1.65	2.05	2.06	1.80	2.05
DHA/EPA	0.54	0.56	0.26	0.28	0.69	0.47	0.56	0.91	0.47	0.69	0.98	0.53	0.89

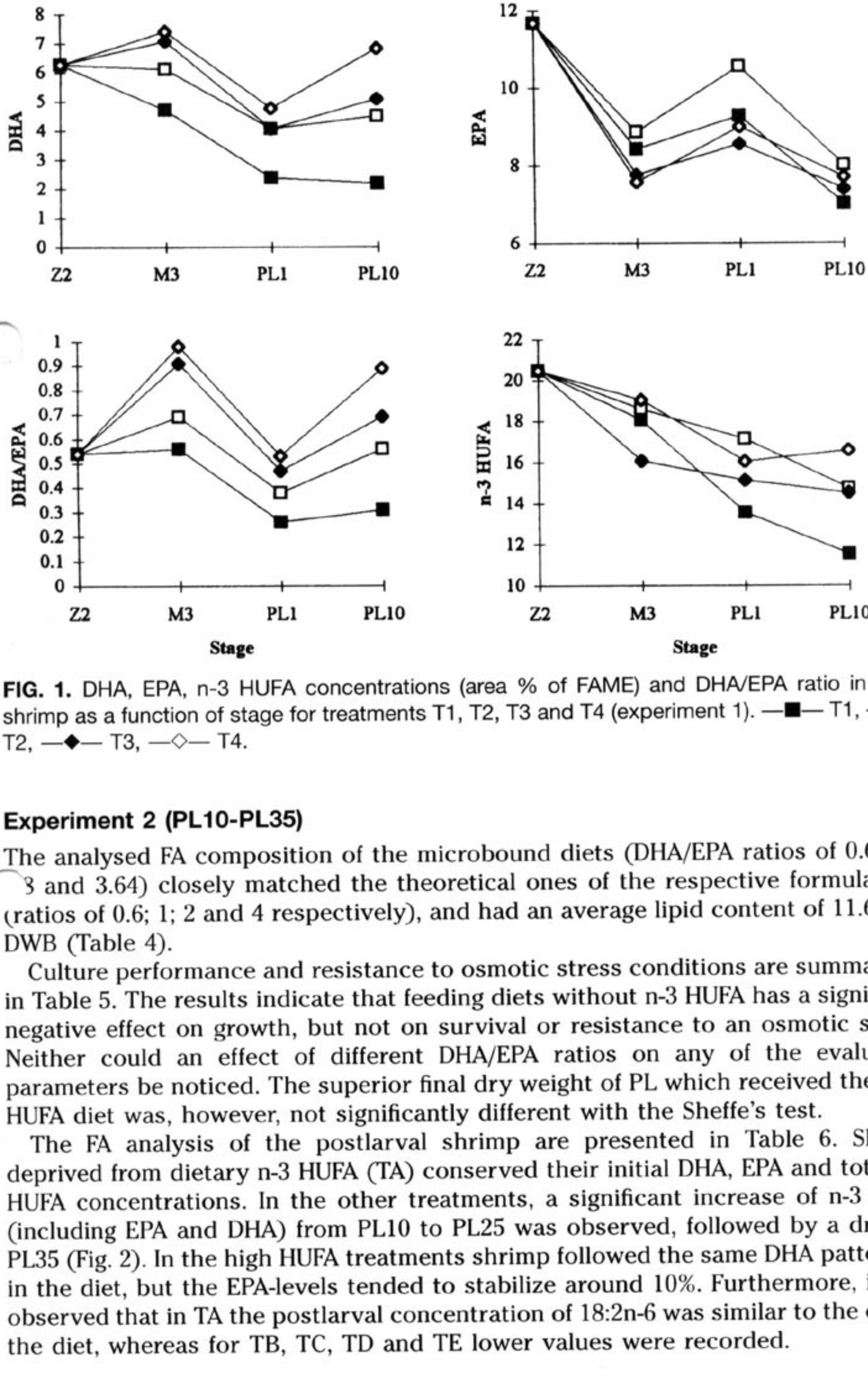

FIG. 1. DHA, EPA, n-3 HUFA concentrations (area % of FAME) and DHA/EPA ratio in white shrimp as a function of stage for treatments T1, T2, T3 and T4 (experiment 1). —■— T1, —□— T2, —◆— T3, —◇— T4.

Experiment 2 (PL10-PL35)

The analysed FA composition of the microbound diets (DHA/EPA ratios of 0.6; 0.9; 3 and 3.64) closely matched the theoretical ones of the respective formulations (ratios of 0.6; 1; 2 and 4 respectively), and had an average lipid content of 11.6% on DWB (Table 4).

Culture performance and resistance to osmotic stress conditions are summarized in Table 5. The results indicate that feeding diets without n-3 HUFA has a significant negative effect on growth, but not on survival or resistance to an osmotic stress. Neither could an effect of different DHA/EPA ratios on any of the evaluation parameters be noticed. The superior final dry weight of PL which received the high HUFA diet was, however, not significantly different with the Sheffe's test.

The FA analysis of the postlarval shrimp are presented in Table 6. Shrimp deprived from dietary n-3 HUFA (TA) conserved their initial DHA, EPA and total n-3 HUFA concentrations. In the other treatments, a significant increase of n-3 HUFA (including EPA and DHA) from PL10 to PL25 was observed, followed by a drop at PL35 (Fig. 2). In the high HUFA treatments shrimp followed the same DHA pattern as in the diet, but the EPA-levels tended to stabilize around 10%. Furthermore, it was observed that in TA the postlarval concentration of 18:2n-6 was similar to the one in the diet, whereas for TB, TC, TD and TE lower values were recorded.

TABLE 4. Selected fatty acid composition (area % of FAME), n-3/n-6 and DHA/EPA ratios in the experimental diets (experiment 2)

	Experimental Diets				
	TA	TB	TC	TD	TE
Fatty Acids					
12:0	39.38	0.23	0.56	0.26	0.22
16:0	11.91	15.54	16.05	17.62	19.26
18:0	3.66	4.49	4.51	5.00	5.63
18:1n-9	11.37	17.29	16.60	16.91	17.17
18:2n-6	13.34	14.99	13.57	13.97	13.78
18:3n-3	1.57	3.59	3.48	3.07	2.97
20:3n-6 +22:1n-11	nd	2.00	1.94	2.04	2.01
20:5n-3	–	10.99	10.02	7.03	4.14
22:6n-3	–	6.62	8.97	12.43	15.03
n-3	1.69	27.27	27.69	27.39	26.34
n-6	12.75	16.69	17.31	17.69	17.84
n-3 HUFA	nd	23.82	22.43	23.08	22.40
n-3/n-6	0.13	1.63	1.60	1.55	1.48
DHA/EPA	–	0.60	0.90	1.80	3.64

The DHA/EPA ratio of the postlarvae, which was initially 0.85, did slightly fluctuate during postlarval development according to the ratios in the diets. In TE, for example, an increase of this ratio up to 1.39 could be observed. This value, however, was still way below the dietary ratio of 3.64.

DISCUSSION

A remarkable difference was noticed between rotifers and *Artemia* in their ability to reach high DHA/EPA ratios through enrichment with self-emulsifying concentrates. Lower ratios and higher EPA concentrations were obtained after enrichment in

TABLE 5. Dry weight, survival and osmotic stress resistance (OSR, survival after stress) of PL35 (experiment 2)

Treatment	Dry Weight (mg)	Survival (%)	OSR (%)
TA	2.69^{a}(0.20)	94.8^{a}(0.7)	64.6^{a}(12.0)
TB	6.73^{b}(0.85)	92.7^{a}(7.2)	59.0^{a}(3.8)
TC	5.69^{b}(1.64)	89.1^{a}(3.0)	46.1^{a}(6.7)
TD	5.97^{b}(1.01)	93.2^{a}(2.9)	65.6^{a}(12.0)
TE	8.29^{b}(0.59)	90.7^{a}(1.7)	62.7^{a}(15.3)

^{a,b}Values within the same column not sharing a common superscript, are significant different ($p < 0.05$, Scheffe's multiple range test).

TABLE 6. Selected fatty acid composition (area % of FAME), n-3/n-6 and DHA/EPA ratios in postlarvae (PL25, PL35) of *P. vannamei* as a function of treatments (experiment 2)

		TA		TB		TC		TD		TE	
	PL10	PL25	PL35	PL25	PL35	PL25	PL35	PL25	PL35	PL25	PL35
Fatty acids											
12:0	nd	0.31	nd	nd	0.23	1.35	0.14	2.23	0.13	nd	0.13
16:0	15.25	14.81	14.63	15.81	16.86	15.30	15.80	13.97	15.66	13.75	15.66
18:0	9.25	4.99	5.06	7.75	8.17	6.92	7.25	5.40	7.58	5.85	8.02
18:1n-9	22.66	19.61	19.09	16.25	19.09	16.27	17.86	15.86	17.20	15.35	17.20
18:2n-6	5.41	13.75	12.75	7.99	9.89	8.20	10.91	8.48	9.08	8.08	9.37
18:3n-3	10.25	3.84	3.52	3.41	3.03	2.91	3.21	3.19	3.51	3.16	3.50
20:4n-6 +22:1n-11	3.71	3.50	3.75	3.43	2.74	3.56	3.08	4.50	3.35	4.60	3.71
20:5n-3	7.06	6.86	7.26	10.36	10.24	11.49	10.69	13.11	10.60	10.81	8 .54
22:6n-3	5.98	5.58	6.50	8.41	6.88	9.04	8.52	12.95	10.73	14.97	11.41
n-3	25.84	19.94	21.12	27.61	13.41	26.78	25.83	30.02	26.15	29.54	25.49
n-6	13.86	25.79	26.03	17.78	17.24	19.55	18.94	22.43	17.10	22.56	17.26
n-3 HUFA	15.88	15.60	16.96	23.43	19.72	23.29	21.95	26.06	21.94	25.78	23.43
n-3/n-6	1.86	0.77	0.81	1.55	1.36	1.37	1.36	1.34	1.53	1.31	1.59
DHA/EPA	0.85	0.81	0.91	0.81	0.67	0.79	0.80	0.99	1.01	1.39	1.34

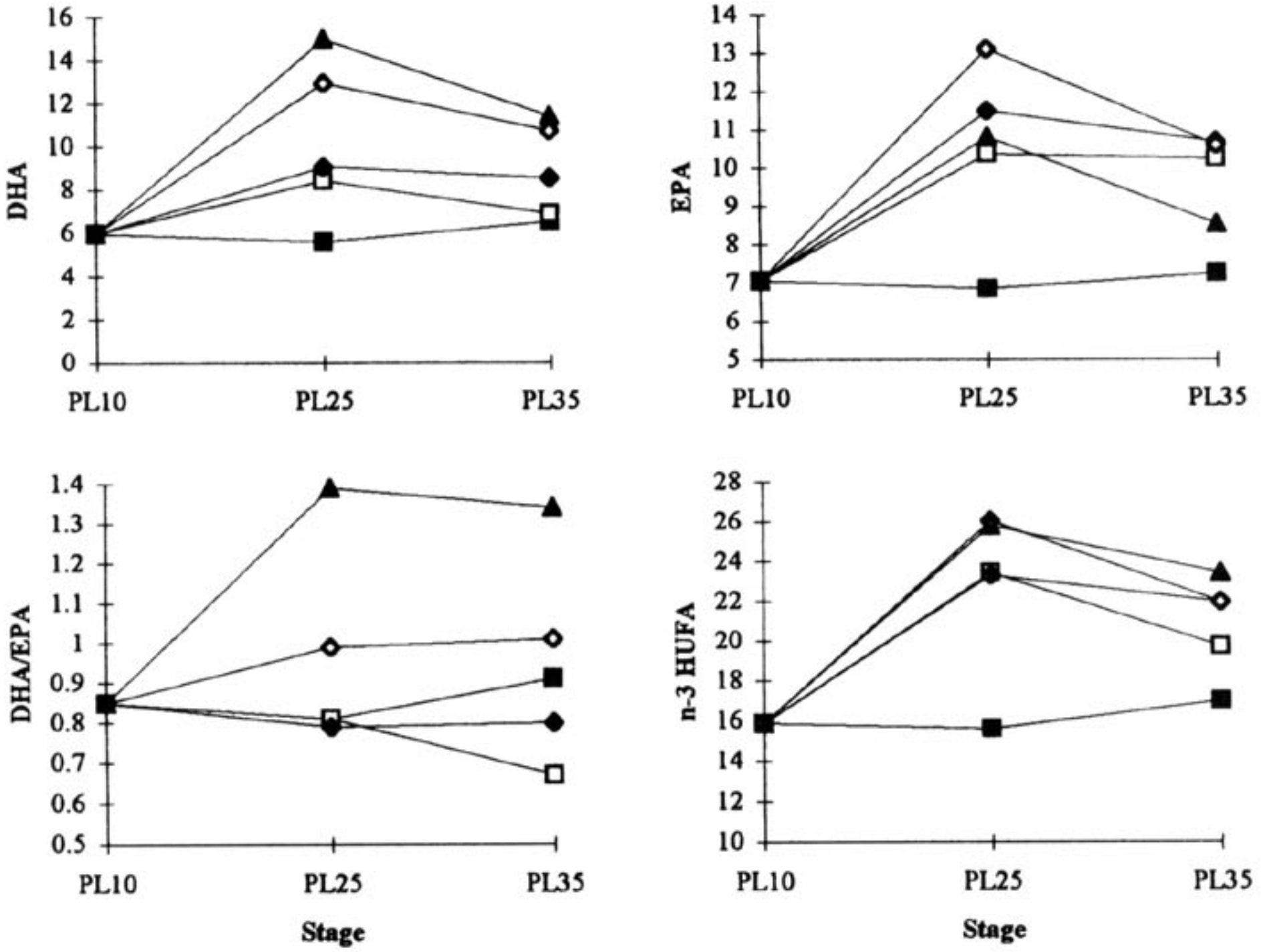

FIG. 2. DHA, EPA, n-3 HUFA concentrations (area % of FAME) and DHA/EPA ratio in white shrimp as a function of stage for treatments TA, TB, TC, TD and TE (experiment 2). —■— TA, —□— TB, —◆— TC, —◇— TD, —▲— TE.

Artemia in comparison with rotifers. Similar observations on the obtained DHA/EPA ratios were made by Dhert *et al.* (1993), but these could not be explained by differences in EPA concentrations; instead they found a higher DHA retention rotifers than in *Artemia*.

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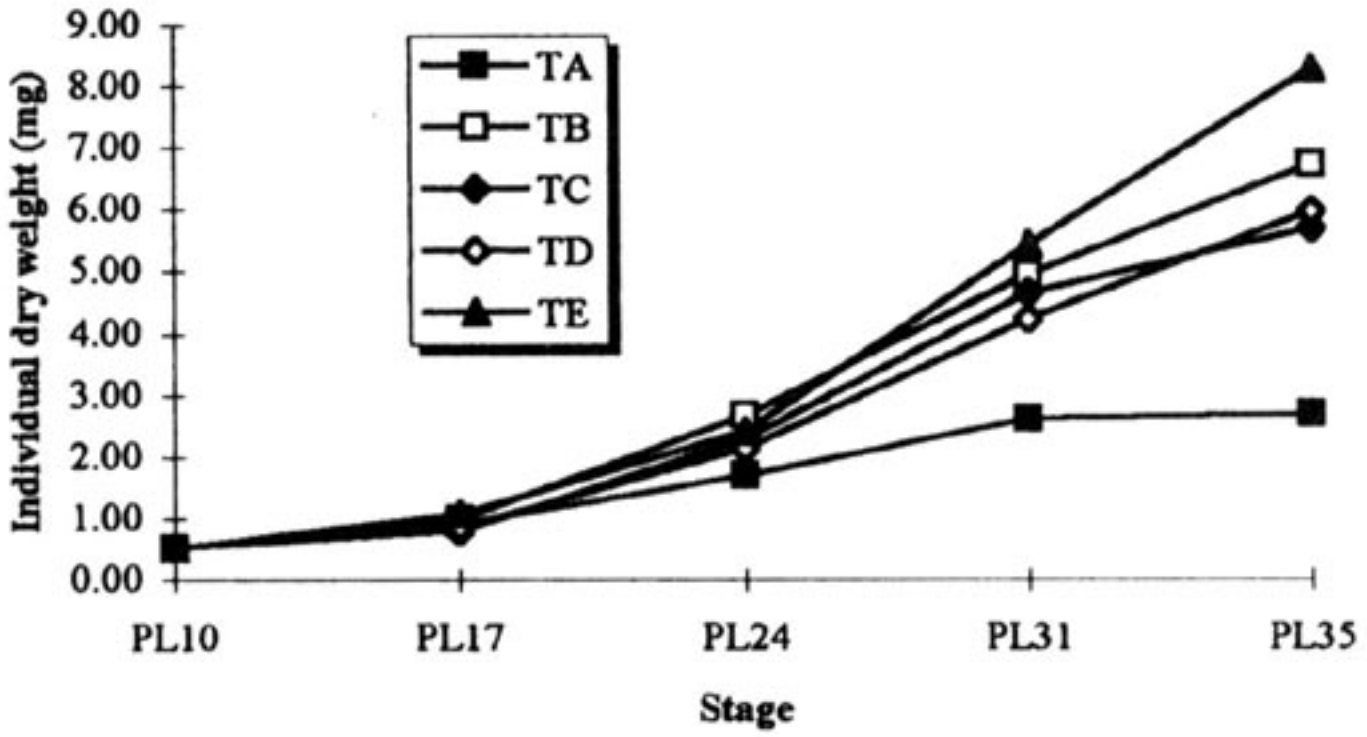

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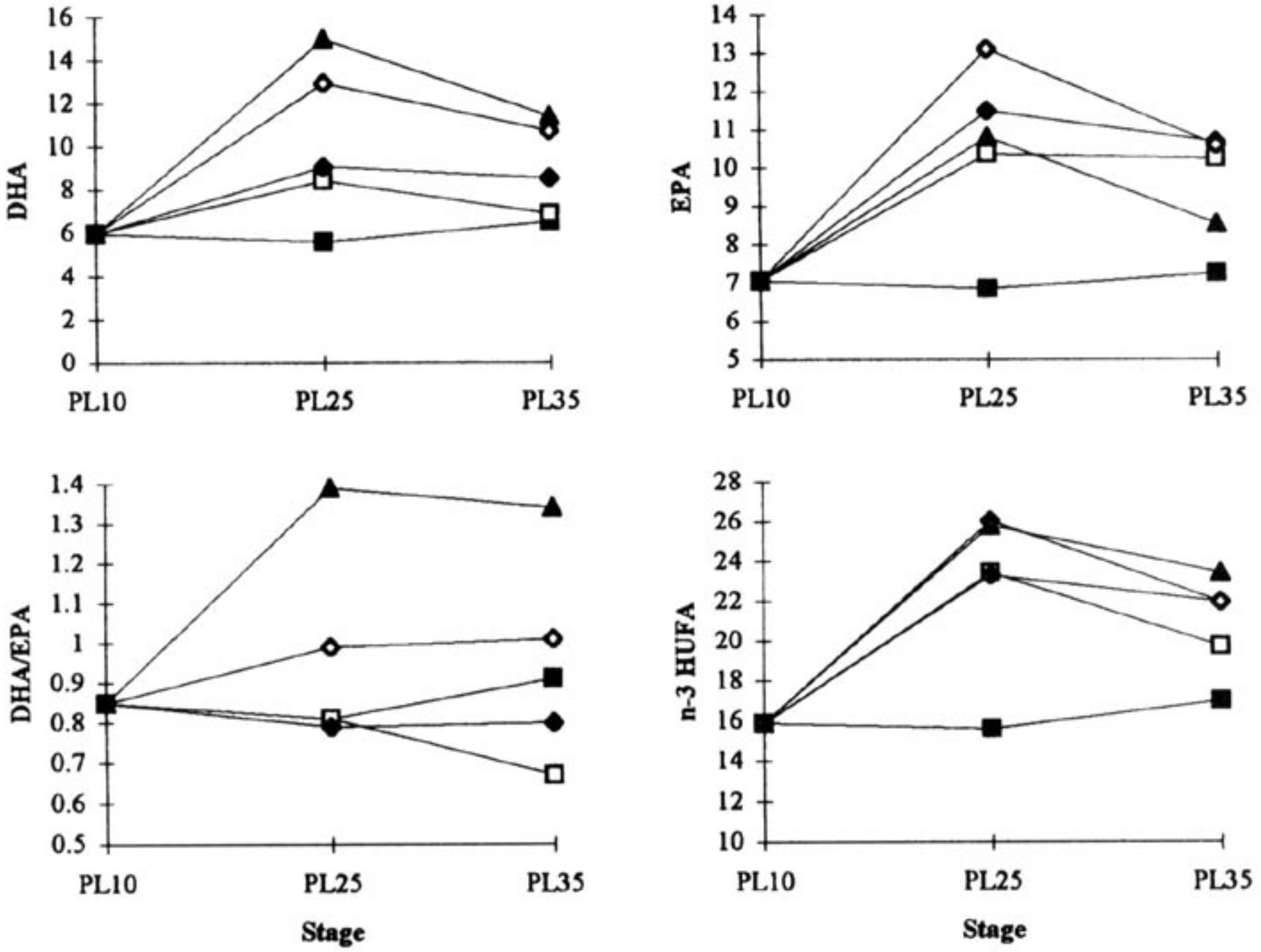
TABLE 6. Selected fatty acid composition (area % of FAME), n-3/n-6 and DHA/EPA ratios in postlarvae (PL25, PL35) of *P. vannamei* as a function of treatments (experiment 2)

		TA		TB		TC		TD		TE	
	PL10	PL25	PL35	PL25	PL35	PL25	PL35	PL25	PL35	PL25	PL35
Fatty acids											
12:0	nd	0.31	nd	nd	0.23	1.35	0.14	2.23	0.13	nd	0.13
16:0	15.25	14.81	14.63	15.81	16.86	15.30	15.80	13.97	15.66	13.75	15.66
18:0	9.25	4.99	5.06	7.75	8.17	6.92	7.25	5.40	7.58	5.85	8.02
18:1n-9	22.66	19.61	19.09	16.25	19.09	16.27	17.86	15.86	17.20	15.35	17.20
18:2n-6	5.41	13.75	12.75	7.99	9.89	8.20	10.91	8.48	9.08	8.08	9.37
18:3n-3	10.25	3.84	3.52	3.41	3.03	2.91	3.21	3.19	3.51	3.16	3.50
20:4n-6 +22:1n-11	3.71	3.50	3.75	3.43	2.74	3.56	3.08	4.50	3.35	4.60	3.71
20:5n-3	7.06	6.86	7.26	10.36	10.24	11.49	10.69	13.11	10.60	10.81	8 .54
22:6n-3	5.98	5.58	6.50	8.41	6.88	9.04	8.52	12.95	10.73	14.97	11.41
n-3	25.84	19.94	21.12	27.61	13.41	26.78	25.83	30.02	26.15	29.54	25.49
n-6	13.86	25.79	26.03	17.78	17.24	19.55	18.94	22.43	17.10	22.56	17.26
n-3 HUFA	15.88	15.60	16.96	23.43	19.72	23.29	21.95	26.06	21.94	25.78	23.43
n-3/n-6	1.86	0.77	0.81	1.55	1.36	1.37	1.36	1.34	1.53	1.31	1.59
DHA/EPA	0.85	0.81	0.91	0.81	0.67	0.79	0.80	0.99	1.01	1.39	1.34



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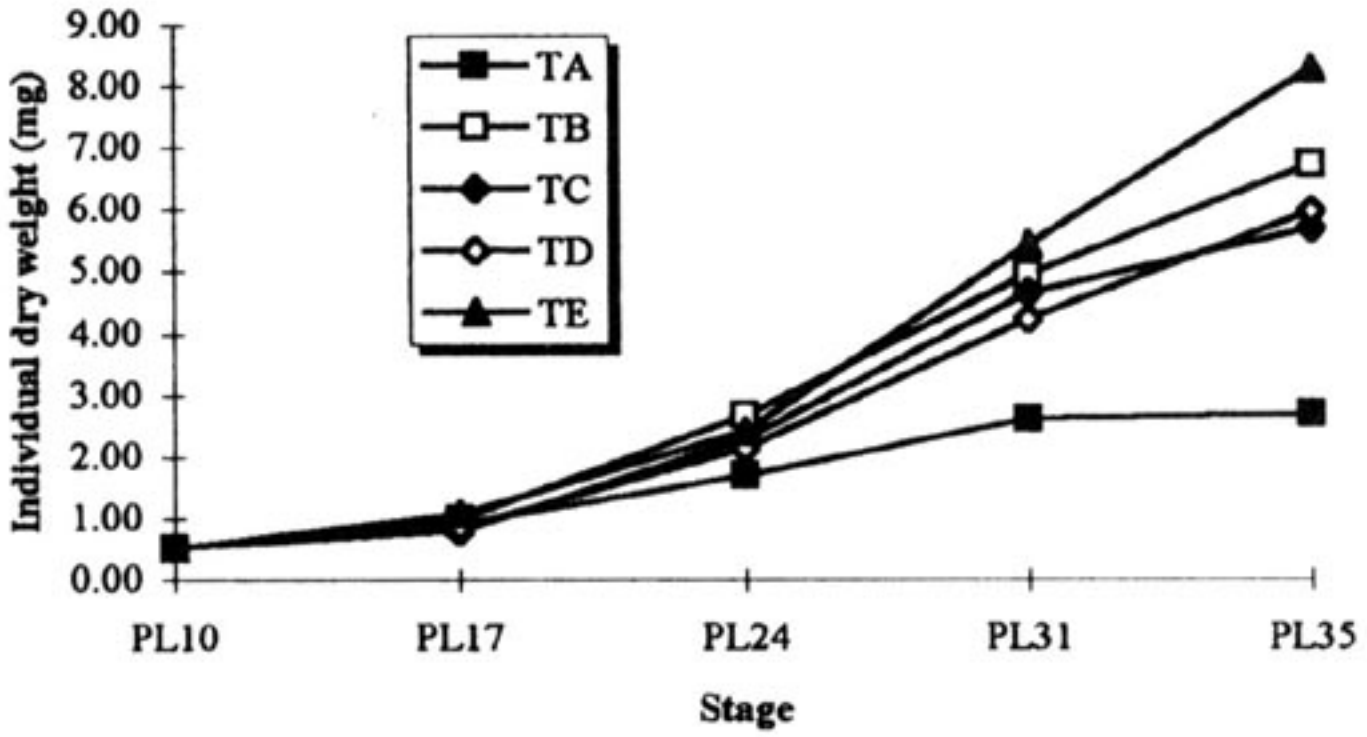

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