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Total replacement of fish meal by enriched-fatty acid *Hermetia illucens* meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles

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Abstract

The study was designed to evaluate the effects of total remplacement of fish meal (FM) and fish oil (FO) by vegetable oil and black soldier fly (BSF) larval meal enriched with fatty acids (FAs) in Nile tilapia juveniles. Fish were fed a FMFO control diet compared to a non-FA-enriched BSF diet (BSF/T0) and diets enriched in linolenic acid-ALA (BSF/T1) or in eicosapentanoic acid-EPA (BSF/T2). After 59 days, the BSF diets did not affect growth except for a decrease by the BSF/T1 diet. However, protein utilization and digestibility were reduced by all the BSF diets. FA-enriched diets did not improve the digestive enzyme activities or immune parameters, while lysozyme and ACH50 values were increased by the BSF/T0 diet. Levels of polyunsaturated FAs in the whole body of fish fed ALA or EPA-enriched BSF diets were comparable to those of FMFO controls. The results demonstrate that BSF meal can totally replace FM without substantially effect on growth or innate immune status. The decrease in fish carcass FA quality induced by the BSF meal can be prevented by a well defined protocol for PUFA enrichment. Nonetheless, investigation of the long-term effects of the BSF meal use during the ongrowing phase is still needed.

KEYWORDS
digestibility, fatty acid enrichment, growth, *Hermetia illucens*, immunity, Nile tilapia

1 | INTRODUCTION

The limited availability of fish by-products is considered as one of the major constraints in the future development of aquaculture (Nguyen et al., 2019; Schalekamp et al., 2016), and finding appropriate their substitutes is currently one of the major issues in fish nutrition. Recently, meals from insects have received great attention as one of the new valuable ingredients for aquafeeds since they have many advantages like low environmental impact, ability to grow on waste and by-products, high feed conversion efficiency and low risk of transmitting zoonotic infections (van Huis et al., 2013; Wang et al., 2007). Insect meals from fly larvae have been identified as high protein meals with a well-balanced essential amino acid profiles (Barroso et al., 2014; Henry et al., 2015). These meals can provide bio-available proteins, fats, minerals and vitamins depending on their rearing conditions (Henry et al., 2015; Looy et al., 2014; van Huis, 2013). Among several insect species able to produce rapidly a large biomass under controlled rearing conditions, the black soldier fly (BSF, *Hermetia illucens* (L. 1758)) has emerged as the most important insect in the world because their larvae can feed on virtually all types of organic wastes, including used frying oil, lignin, cellulose and liquid manure (Müller et al., 2017).

Insects can accumulate fat, especially during their immature stages; nevertheless, they are not able to bioconvert short polyunsaturated fatty acid (PUFAs) chains in long-chain polyunsaturated fatty acids (LC-PUFAs; Barroso et al., 2017). Therefore, insect meals are generally poor in n-3 LC-PUFAs (Akinnawo & Ketiku, 2000; Barroso et al., 2014; Ekpo & Onigbinde, 2007; Finke, 2002; Katayama et al., 2008). The available studies on partial substitution of fishmeal by insect meals report conflicting conclusions depending on the type of insect and fish species (Nogales-Mérida et al., 2019). For example, the partial substitution of fishmeal with *Tenebrio molitor* (L. 1758) had no impact on the meal palatability in Nile tilapia *Oreochromis niloticus* but affects the fatty acid profiles of fish muscles (Sánchez-Muros et al., 2016). Moreover, a decrease in feed utilization and growth compared to fish meal fed Nile tilapia was observed by Sánchez-Muros et al. (2014). On the other hand, the partial substitution of fish meal with larval meal of BSF associated with poultry by-product meal did not affect growth, utilization and feeding efficiency, but altered the whole-body fatty acid composition of Nile tilapia (Devic et al., 2018).

High levels of insect meal can affect the physiological status of fish through various factors such as their low fatty acid quality or reduced digestibility as stated above (Belghit et al., 2018). LC-PUFAs such as arachidonic acid [ARA (20:4n-6)], eicosapentaenoic acid [EPA (20:5 n-3)] and docosahexaenoic acid [DHA (22:6 n-3)] play important roles in fish growth and health, as well as in human health (Arts et al., 2009; Nguyen et al., 2019; Oliva-Teles, 2012; Ruxton et al., 2004; Tocher, 2015; Tocher et al., 2003; Watters et al., 2012). LC-PUFAs are important components of membrane phospholipids, particularly in specialized cells and tissues, such as the brain, retina, testes, heart, liver and kidneys of vertebrates including fish (Arts et al., 2009; Geay et al., 2010, 2015; Oliva-Teles, 2012; Sourabié et al., 2018; Tocher et al., 2003).

A prerequisite for introducing a new ingredient into fish feed is that it will not compromise fish health. In this respect, the feeding of Atlantic salmon *Salmo salar* presmolt with BSF larval meal showed an increased expression of genes indicative of stress response, immune tolerance and increased detoxification activity (Li et al., 2019). However, the effects of fish meal substitution by insect meals on the immune status are not yet well described in fish. The few available results indicate that some insect meals can positively impact the immune system of fish (Cooper & Eleftherianos, 2017) since some components of insects such as silkose or dipterose have been shown to have immunostimulatory actions in mammals (Ohta et al., 2014, 2016). Moreover, it has been reported that low dietary levels of chitin can stimulate the immune system and then increase the disease resistance of gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*; Esteban et al., 2001; Gopalakannan & Arul, 2006). As for growth, the effect of insect meal on the immune system may also vary according to their intrinsic biochemical quality as well as between fish species (Barroso et al., 2017).

Nile tilapia is an economically important freshwater aquaculture species, and its breeding is one of the fastest growing forms of aquaculture for human consumption (Liu et al., 2017; Tonial et al., 2009). Nile tilapia can bioconvert alpha-linolenic acid (ALA) to EPA and then DHA and LA (linoleic acid) to ARA (arachidonic acid) by the enzymatic pathways of desaturation and elongation (Oliva-Teles, 2012). But in the case of a complete replacement of FM by insect meal associated with the use of vegetable oil, the low PUFA dietary contents may affect this endogenous bioconversion into LC-PUFAs.

This study aimed to evaluate, on the one hand, the fatty acid accumulation capacity by the BSF larvae using different types of substrates and, on the other hand, to determine how the use of BSF larval meal enriched with either PUFAs or LC-PUFAs affect growth performance, physiological status and innate immunity of Nile tilapia. Namely, the effects of total substitution of fish oil by vegetable oil and fish meal by BSF larval meals enriched or not in ALA or EPA were determined on growth performance, nutrient digestibility, digestive enzyme activities, fatty acid composition and innate immune parameters of Nile tilapia juveniles.

2 | MATERIALS AND METHODS

2.1 | Insect meal enrichment

The production of insect meals and the in vivo fish feeding trial were conducted in the facilities of the research unit of Hydrobiology and Aquaculture (LHA) of the Faculty of Agricultural Sciences of the University of Abomey-Calavi (FSA/UAC) in Benin in accordance with the institutional considerations and international requirements for laboratory fish welfare.

Larvae of BSF were reared in an insectarium facility. In order to modulate the PUFA composition of the insect meal, three substrates

of different biochemical compositions were used (Table 1). T0: a substrate containing only vegetable by-products (cotton cake and corn bran), and rich in LA. T1: a substrate consisting of a commercial starter feed for the chicks, and rich in LA and ALA. This commercial chicken feed starter was composed of soybean seeds, corn meal, butylhydroxytoluene (BHT), methionine and lysine (Veto Service group). T2: a substrate composed of fish waste (Benin's mainland fisheries) and corn bran, rich in LA and EPA. The insect larvae were grown in triplicate polyvinyl chloride (PVC) tanks (76.50 × 56.50 × 30.50 cm, Auer Packaging). The tank system was sloping down to facilitate the removal of the prepupes from the substrates and equipped with a channel made of pvc tubing to allow automatic collection of the prepupae. Each ingredient was ground using an electric mill (Novital Molino David 4v). Then, the flours were mixed according to the proportions of each diet and moistened with water to 70%. Six kilogram of feed was introduced into each feed container. The initial density was 2 larvae/g of feed. All substrate diets were tested in triplicate. Every 2 days, 30 larvae were sampled per tank and weighed with an electronic balance (Sartorius, d = 0.001 g). The larval growth experiment was stopped when the first prepupae escape from the substrate.

After harvesting, the larvae were rinsed in sterilized water and then dried in an oven (Heraeus) at 60°C for 6 h until weight stabilized. After drying, the larvae were ground to produce the BSF meals. These larval meals were stored at −20°C, and their biochemical composition was determined (Table 2) before their incorporation in the experimental fish diets.

TABLE 1 Ingredient composition of the substrates (T0, T1 and T2) used for the production and enrichment of insect larvae and fatty acid composition analysed in the respective substrates

Ingredients (g/kg of dry matter)	Fish waste[a]	T0	T1	T2
Chicken feed[b]		0	1000	0
Fish waste[a]		0	0	230
cotton cake		400	0	0
corn bran		600	0	770
Protein		225	210	224.83
Lipid		53.8	56	53.81
Fatty acid composition (% of total identified fatty acids)				
C4:0	34.79	0	0	0.39
C14:0	3.38	0	0	0
C16:0	18.73	18.68	14.65	15.89
C18:0	7.69	2.55	3.35	4.07
C20:0	0.38	0.52	0.40	0.62
C22:0	0.12	0	0	0.22
C24:0	1.26	0	0.19	0.60
Total satured	66.36	22.03	18.89	21.79
C16:1n−7	3.31	0	0	0
C18:1n−9	7.67	29.04	26.86	31.70
C20:1n−9	0.16	0.24	0.25	0.03
C22:1n−9		0.02	0.06	0.11
Total monoenes	11.14	29.30	27.16	31.84
C18:2n−6 (LA)	3.26	48.53	49.20	40.26
C18:3n−6	0.43	0	0	0.12
C20:2	0.25	0.02	0	0.05
C22:2	0	0.02	0	0
C20:3n−6	0.11	0	0	0.08
C20:4n−6 (ARA)	0.04	0	0	0.03
Total n−6 PUFA	4.09	48.57	49.20	40.54
C18:3n−3 (ALA)	0.53	0.10	4.64	0.85
C20:3n−3	2.18	0	0	0.60
C20:5n−3 (EPA)	3.97	0.00	0.00	1.09
C22:6n−3 (DHA)	11.59	0	0	3.25
Total n−3 PUFA	18.27	0.10	4.64	5.79
n−3 / n−6 ratio	4.47	0.01	0.10	0.14

[a]Fish waste from Benin's mainland fisheries.

[b]T1 was composed of roasted soybean seeds, corn meal, butylhydroxytoluene (BHT), methionine and lysine.

2.2 | Fish experiment

2.2.1 | Experimental diets

Four isonitrogenous (320 g proteins/kg) and isolipidic (95 g lipids/kg) diets were formulated. The control diet (FMFO) contained fish meal (FM) and fish oil (FO) as protein and lipid sources, respectively. In the three BSF-based diets, FO was replaced by palm oil, while FM was totally replaced by BSF larval meals enriched in ALA (BSF/T1) or EPA (BSF/T2) compared to a non-FA-enriched diet (BSF/T0). The formulation and the chemical composition of these diets are shown in Tables 3 and 4. Meals of vegetable ingredients were heat-cooked, and all feed ingredients were homogenized and water was added gradually (35–40% of the dry ingredients) to the premixed ingredients and mixed for another 10 min. The mixture was then passed through an extruder (BEDO) with an appropriate diameter (1.2–2.2 mm) to prepare the extruded pellets. Pellets were then dried under a hood at room temperature for 12 h and then stored at −4°C.

2.2.2 | Fish rearing and feeding

The feeding experiment was conducted in an aquaculture recirculating system (RAS) including mechanical and biological water filtration systems. Water was equally aerated and exchanged at a flow rate of 3–4 L/min. Water temperature and dissolved oxygen were checked daily and ranged from 28.74 ± 1.33°C to 6.80 ± 1.46 mg/L, respectively. Nitrite and ammonia were monitored weekly and averaged 0.09 ± 0.03 and 0.08 ± 0.004 mg/L, respectively.

Male sex-reversed Nile tilapia at early juvenile stage were acclimated to the experimental conditions for 3 weeks in fibreglass tanks. During this period, all fish were fed firstly with commercial

TABLE 2 Chemical composition of the BSF larval meals produced on different substrates (mean ± SE, n = 3).

	T0 BSF	T1 BSF	T2 BSF	*F*, *p* values
Chemical composition of BSF larval meal (g/kg DM)				
Dry matter (DM)	824.60 ± 0.6^{b}	845.60 ± 2^{a}	804.30 ± 1.2^{c}	$F_{2;6}$ = 215.9; p = 257 × 10^{-6}
Ash	53 ± 0.1^{b}	108.88 ± 0.3^{a}	59.80 0.03^{ab}	H_2 = 7.2; p = .03
Crude protein	471 ± 0.43^{ab}	433.9 ± 0.40^{b}	478.9 ± 0.43^{a}	H_2 = 7.2; p = .03
Crude fat	277 ± 0.5^{a}	226.5 ± 0.7^{b}	267.5 ± 1.18^{ab}	H_2 = 7.2; p = .03
Fatty acid composition (% of total identified fatty acids) in the BSF larval meals				
Decanoic 10:0	0.97 ± 0.007^{a}	0.83 ± 0.01^{b}	0.69 ± 0.006^{c}	$F_{2;6}$ = 136; p = 10^{-5}
Lauric 12:0	50.16 ± 0.15^{a}	43.93 ± 0.11^{ab}	43.35 ± 0.14^{b}	H_2 = 6.49; p = .04
Myristic 14:0	8.82 ± 0.03^{b}	8.49 ± 0.02^{c}	9.13 ± 0.01^{a}	$F_{2;6}$ = 119.6; p = 10^{-5}
Palmitic 16:0	15.39 ± 0.09^{a}	13.71 ± 0.05^{c}	15.00 ± 0.06^{b}	$F_{2;6}$ = 82.15; p = 4 × 10^{-4}
Stearic 18:0	1.80 ± 0.003^{b}	2.77 ± 0.01^{ab}	3.09 ± 0.01^{a}	H_2 = 7.2; p = .03
C22:0	0.41 ± 0.005^{a}	0.00^{b}	00^{b}	
Total satured	77.57 ± 0.04^{a}	69.73 ± 0.18^{b}	71.26 ± 0.06^{ab}	H_2 = 7.2; p = .03
Palmitoleic 16:1	3.85 ± 0.00^{a}	2.28 ± 0.005^{b}	2.79 ± 0.005^{ab}	H_2 = 7.58; p = .02
Heptadecenoic 17:1	0.53 ± 0.002^{ab}	0.15 ± 0.002^{b}	0.59 ± 0.003^{a}	H_2 = 7.32; p = .02
Oleic 18:1	10.62 ± 0.01^{c}	12.97 ± 0.05^{b}	14.81 ± 0.006^{a}	$F_{2;6}$ = 1908; p = 3.87 × 10^{-9}
Total monoenes	15.01 ± 0.01^{b}	15.41 ± 0.06^{ab}	18.20 ± 0.004^{a}	H_2 = 7.32; p = .03
C18:2n−6 (LA)	7.13 ± 0.02^{b}	13.58 ± 0.12^{a}	8.29 ± 0.01^{ab}	H_2 = 7.2; p = .03
C20:4n−6 (ARA)	0.02 ± 0.005^{b}	0.05 ± 0.004^{a}	0.03 ± 0.002^{ab}	$F_{2;6}$ = 9.37; p = .01
Total n−6 PUFA	7.14 ± 0.02^{b}	13.62 ± 0.11^{a}	8.32 ± 0.01^{ab}	H_2 = 7.2; p = .03
C18:3n−3 (ALA)	0.12 ± 0.004^{b}	1.19 ± 0.04^{a}	0.23 ± 0.006^{ab}	H_2 = 7.2; p = .03
C20:3n−3	0.06 ± 0.002^{ab}	0.04 ± 0.004^{b}	0.23 ± 0.004^{a}	H_2 = 7.2; p = .02
C20:5n−3 (EPA)	0.00^{b}	0.00^{b}	1.38 ± 0.03^{a}	H_2 = 7.51; p = .02
C22:6n−3 (DHA)	0.00^{b}	0.00^{b}	0.37 ± 0.03^{a}	$F_{2;6}$ = 54.01; p = 10^{-4}
Total n−3 PUFA	0.7 ± 0.02^{c}	1.23 ± 0.04^{b}	2.21 ± 0.05^{a}	$F_{2;6}$ = 198.1; p = 3.32 × 10^{-6}
Ratio n−3/n−6	0.09 ± 0.002^{ab}	0.08 ± 0.002^{b}	0.26 ± 0.006^{a}	H_2 = 6.48; p = .04

Values in the same row with different superscript letters are significantly different (p < .05).

T0 BSF: BSF meal produced from vegetable by-products. T1 BSF: BSF meal produced from roasted soybean seeds and corn meal. T2 BSF: BSF meal produced from fish waste and soybean cake.

tilapia diet (BIOMAR, INICIO Plus, 2 mm) for 2 weeks and then with a mixture of the four experimental diets in the last week, to habituate them to the experimental formulated feeds. Fish biomass was controlled biweekly in each tank, and the daily food rations were adjusted according to the ration table of the food supplier. Feeding rate was 5% of biomass per day for the first 2 weeks, 4.5% until the 4th week, 4% until the 6th week and 3.3% until the end of the 59th day for the trial. Fish were hand fed to apparent satiation three times per day at 09.00, 13.00 and 17.00 h. A set of 480 juveniles (initial body weight = 7–12 g) was randomly distributed into 16 tanks at the density of 30 fish per tank, representing four replicate tanks per dietary treatment.

2.2.3 | Growth performance and feed utilization

In order to evaluate the growth performance and feed utilization, all fish were individually weighed at the beginning and at the end of the experiment whereas mortality was recorded daily during the experiment. Growth performance and feed utilization were determined using the following formulas:

$$\text{Relative weight gain (RWG, \%)} = 100 \times (\text{Wf} - \text{Wi})/\text{Wi}$$

$$\text{Specific growth rate (SGR, \% per day)} = 100 \times [\ln(\text{Wf}) - \ln(\text{Wi})]/\Delta t$$

$$\text{Feed intake (FI)} = \text{FC}/(N \times \Delta t)$$

$$\text{Feed efficiency (FE)} = (\text{FB} - \text{IB})/\text{TFI}$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)/protein intake (g)}$$

$$\text{Protein productive value (PPV)} = \text{body protein gain/protein intake}$$

$$\text{Retention of FA (\%)} = 100 \times ((W_f \times \text{FA}_f) - (W_i \times \text{FA}_i))/((W_f - W_i) \times (\text{FCR} \times \text{Fad})),$$

$$\text{Survival (\%)} = 100 \times (\text{number of final survivors per tank/initial number per tank})$$

TABLE 3 Ingredient composition (g/kg of dry matter) and nutrient contents of the experimental diets

Diets	FMFO	BSF/T0	BSF/T1	BSF/T2	
Black soldier fly meal	0	207.9	225.6	204.3	
Fish meal[a]	110	0	0	0	
Corn bran[b]	219.6	116.7	110	120.3	
Wheat bran[c]	150	180	160	180	
Soybean cake[d]	335	350	359	350	
Betaine[e]	20	20	20	20	
Menhaden fish oil[f]	30	0	0	0	
Palm oil[g]	30	20	20	20	
Blood meal[h]	30	30	30	30	
Carboxymethyl cellulose[i]	20	20	20	20	
Mineral Premix[j]	25	25	25	25	
Vitamin premix[k]	25	25	25	25	
BHT[l]	0.2	0.2	0.2	0.2	
BHA[m]	0.2	0.2	0.2	0.2	
L-Methionine[n]	5	5	5	5	
Dry matter (g/kg)	926	923	921	927	
Ash (g/kg dry matter)	83	67	75	70	
Protein (g/kg dry matter)	314	316	314	310	
Lipid (g/kg dry matter)	93	103	97	101	
					[1;2]Requirements
Amino acids (units)[0]					
Lysine	20	19	19.1	19	10.4 – 17.4
Methionine	10.7	10	10	10	8.04
Thréonine	12.3	11.7	11.8	11.8	2 – 16
Tryptophane	3.6	2.7	2.7	2.7	0.5 – 4.5
Arginine	20.1	19.9	20	19.9	6 – 18
Histidine	8.8	9.3	9.3	9.3	2 – 8
Isoleucine	12.4	13.3	13.4	13.3	4 – 11
Leucine	24.2	24.4	24.4	24.4	6 – 12
Valine	15.8	18.7	18.7	18.7	0.4 – 14
Phenylalanine	14.3	10.6	10.6	10.6	6 – 18

FMFO = control diet containing fish meal and fish oil as protein and lipid sources. BSF/T0, BSF/T1 and BSF/T2 = diets containing the BSF larval meals produced from the substrates T0 BSF, T1 BSF and T2 BSF, respectively.

[a,e,f,i,l,m,n]Sigma-Aldrich, St Louis, MO, USA.

[b,c,d,g,h]Veto Service group, Cotonou, Bénin.

[0,1,2]Indispensable amino acid values were theoretically calculated and compared to tilapia requirements (NRC, 2011; Ng & Romano, 2013). BHA, butylated hydroxyanisole; BHT, butylated hydroxyl toluene.

[j]Mineral premix (g/kg of mix) was prepared in the lab. from ($CaHPO_4$) $2H_2O$. 727.77; ($MgSO_4$) $7H_2O$. 127.50; NaCl. 60.00; KCl. 50.00; ($FeSO_4$) $7H_2O$. 25.00; ($ZnSO_4$) $7H_2O$. 5.50; ($MnSO_4$) $4H_2O$. 2.54; ($CuSO_4$) $5H_2O$. 0.78; ($CoSO_4$)$7H_2O$. 0.48; ($CaIO_3$) $6H_2O$. 0.29; ($CrCl_3$) $6H_2O$. 0.13.

[k]The vitamin premix was formulated following (Abboudi *et al*., 2009), (to provide g/kg mixture, except as noted): retinyl acetate (1,500,000 IU/g), 0.67; ascorbic acid, 120; cholecalciferol (4,000,000 IU/g), 0.1; tocopheryl acetate (1000 IU/g), 34.2; menadione, 2.2; thiamin, 5.6; riboflavin, 12; pyridoxine, 4.5; calcium-panthotenate, 14.1; p-aminobenzoic acid, 40; vitamin B12, 0.03; niacin, 30; biotin, 0.1; choline chloride, 350; folic acid, 1.5; inositol, 50; canthaxanthin, 5; astaxanthin, 5; butylated hydroxytoluene, 1.5; butylated hydroxyanisole, 1.5; αcellulose, 325.

where W_i and W_f are the initial and final body mass (g), Δt is the duration of the experiment, FC is the feed consumption per tank (g), N is the number of fish per tank, IB and FB are the initial and final biomass per tank (g), TFI is the total feed intake (g), FA_i, FA_f and Fad are the initial and final FA content in mg/g in fish and diet.

2.2.4 | Sample collection

At the beginning of the experiment, 10 fish were randomly euthanized by immersion in ethyl-aminobenzoic acid (MS222: 240 mg/L) solution for analysis of initial whole-body composition. At the middle (day 29)

TABLE 4 Fatty acid composition (% of total fatty acids identified) analysed in the experimental diets

Fatty acid	FMFO	BSF/T0	BSF/T1	BSF/T2
C4:0	7.57	0.01	4.93	0.06
C10:0	0	0	0	0.91
C12:0	0	33.13	36.10	38.75
C14:0	1.49	5.29	5.52	5.93
C16:0	24.12	18.67	15.38	15.41
C18:0	3.45	2.36	2.13	2.19
C20:0	0.34	0.17	0.08	0.16
C22:0	0.09	0.07	0	0
C24:0	0.37	0	0.08	0
Total satured	37.44	59.70	64.22	63.41
C16:1n−7	0	1.57	1.45	1.54
C18:1n−9	33.99	19.29	15.58	16.61
C20:1n−9	1.54	0.45	0	0.32
C22:1n−9	0.72	0.01	0	0
Total monoenes	36.24	21.31	17.03	18.47
C18:2n−6 (LA)	21.98	18.07	17.14	16.20
C18:3n−6	0	0	0	0.12
C20:2	0.25	0.01	0	0
C22:2	0	0.22	0.29	0
C20:3n−6	0.08	0	0	0
C20:4n−6 (ARA)	0.05	0	0	0.02
Total n−6 PUFA	22.36	18.31	17.44	16.34
C18:3n−3 (ALA)	2.09	0.56	1.18	0.63
C20:3n−3	0.10	0.04	0	0.13
C20:5n−3 (EPA)	0.80	0.05	0	0.75
C22:6n−3 (DHA)	0.87	0	0.03	0.24
Total n−3 PUFA	3.87	0.61	1.22	1.75
n−3 / n−6 ratio	0.17	0.03	0.07	0.11

and end (day 59) of the feeding trial, the total fish number and the body weight were recorded to determine the survival rate (SR) and specific growth rate (SGR), respectively. At the end of the growth trial, fish were starved for 24 h prior to the final sampling. Seven fish were randomly sampled in each tank and anesthetized with MS222 (120 mg/L, Sigma). Among them, three fish were frozen at −20°C for the analysis of whole-body composition. Using heparinized or non-heparinized seringues, blood was collected from the caudal vein for both plasma or serum of four fish in each replicate tank. Plasma samples were obtained by centrifugation at 4000×*g* for 15 min using a high-speed refrigerated microcentrifuge and kept at −80°C until subsequent analysis of innate immune parameters (lysozyme activity, alternative complement activity and total peroxidase activity). Muscle, stomach and intestine were frozen on dry ice after dissection and then stored at −80°C until analysis in order to determine the digestive enzyme activities.

2.2.5 | Proximate analysis

Crude protein content in the feed and whole-body fish was estimated according to the Kjeldahl distillation method after digestion of the samples by sulphuric acid (Kirk, 1950). A protein-to-nitrogen conversion factor of 6.25 was used for the feed and whole-body fish samples (Mariotti et al., 2008). For the BSF larval meals, a protein-to-nitrogen conversion factor of 4.76 was used, as suggested by Janssen et al. (2017), to avoid overestimating the protein content due to the presence of non-protein nitrogen from chitin in insects. Dry matter and ash were identified from three fish per tank pooled and homogenized with a mixer. Dry matter in fish homogenate was calculated from weight loss after drying in an oven at 105°C for 24 h, while ash content was calculated from weight loss after combustion of samples in a muffle furnace at 550°C for 12 h.

2.2.6 | Lipid and fatty acid analyses

The experimental diets were homogenized, and the lipids were extracted with chloroform/methanol (2:1, *v*:*v*) according to the Folch method (Folch et al., 1957), edited by Christie (1982), while lipids of fish homogenate (3 fish per tank) were extracted by chloroform/methanol/water (2:2:1.8, *v*:*v*:*v*) following a method adapted from Classics Bligh and Dyer (1959). Briefly, the extracted lipids were converted into FA methyl esters via methylation and subsequently separated by gas chromatography (GC) and quantified following Mellery et al., (2017). The GC trace (Thermo Scientific) was equipped with a capillary column of 100 m × 0.25 mm, 0.2 μm film thickness (RT 2560, Restek). The gas vector (hydrogen) was injected at a pressure of 200 kPa. The flame ionisation detector (FID, Thermo Scientific) was kept at a constant temperature of 255°C. The oven temperature program was as detailed in Mellery et al. (2017). Each peak was identified by comparison of retention times with those for pure methyl ester standards (Larodan) and Nu-Check Prep (Elysian). Data were processed using ChromQuest software 3.0 (Thermo Finnigan). The final results are expressed in percentage of total identified fatty acids.

2.2.7 | Evaluation of immune parameters

Serum lysozyme activity (LA) was determined according to the protocol of Ellis (1990) adapted for Nile tilapia. Thirty microlitres of samples was put into wells of microplate and then added with 100 μL of substrate of freshly prepared *Micrococcus luteus* (Sigma-Aldrich) solution (0.6 mg/mL of Na_2HPO_4 0.05 M, pH 6.2) in triplicate. Absorbance corresponding to *Micrococcus luteus* lysis was measured at 450 nm during 30 min at regular intervals (5 min). One unit (U) of lysozyme was determined as an absorbance decrease of 0.001 per min.

The total peroxidase activity (PA) in the serum was measured according to Hossain and Koshio (2017), with some modifications. Briefly, 5 μL of serum was diluted with 70 μL of Hank's balanced salt solution (HBSS) without Ca^{+2} or Mg^{+2} in flat-bottomed 96-well plates. Then, 25 μL of peroxidase substrate (3,3′,5,5′ tetramethyl benzidine hydrochloride; TMB; ThermoScientific Inc.) was added. The serum mixture was incubated for 2 min. The colour-developing reaction in the serum samples was stopped by adding 25 μL of 4 M sulphuric acid, and the OD (450 nm) was measured in a plate reader. PBS was used as a blank instead of serum.

Plasma haemolytic alternative complement activity (ACH50) was assayed following Sunyer and Tort (1995), later modified by Milla et al. (2010). Briefly, 10 μL of rabbit red blood cells suspension (RRBC, Biomerieux) suspended at 3% in veronal buffer was mixed with serial dilutions of plasma (50 μL of total volume). Haemolysis 100% was obtained by adding 60 μL of distillate water to 10 μL of RRBC. Negative control (fresh water) was obtained by adding 60 μL of veronal buffer to 10 μL of RRBC. Samples were incubated 100 min at 27°C and centrifuged (3000×*g*, 5 min, 4°C). Then, 35 μL of supernatant was transferred to a new microplate in order to measure the absorbance at 405 nm. The ACH50 value was defined as the reciprocal of the plasma dilution which induced the haemolysis of 50% RRBC.

2.2.8 | Digestive enzyme activities

Stomach and intestine samples from four fish of each tank were homogenized in 10 volumes (v/w) cold distilled water. Intestine alkaline phosphatase (AP) and aminopeptidase (N) activities were assayed according to Bessey et al. (1946) and Maroux et al. (1973) using p-nitrophenyl phosphate (Sigma-Aldrich) and L-leucine p-nitroanalide (Sigma-Aldrich) as substrates, respectively. Intestine trypsin activity was assayed according to Holm et al. (1988) & Métais (1968), such as described by Gisbert et al. (2009). Stomach amylase activity of the crude extract was determined by the starch hydrolysis method, described by Bernfeld (1951). Stomach pepsin activity was assayed by the method of Worthington and Manual (1982) modified by Cuvier-Péres and Kestemont (2001). Protein was determined using the Bradford (1976) procedure. Enzyme activities are expressed as specific activities (U or mU mg protein^{-1}).

2.2.9 | Digestibility assessment

A digestibility trial was conducted at the same time of the growth trial. 320 fish (20.90 ± 4 g) were distributed randomly into 8 tanks at the density of 40 fish per tank, whereas two replicate tanks per dietary treatment were used. Fish were fed a diet containing chromium oxide (Sigma, Adrich) as the inert marker at a level of 1% (Cr_2O_3, 10 g/kg) to the previous formulation and fed to the fish under the same conditions as the growth experiment. One week after acclimation to the new diets, faeces were collected during three weeks and twice daily in the morning and afternoon using a siphon. Faeces of *O. niloticus* very rapidly settled to the bottom of the tank and did not easily break up in the water, so that nutrient and marker losses were minimized. Sufficient amount of faeces were collected, freeze dried and immediately kept at −20°C until analysis. Concentration of chromium oxide in diets and faeces was determined according to Furukawa and Tsukahara (1966)

$$ADC_{DM}\text{: Apparent dry matter digestibility} = 100 - 100\,(\%\ \text{marker in diet}/\%\ \text{marker in faeces})$$

$$ADC_{protein}\text{: Apparent digestibility of protein (\%)} = 100 - 100\,((\%\ \text{marker in diet}/\%\ \text{marker in faeces}) \times (\%\ \text{protein in faeces}/\%\ \text{protein in diet}))$$

$$ADC_{lipid}\text{: Apparent digestibility of lipid (\%)} = 100 - 100\,((\%\ \text{marker in diet}/\%\ \text{marker in faeces}) \times (\%\ \text{lipid in faeces}/\%\ \text{lipid in diet})).$$

2.3 | Statistical analysis

All data were presented as mean values ± standard error of mean (SEM, n = 4). Normality and homogeneity of variances of data were assessed with Shapiro–Wilk and Bartlett tests, respectively. When data were heterogeneous or did not have a normal distribution, a log-transformation of the data was applied. Values in percentages were also log-transformed before calculations. Husbandry data, composition of insect larvae and fish, digestibility and digestive enzyme activities were subjected to a unidirectional analysis of variance (ANOVA 1). A bi-directional analysis of variance (ANOVA 2, with diet and sampling time as factors) was performed on the immune parameters, followed by a LSD post hoc test using tank replicate as the statistical unit (n = 4). Probabilities of $p < .05$ were considered significant. Statistical analyses were performed using R 3.03 software.

3 | RESULTS

3.1 | Chemical composition of the BSF larval meals and PUFA enrichment efficiency

BSF larvae fed the chicken feed substrate (T1 = 0.21 ± 0.007 g) displayed higher final weight after 14 days of culture ($F_{2,6}$ = 60.21, $p < .001$) than those produced on the agricultural by-products (T0 = 0.14 ± 0.006 g) or fish waste (T2 = 0.14 ± 0.006 g). Moreover,

these T1 larvae took 10 days to reach the prepupal stage, while those reared on T0 and T2 substrates took 12 and 14 days, respectively.

Data of chemical composition of the BSF larval meals were significantly different ($p < .05$) depending on the type of substrate (Table 2). The highest values ($F_{2;6} = 215.9$; $p < .05$) of dry matter and ash were obtained in the T1 BSF larval meal, and the lowest ones in the T2 and T0 ones, respectively. The highest level of crude proteins was obtained with the T2 substrate and the lowest values with the T1 one ($H_2 = 7.2$; $p < .05$). The highest total lipid level was obtained in the T0 BSF larval meal and the lowest values in the T1 BSF larval meal ($H_2 = 7.2$; $p < .05$).

Concerning the PUFA enrichment efficiency, saturated fatty acid levels were comparable among the three BSF larval meals regardless of the rearing substrate (Table 2). LA and ALA were also found in all the three larval meals, but the highest values for these two PUFAs ($H_2 = 7.2$, $p < .05$) were observed in the T1 BSF larval meal. EPA and DHA were only found in the T2 BSF larval meal as expected. Especially, the EPA content in the T2 BSF larval meal was comparable to that estimated in the T2 substrate (Table 1), indicating a good accumulation during the larval culture.

Thus, this improvement of the BSF larval meals in essential fatty acids allowed us formulating three BSF meal-based diets contrasted in PUFAs or LC-PUFAs (Tables 3A and 4). (1) BSF/T0: a diet containing BSF larval meal with a low LA and ALA level, and without LC-PUFAs. (2) BSF/T1: a diet containing BSF larval meal rich in LA and ALA but without LC-PUFAs. (3) BSF/T2: a diet with low LA and ALA level but with LC-PUFAs, especially a high level of EPA comparable to that in the FMFO control diet.

3.2 | Growth performance and feed utilization

Data on growth, feed utilization and survival are presented in Table 5. Growth parameters (final body weight, RWG and SGR) did not differ statistically between fish fed the FMFO diet and those fed the BSF larval meals, except for a significant decrease in fish received the BSF/T1 diet ($p < .05$). The same trend was observed for the feed efficiency (FE) data, which were not significantly different between fish fed the BSF/T0, BSF/T2 and FMFO control diets, but decreased ($H_3 = 9.17$; $p < .05$) in the BSF/T1 group, representing a 17% decrease compared to the FMFO control group. Values for feed intake (FI) and protein efficiency (PER) were significantly higher ($F_{3;12} = 21.56$; $p < .05$ and $F_{3;12} = 115.3$; $p < .05$) in FMFO control fish than in fish fed the BSF larval meals. No differences were observed between the fish groups fed the three BSF larval meal-based diets in the growth characteristics, feed utilization or protein efficiency. Survival rate was not significantly affected by the experimental diets.

3.3 | Nutrient digestibility

The ADC values of dry matter (ADC_{DM}), crude protein ($ADC_{protein}$) and lipid (ADC_{lipid}) are presented in Table 6. ADC_{DM} was significantly higher ($F_{3,4} = 70.33$; $p < .05$) in FMFO and BSF/T1 fish than in the BSF/T0 and BSF/T2 groups, with the highest values in FMFO controls. No significant difference of ADC_{DM} was observed between BSF/T0 and BSF/T2 fish. Values for $ADC_{protein}$ were also significantly higher ($F_{3;4} = 354.36$; $p < .05$) in FMFO controls than all the fish groups fed insect meals, with the lowest values in the BSF/T2 groups. BSF/T0 and BSF/T1 fish showed no significant differences

TABLE 5 Growth performance, feed utilization and survival of *Oreochromis niloticus* juveniles fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 4)

Parameters	FMFO	BSF/T0	BSF/T1	BSF/T2	*F*, *p* values
Initial body weight (g)	9.17 ± 0.02	9.18 ± 0.01	9.17 ± 0.01	9.19 ± 0.01	$F_{3;12} = 0.82$; $p = .507$
Final body weight (g)	53.14 ± 0.62^{a}	42.56 ± 0.41^{ab}	41.44 ± 0.44^{b}	42.69 ± 0.23^{ab}	$H_3 = 10.74$; $p = .01$
Relative weight gain (%)	479.35 ± 7.43^{a}	363.68 ± 4.38^{ab}	351.90 ± 4.93^{b}	364.45 ± 2.98^{ab}	$H_3 = 10.15$; $p = .02$
Specific growth rate (% d^{-1})	2.98 ± 0.02^{a}	2.60 ± 0.02^{ab}	2.56 ± 0.02^{b}	2.60 ± 0.01^{ab}	$H_3 = 10.14$; $p = .02$
Feed intake (FI, g/fish/day)	0.79 ± 0.01^{a}	0.70 ± 0.01^{b}	0.72 ± 0.006^{b}	0.71 ± 0.009^{b}	$F_{3;12} = 21.56$; $p = .00004$
Feed efficiency	0.94 ± 0.01^{a}	0.79 ± 0.01^{ab}	0.78 ± 0.004^{b}	0.79 ± 0.01^{ab}	$H_3 = 9.17$; $p = .03$
Protein efficiency ratio	3.00 ± 0.03^{a}	2.51 ± 0.03^{b}	2.49 ± 0.01^{b}	2.54 ± 0.02^{b}	$F_{3;12} = 115$; $p = 4.10^{-6}$
Protein productive value	43.54 ± 1.4^{a}	36.92 ± 0.90^{b}	37.26 ± 1.03^{b}	34.34 ± 0.79^{b}	$F_{3;12} = 13.85$; $p = .000334$
Survival (%)	100 ± 0.00	99.16 ± 0.83	99.17 ± 0.83	98.33 ± 0.96	$F_{3;12} = 0.8$; $p = .80$

Values in the same row with different superscript letters are significantly different ($p < .05$).

TABLE 6 Apparent digestibility coefficients (ADC) of nutrients in Nile tilapia juveniles fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 2)

Parameters	FMFO	BSF/T0	BSF/T1	BSF/T2	*F*, *p* values
ADC_{DMa} (%)	66.32 ± 1.40[a]	57.32 ± 0.88[b]	64.32 ± 0.23[a]	57.09 ± 0.33[b]	$F_{3;4}$ = 31.33; p = .003
$ADC_{protein}$ (%)	88.33 ± 0.20[a]	81.24 ± 0.63[b]	81.22 ± 0.75[b]	77.90 ± 0.16[c]	$F_{3;4}$ = 75.83; p = .0005
ADC_{lipid} (%)	90.39 ± 0.12	90.80 ± 1.79	89.61 ± 0.28	90.06 ± 0.25	$F_{3;4}$ = 0.72; p = .60

Values in the same row with different superscript letters are significantly different (p < .05).

of $ADC_{protein}$. Values for ADC_{lipids} were high and were not affected by the type of diet.

3.4 | Digestive enzyme activities

Data on digestive enzyme activities are presented in Table 7. The BSF larval meal diets lowered the amylase activity level with significantly lower ($F_{3;4}$ = 5.28; p < .05) values in fish fed the BSF/T0 or BSF/T2 diets compared to fish fed the FMFO control diet. Amylase values were also lower (p < .05) in fish fed the BSF/T1 or BSF/T2 diet than those receiving the BSF/T0 diet. No differences were observed concerning pepsin and aminopeptidase activities. Trypsin activity was elevated in fish fed the BSF larval meals, especially in fish receiving the BSF/T1 diet ($F_{3;4}$ = 3.64; p < .05), but values did not differ between fish fed the three BSF larval meal-based diets. Alkaline phosphatase activity was significantly elevated in ($F_{3;4}$ = 7.95; p < .05) in fish fed the BSF/T1 diet compared to FMFO controls and other BSF fed fish.

3.5 | Whole-body biochemical composition

3.5.1 | Protein and total lipid contents

Data on the chemical composition of fish whole body are presented in Table 8. Dry matter and protein contents did not differ between fish of all the experimental groups. Total lipid content was significantly lower in fish fed the FMFO diet than those receiving the BSF larval meal-based diets ($F_{3;12}$ = 4.35; p < .05), while ash content was reduced by the latter diets ($F_{3;12}$ = 15.52; p < .05), with the lowest value observed in BSF/T2 fish.

3.5.2 | Fatty acid profiles, LA and ALA retention coefficients

Total saturated fatty acid (SFA) content in fish whole body was comparable between fish fed the BSF larval meal-based diets and significantly higher ($F_{3;12}$ = 12.64; p < .05) than FMFO control fish, while the opposite was observed for MUFA (Figure 1a). Regarding the PUFAs and LC-PUFAs concentrations, only fish fed the diet BSF/T0 (from vegetable substrate) resulted in significantly lower (p < .05) proportions of LA, ALA, EPA, DHA, total PUFAs and LC-PUFAs and n3/n6 compared to FMFO fish, while ARA level was not affected (Figure 1a–c). There were no significant differences in LA and total PUFAs concentrations between fish fed the BSF/T0 and BSF/T1 diets or between the BSF/T1 and BSF/T2 diets as for ALA for the latter fish groups.

Retention efficiency of dietary OA, LA and ALA was calculated for some essentials PUFAs using BW gain values, FA contents in feeds and whole body expressed as mg/g and estimated feed intake using FCR per tank. In this trial, net production (retention efficiency values >100%) of AO was found in all diet groups and was significantly higher

TABLE 7 Digestive enzyme activities of Nile tilapia juveniles fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 4)

Parameters	FMFO	BSF/T0	BSF/T1	BSF/T2	*F*, *p* values
Amylase (U mg. protein^{-1})	50.40 ± 2.34[a]	46.45 ± 2.13[a]	30.00 ± 5.57[b]	27.43 ± 7.74[b]	$F_{3;12}$ = 5.28; p = .01
Pepsin (mU mg. protein^{-1})	58.84 ± 5.23	62.38 ± 7.80	59.48 ± 7.88	70.14 ± 8.59	$F_{3;12}$ = 0.48; p = .70
Trypsin (mU mg. protein^{-1})	60.58 ± 18.15 [b]	120.4 ± 23.1[ab]	186.7 ± 39.1[a]	98.29 ± 26.2[ab]	$F_{3;12}$ = 3.64; p = .04
Aminopeptidase (mU mg. protein^{-1})	10.78 ± 0.32	9.30 ± 1.82	6.33 ± 0.76	7.42 ± 1.35	$F_{3;12}$ = 2.69; p = .09
Alkaline phosphatase (mU mg.protein^{-1})	82.32 ± 0.67[b]	71.04 ± 7.71[b]	116.13 ± 10.42[a]	82.15 ± 4.84[b]	$F_{3;12}$ = 7.95; p = .0035

Values in the same row with different superscript letters are significantly different (p < .05).

TABLE 8 Whole-body composition of Nile tilapia juveniles fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 4).

Composition (g/kg^{-1} of dry matter)	FMFO	BSF/T0	BSF/T1	BSF/T2	*F*, *p* values
Dry matter	258.7 ± 5.07	261.85 ± 5.83	272.02 ± 3.66	256.95 ± 7.97	$F_{3;12}$ = 1.33; p = .31
Protein	577.3 ± 5	576.9 ± 10.7	567.6 ± 8.9	564.2 ± 40	$F_{3;12}$ = 0.75; p = .54
Total lipid	212.6 ± 5.7^{b}	264.6 ± 7.4^{a}	275.4 ± 18.5^{a}	256.7 ± 16.5^{a}	$F_{3;12}$ = 4.35; p = .03
Ash	37.91 ± 1.52^{a}	31.05 ± 1.35^{b}	25.98 ± 1.53^{bc}	22.56 ± 2.24^{c}	$F_{3;12}$ = 15.52; p = .00019

Values in the same row with different superscript letters are significantly different (p < .05).

($F_{3;12}$ = 10.89; p < .05) in the BSF/T1 and BSF/T2 groups (Figure 2a). OA retention values were comparable between the BSF/T0 and FMFO control groups. LA retention was comparable between the groups of fish fed the BSF/T1, BSF/T2 and FMFO diets, but significantly lower ($F_{3;12}$ = 9.18; p < .05) in the group of fish fed BSF/T0 (Figure 2b). ALA retention was significantly higher ($F_{3;12}$ = 8.74; p < .05) in the BSF/T1 and BSF/T2 fish groups, and there was no significant difference between the BSF/T0 and FMFO control groups (Figure 2c).

3.6 | Innate immune status

The innate immune status was characterized by the levels of serum lysozyme and peroxydase activities as well as by the plasma haemolytic alternative complement activity (ACH50; Figure 3a–f) evaluated after 29 and 59 days of feeding. Regardless of the feeding period, lysozyme levels (Figure 3a) increased with significantly higher values ($F_{3;12}$ = 4.72; p < .05) in BSF/T0 or BSF/T2 fish than in FMFO controls. Neither significant differences in lysozyme values were observed between BSF/T1 and BSF/T2 nor BSF/T1 and FMFO fish. It should also be noted that lysozyme levels were higher ($F_{3;12}$ = 4.93; p < .05) after 29 days (769.35 ± 115.50) of feeding than after 59 days (547.53 ± 83.35), regardless of the diet (Figure 3b). Whatever the duration of feeding, ACH50 values (Figure 3c) were significantly higher ($F_{3;12}$ = 4.07; p < .05) in fish fed the BSF/T0 diet compared to those fed the BSF/T2 diet and the FMFO control groups. No significant differences in ACH50 values were observed between BSF/T0 and BSF/T1 fish and also between BSF/T1, BSF/T2 and FMFO control groups and at both days 29 and 59 (Figure 3d). Values for peroxidase activity (Figure 3e) did not significantly differ between fish groups and on both days 29 and 59 (Figure 3f).

4 | DISCUSSION

4.1 | PUFA enrichment of BSF larval meal

The final composition of animal lipids generally reflects that of the fatty acid composition of the diet, which influences the quality and healthy properties of the product (Sánchez-Muros et al., 2014). It has been also shown that terrestrial insects like *H. illucens* have high levels of saturated fatty acids (SFA) being especially rich in the medium-chained FA C12:0 lauric acid and are very low in LC-PUFAs, which could limit their use as fish feed (Barroso et al., 2017; Liland et al., 2017). This was confirmed by the results of the current study since the BSF larvae T0 produced using a substrate composed of vegetable by-products were low in PUFAs and contained no LC-PUFAs. But the current study also showed that the BSF larvae are able to accumulate sufficient amounts of PUFAs or LC-PUFAs depending on the fatty acid composition of the rearing substrate. Indeed, the BSF larvae T1 produced using a soybean and corn meal-based substrate, rich in LA and ALA contained higher levels of LA and ALA than those produced using other substrates. Moreover, only the BSF larvae T2 produced using a fish waste-based substrate contained LC-PUFAs, especially with substantial EPA amount. These results may indicate that the accumulation process in the BSF larvae is variable and would depend on the intrinsic quality of the rearing substrate. Indeed, the LA level was comparable between the three tested substrates, but the BSF larvae T1 was more richer in LA than the BSF larvae T0 and T2 (Tables 1 and 2). A better protection of FA structures in this T1 substrate relating to the addition of an antioxidant (BHT) could explain its better rearing quality. Also, a eventual alteration of the structure of some FAs could explain the low DHA level in the BSF larvae T2 despite the high level this FA in the substrate T2.

Although it is well known that insect meals are poor in essential fatty acids, studies on their enrichment in PUFAs or LC-PUFAs are still limited. It has recently been demonstrated that feeding BSF larvae with seaweed (brown algae *Ascophyllum nodosum*) enriched the larvae in EPA (Liland et al., 2017). Feeding BSF larvae with a diet containing 40% fish meal and 60% laying hen feed for 4 days increased the concentration of EPA and DHA in the larvae (Barroso et al., 2017). The enrichment protocol used in the present study showed that it is possible to obtain BSF larval meals differentially enriched in PUFAs or CL-PUFAs (Table 2) from available and less expensive food ingredients (Table 1).

4.2 | Effects of PUFAs enriched larval meals on growth related parameters

Survival was high in all fish groups and was not affected by BSF meals. BSF diets did not significantly affect growth parameters and feed efficiency, except for significant decrease by the BSF/T1 diet,

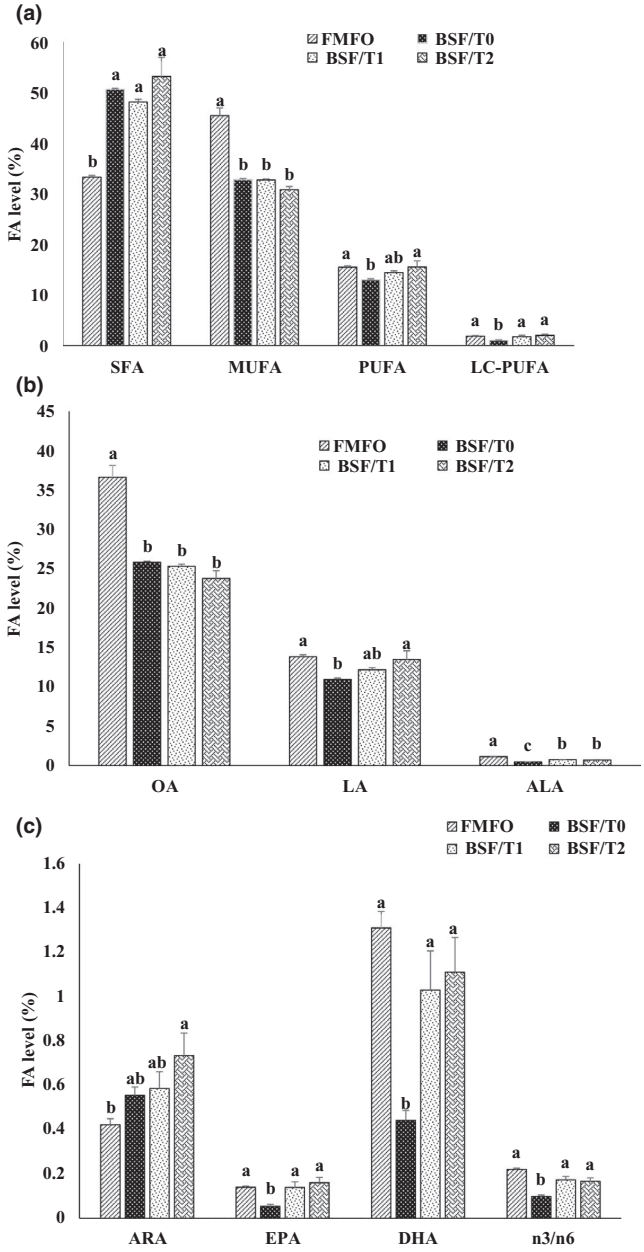

FIGURE 1 Fatty acid composition (% of total identified fatty acids) for (a) all fatty acid groups, (b) selected C18 unsaturated fatty acids and (c) LC-PUFA in *Oreochromis niloticus* whole body of fish fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 4). SFA: saturated fatty acids (H_3 = 12.64; p = .0054), MUFA: monounsaturated fatty acids (H_3 = 12.73; p = .0052), PUFA: C18-polyunsaturated fatty acids ($F_{3;12}$ = 15.89; p = .002), LC-PUFA: long-chain polyunsaturated fatty acids ($F_{3;12}$ = 4.58; p = .02), OA: oleic acid (H_3 = 12.51; p = .0058), LA: linoleic acid ($F_{3;12}$ = 5.32; p = .0145), ALA: α-linolenic acid ($F_{3;12}$ = 63.17; $p = 1.27 \times 10^{-7}$), ARA: arachidonic acid ($F_{3;12}$ = 4.8; p = .02), EPA: eicosapentanoic acid ($F_{3;12}$ = 6.79; p = .0063), DHA: docosahexanoic acid ($F_{3;12}$ = 8.76; p = .00237). Columns with no common letter within the same group denote significant differences (p < .05)

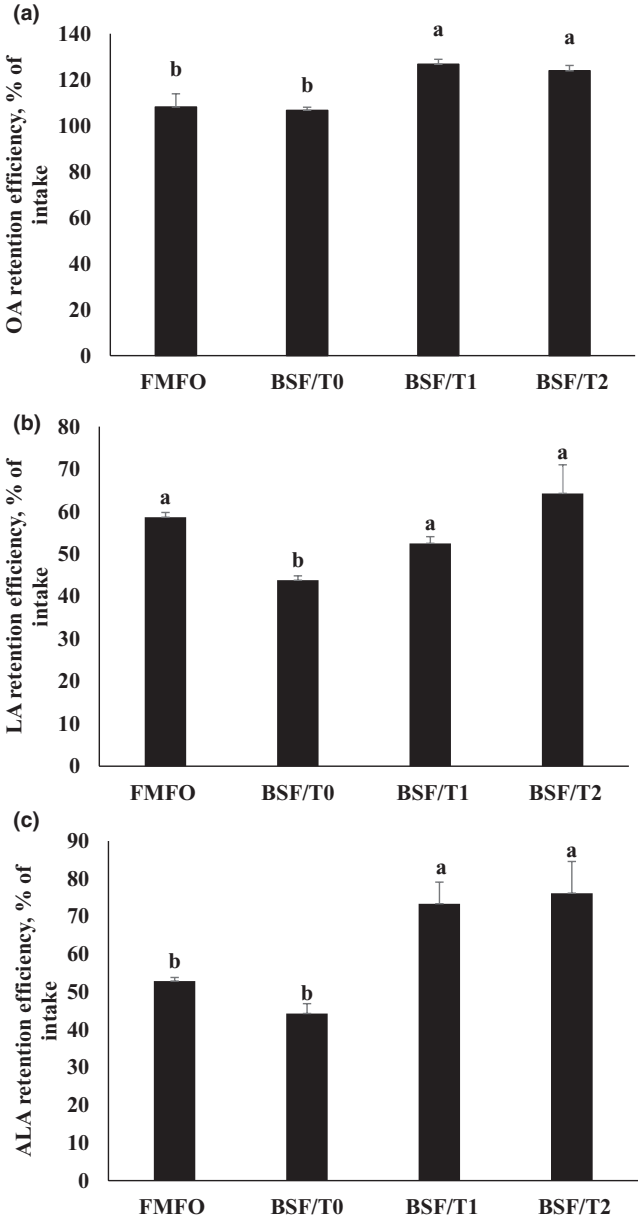

FIGURE 2 Retention efficiency of fatty acid (% of intake) for (a) OA ($F_{3;12}$ = 10.89; $p = 9 \times 10^{-4}$), (b) LA ($F_{3;12}$ = 9.18; p = .002) and (c) ALA ($F_{3;12}$ = 8.74; p = .0024) in *Oreochromis niloticus* fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 4). Columns with no common letter within the same group denote significant differences (p < .05).

indicating no positive effect of FA enrichment. In a previous study, the total replacement of FM with BSF protein meal but associated with fish oil showed no negative growth impact on Atlantic salmon (*Salmo salar*; Belghit et al., 2019). Other research on tilapia juveniles has shown that both meals from housefly larvae (*Musca domestica*) and blowfly larvae (*Chrysomya megacephala*) can replace up to 100% of the FM in practical diets for tilapia fingerlings without affecting fish performance compared to FM- based control diets (Ogunji, Kloas, Wirth, Neumann, et al., 2008; Ogunji, Kloas, Wirth, Schulz, et al., 2008; Ogunji, Schulz, et al., 2008; Sing et al., 2014). In the current study, the negative effect on growth rate by the BSF/T1 diet may be explained by the relatively high incorporation of BSF larval meal (226 g/kg dry matter compared to 204–208 g/kg in other BSF meal diets) which was due to the low protein content of the BSF

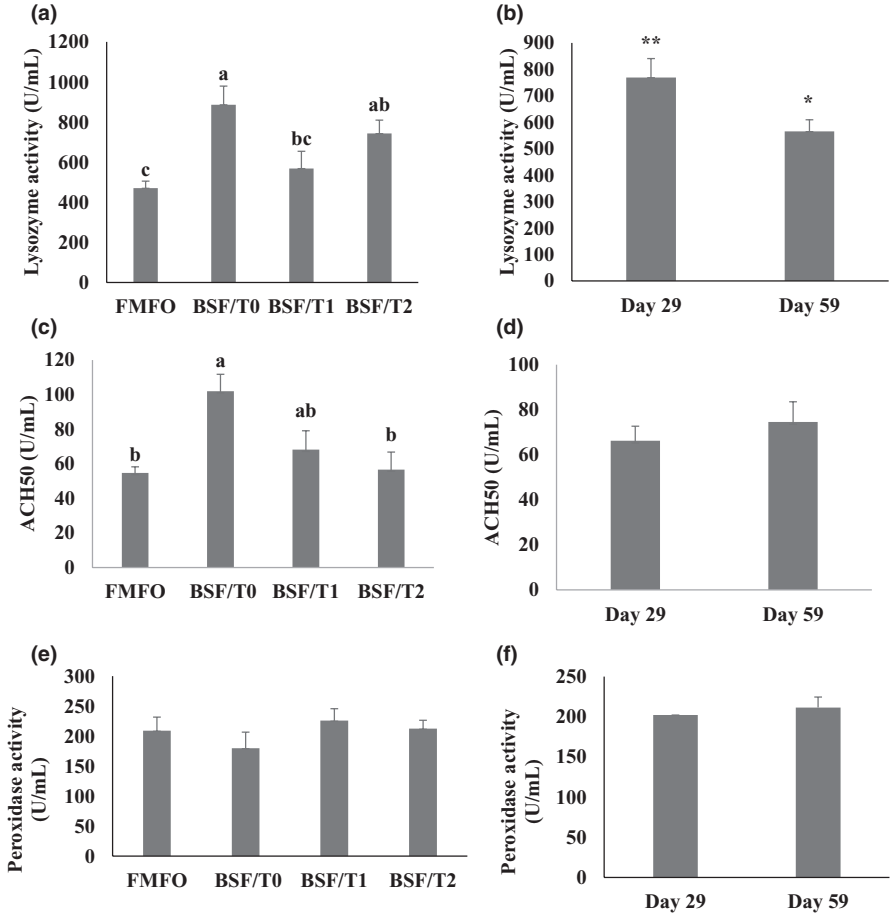

FIGURE 3 Immune parameters (U/ml) for (a and b) serum lysozyme activity level (($F_{3;24}$ = 6.01; p = .002) and ($F_{1;24}$ = 5.49; p = .03)), (c and d) plasma alternative complement pathway activity (ACH50; $F_{3;24}$ = 5.02; p = .008) and ($F_{1;24}$ = 1.11; p = .30) and (e and f) serum peroxidase activity ($F_{3;24}$ = 1.33; p = .29) and ($F_{1;24}$ = 0.11; p = .74) in *Oreochromis niloticus* fed fish meal or BSF larval meal diets enriched in ALA or EPA during 59 days (mean ± SE, n = 4). Columns with no common letter within the same group denote significant differences (p < .05)

larval meal produced with the substrate T1. A considerable reduction in growth was also observed in blue tilapia (*Oreochromis aureus*) when fed with fresh larvae of the whole or chopped BSF (Bondari & Sheppard, 1987). The same trend was observed by Kroeckel et al. (2012) who reported a negative effect on growth of turbot juveniles (*Psetta maxima*) when the amount of BSF meal in the diet increased from 330 to 756 g/kg. High incorporation of insect meal in fish diet can result in a high level of chitin (Barroso et al., 2014) which may interfere with the digestive processes and there by decreases feed efficiency and food intake with subsequent negative effect on growth.

Regarding the lack of positive effect of FA-enriched BSF meal on growth, other studies using other types of ingredients have shown that dietary supplementation of flaxseed oil rich in ALA for 45 days has no effect on growth performance in adult tilapia (Tonial et al., 2009). It has been shown that high dietary n-3 PUFA reduced the growth of tilapia (Huang et al., 1998; Kanazawa et al., 1980) and that a >1% dietary α-linolenic lowered the growth of blue tilapia (Stickney & McGeachin, 1983). There was no growth improvement in tilapia when either dietary α-linolenic or n-3 PUFA were provided (Takeuchi et al., 1983) and the use of conjugated α-linolenic between 0.5–1.0% and 0.5–2.5%, at the expense of soybean oil and fish oil, respectively, had no significant effect on the growth performance or feed utilization of Nile tilapia or their GIFT strain (Luo et al., 2012; Santos et al., 2011). Similar results were observed in juvenile common carp whose growth performance increased with the total replacement of fish oil by sesame oil deficient in ALA and DHA (Nguyen et al., 2020). The lack of positive effects of dietary supplementation in PUFAs or LC-PUFAs on growth could be due to the differences in the doses used or to the interference with the high endogenous hability of the latter species for LC-PUFAS biosynthesis. In salmonids such as rainbow trout, it has been reported that a DHA-enriched diet improved the growth performance of juvenile compared to a diet enriched with ALA (Cornet et al., 2018).

4.3 | Fatty acid profiles in fish whole body and LA and ALA retention coefficients

In the present study, the whole body of fish receiving the BSF/T0 diet were poorer in LA and ALA compared to those of the FMFO controls or fish fed FA-enriched BSF diets, and despite the recommended adequate dietary supply (NRC, 2011). Nonetheless, the whole body of these BSF/T0 fish displayed a comparable ARA level as that of the FMFO controls fish or the BSF/T1 ones indicating a high endogenous hability of production of ARA from the bioconversion of dietary LA contained (Tocher, 2003). Although the BSF/T0 diet did not contain EPA and DHA, the carcasses of fish fed this diets contained these essential fatty acids, confirming an efficient endogenous production capacity of EPA and DHA from ALA reserves. In addition, EPA and DHA levels in the carcasses of

fish fed the BSF/T1 diet (rich in LA and ALA) were not significantly different from those of fish fed the FMFO control diet. These observations confirm that Nile tilapia has a high active capacity for biosynthesis of LC-PUFAs from dietary precursors or endogenous reserves as previously reported by Stoneham et al. (2018) on the same species fed with algae.

Concerning the FA enrichment, the BSF/T2 diet induced higher ARA level in the whole-body fish compared to fish fed the FMFO diet or the BSF/T0 one. Moreover, EPA and DHA levels in the whole-body fish were higher for fish fed the BSF/T1 and BSF/T2 diets than fish fed the BSF/T0 and comparable to that of fish fed the FMFO control diet, indicating an advantage of the FA enrichment of the BSF meal in terms of human health. Our results also demonstrated that the low level of LC-PUFAs in fish fed the non-FA-enriched BSF meal could be explained by a low retention of PUFAs because the retention level of AO, LA and ALA was significantly higher in fish fed the BSF/T1 diet and/or BSF/T2 compared to BSF/T0 fish.

4.4 | Effects of PUFAs enriched larval meal on digestibility and digestive enzyme activities

4.4.1 | Digestibility

In this study, the total substitution of FM by BSF meal induced a decrease in the digestibility of dry matter and proteins whatever the enrichment in PUFAs or LC-PUFAs. Previous studies reported that the exoskeleton of BSF prepupae contains high polysaccharide chitin (approximately 87.0 g/kg, DM) which might affect the digestibility and the utilization of other nutrients (Kroeckel et al., 2012; Shiau & Yu, 1999). As we found, Ogunji et al. (2009) reported lower values of ADCprotein for Nile tilapia fed housefly maggot meal compared to FM fed fish. It has been also reported that incorporation of chitin at inclusion levels of 1% or higher into diets was reported to decrease feed intake and growth in carp, hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) and Atlantic salmon (Gopalakannan & Arul, 2006; Olsen et al., 2006; Shiau & Yu, 1999). So, an eventual high presence of chitin could have affected not only the protein digestibility but also the protein sparing activity of lipids in this study as no effects of ALA or EPA enrichment were observed. The sparing effect of lipid to maximize the use of protein for growth and improved PER has been reported earlier in various fish species (Chatzifotis et al., 2010; Giri et al., 2011; Han et al., 2014; Luo et al., 2005; Nayak et al., 2018; Song et al., 2009). In contrast to some previous reports (Ogunji et al., 2009; Shiau & Yu, 1999), values of ADC_{lipid} in this study did not significantly differ from any fish group. Therefore, the BSF larval meal enriched with either PUFAs or LC-PUFAs did not influence the lipid digestibility. However, lipid digestibility values were, in general, high and comparable or even higher than values obtained for tilapia fed with other protein sources, such as soybean meal, rapeseed meal, sunflower meal, corn gluten meal, dehulled flax, poultry by-product meal and distillers grains (Borgeson et al., 2006; Tran-Ngoc et al., 2019).

4.4.2 | Digestive enzyme activities

In the present study, the BSF meal-based diets induced a trend of increase in trypsin activity level whatever the FA enrichment and an increase in alkaline phosphatase was observed for fish fed the BSF/T1. The trend of increase in these proteolytic enzymes activities in fish fed the BSF meal diets may be related to the low proteins digestibility. It has been reported that the digestibility of proteins/AAs of fish fed insect meal depends on how much of the AAs are bound to chitin or scleroprotein that is mainly present in insect cuticles (Becker & Yu, 2013; Finke, 2007). I was also reported that trypsin can be increased in fish species to compensate for possible nutritional deficiencies (Cara et al., 2007; Kumar et al., 2007), and an increase in trypsin activity has been reported in Nile tilapia when the dietary protein level was reduced (Santos et al., 2016). Papoutsoglou and Lyndon (2006) found also an increase in chymotrypsin activity in Spotted wolfish, *Anarhichas minor*, when the dietary protein concentration was reduced, indicating an adaptation of this species to low protein concentrations as a way to better absorb nutrients from the diet. In the present study, the ALA-enriched BSF diet induced an increased alkaline phosphatase activity but this effect may not be a specific result of the FA enrichment as it was not observed for the EPA-enriched BSF diet (BSF/T2). In rainbow trout, Ducasse-Cabanot et al. (2007) found that alkaline phosphatase activity decreased with the removal of FO from the diet. The latter authors pointed that the lower digestive capacity in the enterocyte membrane of fish fed low-lipid diets was related to the lower levels of dietary lipids rather than to a modification in the enterocyte brush border membrane FA composition, which remained unchanged.

Regarding amylase activity, a significant decrease was observed in fish fed both the FA-enriched BSF diets not in the non-enriched one, so such effect may not be a direct consequence of the FA enrichment, but perhaps related to an endogenous enzymatic balance because the proportions of the ingredients containing carbohydrates were comparable between the three BSF diets (Table 3). Endogenous production of amylase by the intestinal microflora (Krogdahl et al., 2005; Sugita et al., 1997) might also have interfered with the effects of the treatments.

4.5 | Effects of PUFAs enriched larval meal on immune status

In this study, total FM substitution by non-enriched BSF larval meal or EPA-enriched one induced an increase in lysozyme activity. BSF meal may be a source of antimicrobial peptides that are biologically active fractions against pathogenic bacteria (Jozefiak & Engberg, 2017; Park et al., 2014). In addition to their antimicrobial effects, these peptides may boost the specific innate immune responses and exert selective immunomodulatory effects (Nogales-Mérida et al., 2019). Moreover, some authors have suggested that chitin may be targeted by a positive intestinal microbial community, which improves host organism performance and health status (Karlsen et al., 2017; Ringo et al., 2006).

Additional benefits of incorporating chitin into fish diets have been reported, including an increase in the activity of the innate immune system in European sea bream (*Sparus aurata*; Esteban et al., 2001) or the stimulation of macrophage activity in rainbow trout (Sakai, 1992).

The results of the present study indicate that the total substitution of FM by BSF meals also increased serum ACH50 activity. The increase in ACH50 activity may strongly improve the immune status of fish (Fletcher et al., 2011) and can be considered as an important indicator of a good immune defence in fish (Ellis, 2001). The fact that there were no significant differences in the levels of lysozyme activity between fish fed the BSF/T0 and BSF/T2 diets and the ACH50 activity between fish fed the BSF/T0 and BSF/T1 diets could indicate that the enrichment of the BSF larval meal with PUFAs or LC-PUFAs did not provide any advantage on the immune status of Nile tilapia in the conditions of the current study.

5 | CONCLUSIONS

In conclusion, the total substitution of FM by BSF meal has no substantial effects on growth rate of Nile tilapia but decreases the essential fatty acid contents in the whole-body fish, especially for ALA, EPA and DHA, and this could be prevented by PUFAs and LC-PUFAs enrichment. In addition, BSF meal appears to have a stimulating action on some innate immune functions, namely lysozyme and ACH50 activities. However, the use of BSF meal as complete substitute of fish meal needs more investigation because it can reduce the digestibility of some nutrients or affect the activity of some digestive enzymes such as amylase, whatever the PUFAs enrichment.

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