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Growth performance and immune status in common carp *Cyprinus carpio* as affected by plant oil-based diets complemented with β-glucan

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ABSTRACT

Omnivorous fish species such as the common carp (*Cyprinus carpio*) are able to biosynthesise long chain polyunsaturated fatty acids (LC-PUFAs) from plant oil PUFA precursors, but the influence of the amount and quality of the LC-PUFAs biosynthesised from these oils on the immunocompetence status of the fish has received little attention. This study aims to evaluate whether the conversion of PUFA by carp induces a sufficient biosynthesis of LC-PUFA to maintain a good immunocompetence status in this species. Six iso-nitrogenous (crude protein = 39.1%) and iso-lipidic (crude lipids = 10%) diets containing three different lipid sources (cod liver oil (CLO) as fish oil; linseed oil (LO) and sunflower oil (SFO) as plant oils) were formulated with or without β-glucan supplementation at 0.25 g/kg diet. *Juvenile* carp (16.3 ± 0.6 g initial body weight) were fed a daily ration of 4% body weight for 9 weeks and then infected at day 64 with the bacteria *Aeromonas hydrophyla*. No significant differences in survival rate, final body weight, specific growth rate and feed conversion rate were observed between diets. After bacterial infection, mortality rate did not differ between fish fed CLO and plant oil-based diets, indicating that the latter oils did not affect the overall immunocompetence status of common carp. Plant oil-based diets did not alter lysozyme activity in healthy and infected fish. No negative effects of plant oils on complement activity (ACH50) were observed in healthy fish, even if both plant oil-based diets induced a decrease in stimulated fish two days after infection. Furthermore, the levels of various immune genes (*nk*, *lys*, *il-8*, *pla*, *pge*, *alox*) were not affected by plant oil-based diets. The expression of *pla* and *pge* genes were higher in SFO-fed fish than in CLO ones, indicating that this plant oil rich in linoleic acid (LA) better stimulated the eicosanoid metabolism process than fish oil. In response to β-glucan supplementation, some innate immune functions seemed differentially affected by plant oil-based diets. LO and SFO induced substantial LC-PUFA production, even if fish fed CLO displayed the highest EPA and DHA levels in tissues. SFO rich in LA induced the highest ARA levels in fish muscle while LO rich in α-linolenic acid (ALA) sustained higher EPA production than SFO. A significantly higher fads−6a expression level was observed in SFO fish than in LO ones, but this was not observed for elovl5 expression. In conclusion, the results show that common carp fed plant oil-based diets are able to produce substantial amounts of LC-PUFA for sustaining growth rate, immune status and disease resistance similar to fish fed a fish oil-based diet. The differences in the production capacity of LC-PUFAs by the two plant oil-based diets were associated to a differential activation of some immune pathways, explaining how the use of these oils did not affect the overall immunocompetence of fish challenged with bacterial infection. Moreover, plant oil-based diets did not induce substantial negative effects on the immunomodulatory action of β-glucans, confirming that these oils are suitable for sustaining a good immunocompetence status in common carp.

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1. Introduction

The limited availability of fish meal and fish oil is considered to be one of the major constraints in the future development of aquaculture [1,2]. In this context, strategies for marine fish product replacement with plant products are highly recommended. The plant products or by-products are potential material sources for fish feed production thanks to their low price and high abundance [3]. Most plant-derived oils contain polyunsaturated fatty acids (PUFA) but no long chain PUFAs (LC-PUFA, > 18C) [4]. Some plant-derived oils, such as linseed oil, sunflower oil or sesame oil, provide the PUFA precursors (LA and ALA) for the important n-3 (eicosapentanoic acid (EPA); docosahexaenoic acid (DHA)) and n-6 LC-PUFA (arachidonic acid (ARA)) biosynthesis [5–8]. Most studies demonstrated that partial or total replacement of fish oil by terrestrial plant-derived oils did not influence the growth performance of freshwater fish with omnivorous or herbivorous feeding habits [9–12]. Nonetheless, for some other species, marine and/or carnivorous, although the partial substitution of fish oil by plant oil did not induce a negative effect on fish growth [13–17], the total fish oil replacement in the diet was associated with a significant reduction of growth such as in Eurasian perch [18], rainbow trout [19–22], turbot [23], sea bream [15,24] and European sea bass [17,25]. Moreover, when all fish-based ingredients (including fish meal and fish oil) were replaced by plant ones, poor growth performance was reported in most freshwater species, such as rainbow trout [19] and common carp [26].

The LC-PUFAs, such as ARA, EPA and DHA, play an important role in fish health and in human health [27–29]. The sufficient supplementation of these LC-PUFAs in the diet enhances the immune response of fish [30], while a deficiency of these LC-PUFAs in plant-derived oil diets might induce fish health problems such as digestive tract deformity [31], problems of gut morphology [17], low bacterial resistance [15,32] or a reduction of some immune parameters [33,34].

The effects of LC-PUFA insufficiency on the immune response might be linked to the deficiency in EPA and DHA, or especially ARA for eicosanoid metabolism [35,36]. Eicosanoids are signalling compounds produced by cells that play a wide range of physiological functions, including in inflammatory responses [37,38]. Eicosanoids including prostaglandins and leukotrienes are produced from ARA, EPA and dihomoglinolenic acid (20:3n-6) when these FAs are released from tissue phospholipids (PL) by phospholipase A2 (PLA2) [39]. ARA is the major precursor of highly active eicosanoids while EPA produces much less active eicosanoids [38,40]. A study on humans showed that moderate levels of dietary essential FAs can decrease some markers of endothelial activation, and that this mechanism of action may contribute to the reported health benefits of n-3 FAs [41]. However, when the proportions of LC-PUFA-based eicosanoid actions are higher with n-6 than n-3 mediators, they cause healthy physiology to shift toward pathophysiology [42]. Studies on mammals demonstrated that low ARA-derived prostaglandins E2 (PGE2) are associated with the stimulation of immune function, whereas high concentrations are immunosuppressive [40]. In fish, previous studies have focused principally on the effects of dietary FAs on the modification of the FA profile of tissues [20,43,44] or fish health [17,30,31,34]. It was also demonstrated that an increase in eicosanoid levels, such as thromboxane B2 and prostaglandin E2, was observed in salmon fed a diet rich in LA (known to be the precursor of ARA) [45], and an ARA-enriched diet induced changes in complex lipids and immune-related eicosanoids in zebrafish *Danio rerio* [46]. However, there are few studies focusing on the extent to which omnivorous fish species can get sufficient use of precursors of PUFA from some plant oils to sustain a sufficient growth rate and immune status.

The innate immune system of fish, including cellular and humoral systems, can be stimulated by compounds such as β-glucan [47,48], lipopolysaccharides (LPS) [49,50], bovine lactoferrin [51–53], inulin [54] and chitosan [55]. These substances could enhance immune parameters such as lysozyme, complement, macrophage and peroxidase activities, or upregulate the expression of genes involved in the fish immune system. Among these immunostimulant, β-glucan, a polysaccharide derived from fungi or bacteria, is known to be an immunomodulatory factor [56] enhancing several inflammatory responses [57,58] or playing an anti-inflammatory role in some cases [59–61]. The immunomodulatory actions of immunostimulant compounds may be influenced by the fluidity of cellular membranes, which is itself influenced by the FA composition in the phospholipid layer [62–64]. However, information on the influence of the amount and profile of dietary FAs on the immunomodulatory effects of immunostimulants is rather limited in fish.

The common carp is an important aquaculture species; it is the most cultured fish for human food consumption. In research, this species is an important fish for a wide range of studies focusing on physiology, such as nutrition and farming conditions [65], fish diseases and immunology and fish flesh quality [66–68]. Common carp is a freshwater fish that is able to biosynthesise the LC-PUFAs from PUFA precursors by a series of elongation and desaturation reactions [29]. Previous studies have shown that the utilisation of plant oil sources rich in PUFAs, such as linseed oil, corn oil, rapeseed oil or a blend of plant oils, induced good contents of LC-PUFAs associated with higher expression levels in common carp organs of genes involved in FA metabolism, compared to those of fish fed a fish oil-based diet [26,67–73]. However, to our knowledge, the effects of dietary FA profiles on the immune status, and especially on the eicosanoid metabolism process, have not been investigated in this species so far. Some studies have also demonstrated that immune parameters such as lysozyme, complement, macrophage activity or the expression of genes involved in the immune system of common carp could be stimulated by an immunostimulant supplementation, such as β-glucan, lipopolysaccharide (LPS), nucleotides from yeast RNA, chitosan or plant extracts through injection, oral administration or immersion [74–82]. However, it is not known if the amount and composition of LC-PUFAs produced by omnivorous fish from dietary PUFA precursors are suitable to sustain a good immmunocompetence and modulate the response to immunostimulants, as stated above.

In this context, the present study was conducted in order to answer two questions: (1) Are common carp able to biosynthesise enough LC-PUFAs (ARA, EPA and DHA) from PUFA precursors (LA and ALA) of some plant oils to sustain a good physiological and immune status, and (2) to what extent the total replacement of fish oil by plant oils can affect the response to supplementation with an immunostimulatory compound. Based on these questions, this study aims to evaluate the influence of different lipid sources in association with β-glucans on immune parameters, tissue FA composition and expression of genes involved in FA biosynthesis, the immune system and eicosanoid metabolism processes of the common carp.

2. Materials and methods

2.1. Experimental diets

Six iso-nitrogenous (crude protein = 39.1%) and iso-lipidic (crude lipids = 10%) diets containing three different lipid sources (cod liver oil (CLO) as fish oil; linseed oil (LO) and sunflower oil (SFO) as plant-derived oils) were formulated with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) MacroGard β-glucan supplementation (0.25 g/kg diet). Each diet contained soluble fish protein concentrate (SFPC), wheat gluten and gelatin as protein sources. The formulation and approximate composition of the experimental diets are shown in Table 1. The studied FA composition of each diet is presented in Table 2. Ingredients were mixed and moistened with fresh water (20%) for pelleting. The 3 mm pellets were then thoroughly air-dried and stored at 4 °C.

2.2. Nutritional trial

Common carp juveniles (Initial body weight, IBW = 16.3 ± 0.7 g/

Table 1
Ingredients and approximate composition of the six experimental diets.

Ingredients (g/kg dry matter, DM)	Experimental diets					
	CLO	LO	SFO	CLO$^+$	LO$^+$	SFO$^+$
Soluble fish protein concentrate (SFPC)[a]	120.0	120.0	120.0	120.0	120.0	120.0
Wheat gluten[b]	300.0	300.0	300.0	300.0	300.0	300.0
Gelatin[c]	60.0	60.0	60.0	60.0	60.0	60.0
Modified starch[d]	345.0	345.0	345.0	344.75	344.75	344.75
Cod liver oil[e]	100.0	0	0	100.0	0	0
Sunflower oil[f]	0	0	100.0	0	0	100.0
Linseed oil[g]	0	100.0	0	0	100.0	0
Vitamin premix[h]	10.0	10.0	10.0	10.0	10.0	10.0
Mineral premix[i]	65.0	65.0	65.0	65.0	65.0	65.0
MacroGard (β-glucans)[j]	0	0	0	0.25	0.25	0.25
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Crude protein, CP (% DM)	39.1	39.1	39.1	39.1	39.1	39.1
Crude fat, CF (% DM)	10.0	10.0	10.0	10.0	10.0	10.0
Gross energy, GE (MJ/Kg DM)	19.1	19.1	19.1	19.1	19.1	19.1
CP/GE (g/MJ)	20.5	20.5	20.5	20.5	20.5	20.5
CF/GE (g/MJ)	5.2	5.2	5.2	5.2	5.2	5.2

Experimental diet nomenclature: CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO$^+$, LO$^+$, SFO$^+$) or without (CLO, LO, SFO) β-glucan supplementation.
The vitamin premix was formulated following [83] (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; α-cellulose, 325.
[a]Cook Carp Concept, 56 Rue de Metz, 57130 Jouy-aux-Arches, France.
[b,c,g]Sigma-Aldrich, St Louis, MO, USA.
[d]Baaboo Food, Ho Chi Minh City, Vietnam.
[e]Mosselman s.a., Route de Wallonie, B-7011 Ghlin, Belgium.
[f]Simply Oil, Cai Lan Oils & Fats Industries Co., Ltd, Vietnam.[i].
[j]The mineral premix was formulated following [83] (to provide g/kg mixture, except as noted): $CaHPO_4{\cdot}2H_2O$, 295.5; $Ca(H_2PO_4)2{\cdot}H_2O$, 217; $NaHCO_3$, 94.5; $Na_2SeO_3{\cdot}5H_2O$, 11 mg; KCl, 100; NaCl, 172.4; KI, 0.2; $MgCl_2$, 63.7; $MgSO_4$, 34.3; $MnSO_4{\cdot}4H_2O$, 2; $FeSO_4{\cdot}4H_2O$, 10; $CuSO_4{\cdot}5H_2O$, 0.4; $ZnSO_4{\cdot}7H_2O$, 10.

fish) were obtained from the Research Institute of Aquaculture N°1 (RIA1), Vietnam. Fish were acclimated for two weeks in an indoor tank system in the wet-lab of the Faculty of Fisheries at the Vietnam National University of Agriculture. During that period, they were fed a commercial pellet for carp juveniles (Cargill, code 7434) containing 35% crude protein. After acclimation, fish were randomly distributed into 18 tanks of 120 L (3 aquariums per diet) at a density of 20 fish per tank. Fish were then fed twice a day (08.00 and 14.00) with the experimental diets at a ration of 4% body weight per day for 9 weeks. Daily feed intake was weighed and recorded to calculate feed conversion rate (FCR).

During the experimental period, the rearing conditions in the experimental system were maintained constant: temperature of 26–28 °C, dissolved oxygen at 5 mg/L, pH of 7.5 and 12 h light: 12 h dark photoperiod. Nitrite, nitrate and NH_3/NH_4^+ values were measured once a week and averaged 0.005, 5 and 0.05 mg/L, respectively. The tanks were siphoned daily to remove fish faeces and about 30% of the water was renewed.

2.3. Challenge test

A strain of *Aeromonas hydrophila* was originally isolated and identified from infected common carp and identified by the Centre of Research and Development in Biotechnology, Hanoi University of Science and Technology, Vietnam, according to the protocol of [84]. The bacterial culture process was described in Ref. [81]. The median lethal dose LD50 was determined by intraperitoneal injection with doses of 10^6, 10^7, 10^8 and 10^9 CFU/fish and the results showed that the LD50 was 5.01×10^8 CFU/fish, for fish of 30 g. One day after the end of the nutritional trial, at day 64, fish were divided into two batches; one group was intra-peripherally injected with *A. hydrophila* with a dose of 5.01×10^8 CFU/fish and the other group with the bacterial medium culture Tryptic Soy broth (TSB; Merck, Darmstadt, Germany) only. Non-supplemented and β-glucan supplemented fish were then monitored over a period of 10 days and the daily mortality was recorded. The bacterial contamination was confirmed by the re-implantation of the infected fish kidney samples on the nutrient agar medium and bacterial colony descriptions were followed [85].

Table 2
Fatty acid composition (% of total identified fatty acids) in the experimental diets.

	Diet					
	CLO	LO	SFO	CLO$^+$	LO$^+$	SFO$^+$
C6:0	0.2	0.2	0.0	0.3	0.5	0.4
C8:0	0.1	0.0	0.0	0.0	0.0	0.0
C10:0	0.1	0.1	0.1	0.1	0.1	0.1
C12:0	0.1	0.1	0.1	0.1	0.1	0.1
C14:0	3.7	0.5	0.5	4.1	0.6	0.9
C15:0	0.3	0.1	0.1	0.3	0.1	0.1
C16:0	12.7	7.9	8.7	12.4	7.9	8.7
C17:0	0.3	0.1	0.1	0.3	0.1	0.1
C18:0	2.7	3.3	3.3	2.5	3.3	3.2
C18:1n-9 (OA)	20.8	21.5	25.4	19.6	21.8	25.3
C18:2n-6 (LA)	11.5	22.0	53.3	9.9	22.8	48.0
C18:3n-3 (ALA)	4.4	39.3	1.5	2.1	37.3	1.6
C20:4n-6 (ARA)	0.5	–	–	0.5	–	–
C20:5n-3 (EPA)	6.5	–	–	7.6	–	–
C22:6n-3 (DHA)	9.0	–	–	10.5	–	–
SFA	20.4	12.8	14.0	20.4	13.1	14.7
MUFA	43.0	23.5	28.6	43.8	23.9	30.9
C18-PUFA	36.6	63.7	57.4	35.8	62.9	54.5
C18-PUFA n-6	32.5	43.5	78.8	29.6	44.6	73.3
C18-PUFA n-3	17.5	61.4	55.0	13.9	60.3	49.9
LC-PUFA	18.8	2.4	2.4	21.7	2.7	4.5
LC-PUFA n-6	1.2	0.3	0.3	1.3	0.3	0.3
LC-PUFA n-3	17.6	2.1	2.1	20.4	2.4	4.2
n3/n6 ratio	1.8	1.9	0.1	2.2	1.7	0.1
ALA/LA	0.4	1.8	0.03	0.2	1.6	0.03

Experimental diet nomenclature: CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO$^+$, LO$^+$, SFO$^+$) or without (CLO, LO, SFO) β-glucan supplementation. OA: oleic acid; LA: linoleic acid; ALA: α-linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosapentaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: C18-polyunsaturated fatty acids; LC-PUFA: long chain polyunsaturated fatty acids.

2.4. Sample collection

After 9 weeks of rearing (D63), the total fish number and body weight were recorded to determine the survival rate (SR) and specific growth rate (SGR), respectively. At the end of the growth trial and after two days (D65) of bacterial challenge test, three fish per aquarium were randomly selected and anesthetised with clove oil (50 μL/L, Sigma-Aldrich). Heparin blood plasma was individually sampled for lysozyme and complement (ACH50) activities, fish liver and dorsal muscle were dissected to analyse the FA composition, while fish kidney and liver were collected for gene expression analyses. The tissue samples were snap frozen in liquid nitrogen and then stored at −80 °C.

2.5. Sample analysis

2.5.1. Fatty acid analyses

The experimental diets were homogenised and the lipids were extracted with chloroform/methanol (2:1, *v:v*) according to the Folch method [86], edited by Christie [87], while lipids of fish liver and dorsal muscle (3 fish per tank) were extracted by chloroform/methanol/water (2:2:1.8, *v:v:v*) following a method adapted from Bligh and Dyer [88]. 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. [20]. The GC trace (Thermo Scientific, Milan, Italy) was equipped with a capillary column of 100 m × 0.25 mm, 0.2 µm film thickness (RT 2560, Restek, Bellefonte, PA, USA). 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 Ref. [20]. Each peak was identified by comparison of retention times with those for pure methyl ester standards (Larodan, Solna, Sweden) and Nu-Check Prep (Elysian, Minnesota, USA). Data were processed using ChromQuest software 3.0 (Thermo Finnigan, Milan, Italy). The final results are expressed in percentageof total identified fatty acids.

2.5.2. Immune parameter analyses

Lysozyme activity was determined according to the protocol of Ellis [89] adapted for common carp. Heparin blood plasma (30 µL) was individually added in triplicate to 30 µL of PBS buffer (phosphate-buffered saline, pH 6.2). The 100 µL-bacterial suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich) (200 mg/L in 0.05 M NaH_2PO_4, pH 6.2) was then added to the mix of plasma and PBS buffer. Two readings at 530 nm wave length were taken with a spectrophotometer after 30 s and 4.5 min of shaking. The lysozyme activity unit (U/mL) was defined as the amount of enzyme causing a decrease in absorbance of 0.001/min.

The protocol to determine the complement activity was described in Ref. [90] and adapted for common carp. Accordingly, blood plasma was added by a series of dilutions with veronal buffer (VCM-F, BioMérieux, Marcy l'Étoile, France) to a 96-well round bottom plate. Wells were then filled with 10 µL of 3% rabbit blood cells (RaRBC, BioMérieux) (70 µL total volume for each well). Samples were incubated at 27 °C for 2 h and centrifuged (3000 × *g*, 5 min, 4 °C) to collect the supernatant. Then, 35 µL of supernatant was measured the absorbance at 405 nm. The haemolysin (HLY) was recorded as the highest dilution of plasma showing complete lysis. The ACH50 value was defined as the reciprocal of the plasma dilution which induced 50% haemolysis of RaRBC.

2.5.3. Gene expression analyses

The total RNA of liver and kidney were individually extracted from a batch of three fish for each tank using 1 mL trizol (Extract-all®, Eurobio, Courtaboeuf, France). The quality of extracted RNA was checked using a Nanodrop 2000 spectrophotometer (Thermo-Fischer Scientific, Waltham, MA, USA) and electrophoresis on a 1.2% agarose gel. Each individual RNA sample was then treated using a RTS DNAse™ kit (MO BIO Laboratories, Carlsbad, CA, USA) to avoid DNA contamination. Then, 1 µg of total RNA was reverse transcribed to cDNA in using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). The cDNA was then diluted with ultrapure water (Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water, ThermoFisher scientific) and used for real-time qPCR to determine gene expression levels. Expression of *nk* (natural killer cell enhancing factor), *lys* (lysozyme), *il8* (interleukin 8), *elovl5* (elongase very long delta 5), *fads6-a* (fatty acid desaturase delta 6), *pla* (phospholipase A2), *pge* (prostaglandin E2 synthase) and *alox* (Arachidonate 5-lipoxygenase) genes were determined using specific primers that were designed on Primer3 software and re-checked for quality on Amplifix software against sequences of the common carp published on Genbank (Table 3). The efficiency of each gene was confirmed before analysis. The *40s* (40S ribosomal protein) and *18s* (18S ribosomal RNA) [91] genes were used as housekeeping genes. The amplification of cDNA was conducted in triplicate using an iQ™ SYBR® Green Supermix kit (Bio-Rad Laboratories, Hercules, CA, USA). Thermal cycles and fluorescence detection were carried out using a StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) under the following conditions: 10 min of initial denaturation at 95 °C, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s. For analysis, a standard curve produced from a pool of cDNA from all samples was included to calculate the PCR efficiency and to normalise the transcript levels. The relative levels of RNA were quantified for each gene by densitometry, which was performed by measuring the photostimulated luminescence values using StepOne Software v2.1. Ratios of candidate gene/housekeeping gene products were subsequently calculated for each gene and used to assess the differences in expression levels between experimental groups.

2.6. Data presentation and statistical analysis

The husbandry parameters were calculated as follows:

Survival rate (SR, %) = 100 × final number of fish/initial number of fish.

Specific growth rate (SGR, %/day) = 100 × (Ln (FBW) – Ln (IBW))/ΔT.where FBW and IBW are final and initial body weights, respectively, and ΔT is the number of days of the growth trial.

FCR = (final biomass – initial biomass + dead biomass)/feed intake.

Mean values of all variables were checked for homogeneity by univariate tests, and then subjected to a two-way analysis of variance (ANOVA 2) followed by a *LSD post-hoc* test using the tank replicate as statistical unit (n = 3). Differences between treatments were considered significant at P value < .05. All data were analysed with the statistical package STATISTICA 5.0 (Statsorf, Inc., East 14 Street, Tulsa, USA).

3. Results

3.1. Growth and feed utilisation

After nine weeks of feeding, the husbandry parameters, namely SGR, SR and FCR, were calculated and results are presented in Table 4. No differences between groups were observed for all parameters. The FBW was two times higher than IBW (33.8 g *vs.* 16.3 g), with a SGR ranging from 1.1 to 1.3%/day. A high SR was observed in all treatments (ranging from 94.7 to 98.7%), suggesting that the rearing conditions were suitable for common carp requirements.

Values were represented by means ± SD. CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. IBW: initial body weight; FBW: final body weight; SGR: specific growth rate; FCR: feed conversion rate; SR: survival rate. Data were transformed in Log for final body weight; Arcsine ($\sqrt{X}$) for survival rate before statistical analysis. Values with no common superscript letter within the same row denote significant differences ($P < .05$) (*)

3.2. Fatty acid composition in carp liver and muscle and expression of genes involved in FA biosynthesis processes

Dietary FA composition varied with the dietary oil sources (Table 2). Of note, LA was abundant in diets containing SFO (four times higher than in CLO diets and two times higher than in LO diets) while ALA was abundant in LO-based diets (11 and 24 times higher than in of CLO- and SFO-based diets). The LC-PUFAs such as ARA, EPA and DHA were only provided for fish fed CLO-based diets. The LO and SFO-based diets were rich in PUFAs whereas CLO-based diets contained a high level of LC-PUFAs. The n-3/n-6 ratio in LO diets was comparable to that

Table 3
Primer sequences for amplification of candidate genes involved in the immune system, FA biosynthesis and eicosanoid metabolism processes in common carp.

Gene nomenclature	Function	Genbank No.	Primer sequence
Immune gene			
il8	Cytokines	EU011243	Fw: CGCTGCATTGAAACTGAGAG Rv: TTAACCCAGGGTGCAGTAGG
nk	Natural killer cell enhancing factor	AB048789	Fw: TGTGATGCCAGATGGACAGT Rv: CCTTGTTTCCGAGGTGTGTT
lys	Lysozyme	AB027305	Fw: GTGTCTGATGTGGCTGTGCT Rv: GAACGCACTCTGTGGGTCTT
Fatty acid biosynthesis genes			
fads6-a	Desaturase delta 6	[26]	Fw: ATCGGACACCTGAAGGGAGCG Rv: CATGTTGAGCATGTTGACATCCG
elovl5	Elongase delta 5	KF924199	Fw: AGGAGAGGCTGACAACAGGA Rv: CAGGAAGGTGATCTGGTGGT
Eicosanoid metabolism genes			
pla	Secreted phospholipase	KF793834	Fw: CTGCATGACAAGTGATGAGCAA Rv: CTGGTGCTCAAATCCATCAGGT
pge	Prostaglandin E synthase 2	XM_019098948	Fw: CAAGGAATTCATGGGAGGCGATCA Rv: CACACGTCGGTACCAGTTCTTCA
alox	Arachidonate 5-lipoxygenase	XM_019066935	
Housekeeping genes			
40s	40S ribosomal protein	AB012087 [91]	Fw: CCGTGGGTGACATCGTTACA Rv: TCAGGACATTGAACCTCACTGTCT
18s	18S ribosomal RNA	FJ710826 [91]	Fw: GAGTATGGTTGCAAAGCTGAAAC Rv: AATCTGTCAATCCTTTCCGTGTCC

of CLO diets (ranging from 1.7 to 2.2) and higher than in the SFO diets (n-3/n-6 = 0.1). The ALA/LA ratio value was the lowest in the SFO (0.03) diets compared to CLO (0.2 and 0.4) and LO (1.6 and 1.8) diets.

At the end of the experimental feeding period, we observed significant differences in the FA levels of carp liver and muscle between experimental conditions ($P < .05$). No influence of β-glucan supplementation was found on the FA profiles of liver and dorsal muscle from common carp.

In liver, a difference was observed in all the FA types (Fig. 1a). The highest level of SFA was found in the CLO group and the same results were recorded for MUFA and LC-PUFA contents ($P < .05$). In contrast, the PUFA contents in LO and SFO groups reached a higher value than in the CLO group ($P < .05$). Regarding the essential PUFA levels, we found significant differences in LA and ALA levels ($P < .05$). In contrast, the major MUFA, namely OA, remained at a similar level in all treatments (Fig. 1b). The highest value of LA levels was observed in SFO fish, while that of the CLO group was the lowest, the LO-fed fish being in an intermediate position. ALA was abundant in LO fish but very low in other treatments ($P < .05$). The major LC-PUFA presented different levels between experimental conditions (Fig. 1c). The level of ARA in the SFO group (1%) was significantly higher than those in the CLO (0.2%) and LO (0.3%) groups. EPA and DHA levels were the highest in CLO fish while the lowest value was found in the SFO group, intermediate value being observed in LO-fed fish ($P < .05$). The n3/n6 ratio varied around 1.2 (Fig. 1c) for fish fed CLO- and LO-based diets, and was significantly higher ($P < .05$) than in the SFO group (around 0.1).

CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: C18-polyunsaturated fatty acids, LC-PUFA: long chain polyunsaturated fatty acids, OA: oleic acid, LA: linoleic acid, ALA: α-linolenic acid, ARA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid. Columns with no common letter within the same group of FAs denote significant differences ($P < .05$) (*)

In dorsal muscle, the FA composition showed similar trends than in the liver, with diet-related differences found in all the FA groups (Fig. 2a), essential PUFA (Fig. 2b) and the main LC-PUFA (Fig. 2c). SFA and MUFA contents in dorsal muscle of fish fed the CLO-based diet were higher than for the groups fed LO and SFO diets; a similar result was observed for LC-PUFA content. In contrast, the contents in C18-PUFA were much higher in the muscle of fish fed plant oil-based diets than CLO-fed fish ($P < .05$, Fig. 2a). Regarding the levels of OA and essential PUFA (Fig. 2b), we observed an increase in OA levels with the plant oil-based diets, the difference being significant in the SFO^+ condition ($P = .03$). Differences were much more striking for LA and ALA levels in muscle. The highest values for LA were observed in the SFO groups, while they were the lowest for fish fed the CLO ($P < .05$). Intermediate levels were observed in LO-fed fish. As for ALA, the muscle of fish fed a LO diet presented much higher levels, as compared to the CLO and SFO conditions for which the ALA levels remained very low ($P < .05$). Results concerning LC-PUFA (ARA, EPA and DHA) levels were also significantly different ($P < .05$) between experimental conditions (Fig. 2c). The ARA levels in the SFO groups were significantly higher ($P < .05$) than those found in the CLO or LO conditions. In contrast, the DHA levels in the muscle of fish fed the SFO and LO diets were significantly lower than for the CLO groups. The EPA contents in the muscle of CLO-fed fish were about 2.5 times higher than in the muscle of LO-fed fish and 7 times higher than in the SFO conditions. Accordingly, the EPA levels in the muscle of fish fed the LO-

Table 4
Husbandry parameters of experimental fish fed different plant oil diets with or without β-glucans after 9 weeks of rearing.

Diet	CLO	LO	SFO	CLO+	LO+	SFO+
Parameters						
IBW (g/fish)	15.6 ± 0.4	16.9 ± 0.5	16.4 ± 0.5	16.0 ± 0.7	16.4 ± 0.8	16.2 ± 0.6
FBW (g/fish)	34.1 ± 2.1	32.7 ± 1.3	33.6 ± 2.2	36.2 ± 7.3	34.7 ± 2.2	31.7 ± 4.4
SGR (%/day)	1.2 ± 0.2	1.0 ± 0.1	1.1 ± 0.2	1.3 ± 0.3	1.2 ± 0.1	1.3 ± 0.2
FCR	1.84 ± 0.4	2.01 ± 0.3	2.23 ± 0.5	2.03 ± 0.5	1.97 ± 0.3	1.81 ± 0.3
SR (%)	98.7 ± 2.3	94.7 ± 2.3	97.3 ± 2.3	97.3 ± 2.3	96.0 ± 6.9	97.3 ± 4.6

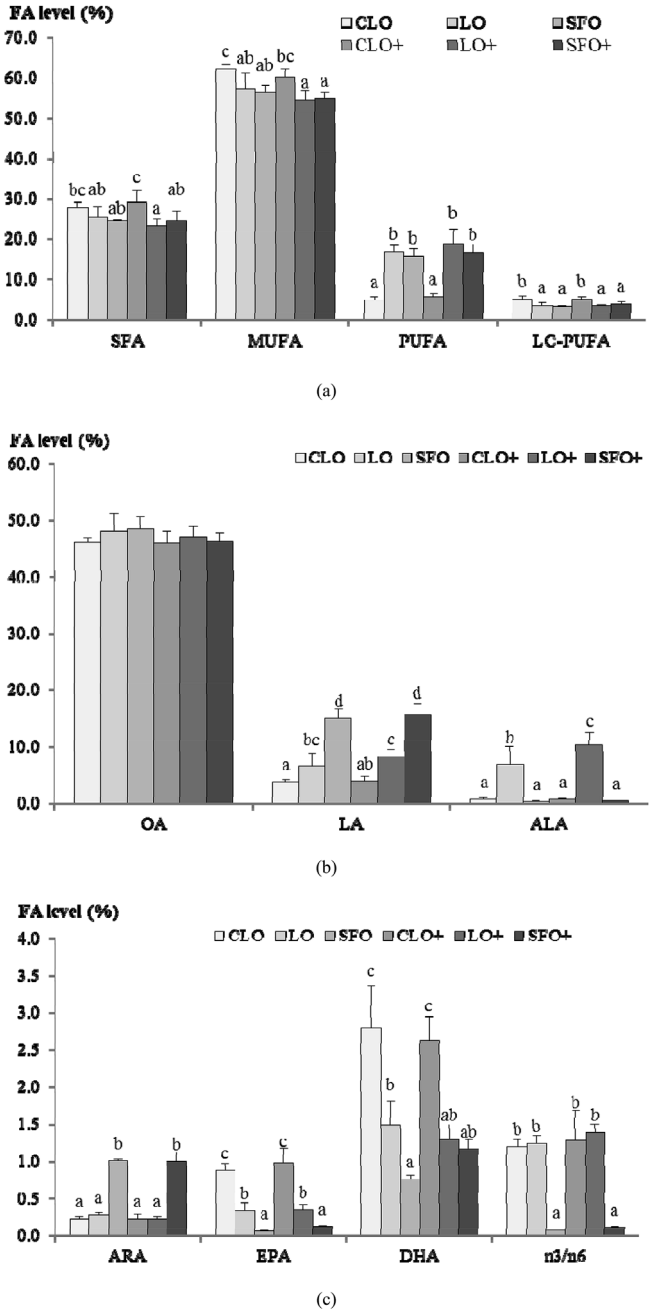

Fig. 1. Fatty acid composition (% of total identified fatty acids) for (a) all fatty acid groups, (b) selected C18 unsaturated fatty acids and (c) LC-PUFA in common carp liver after 9-week feeding period.

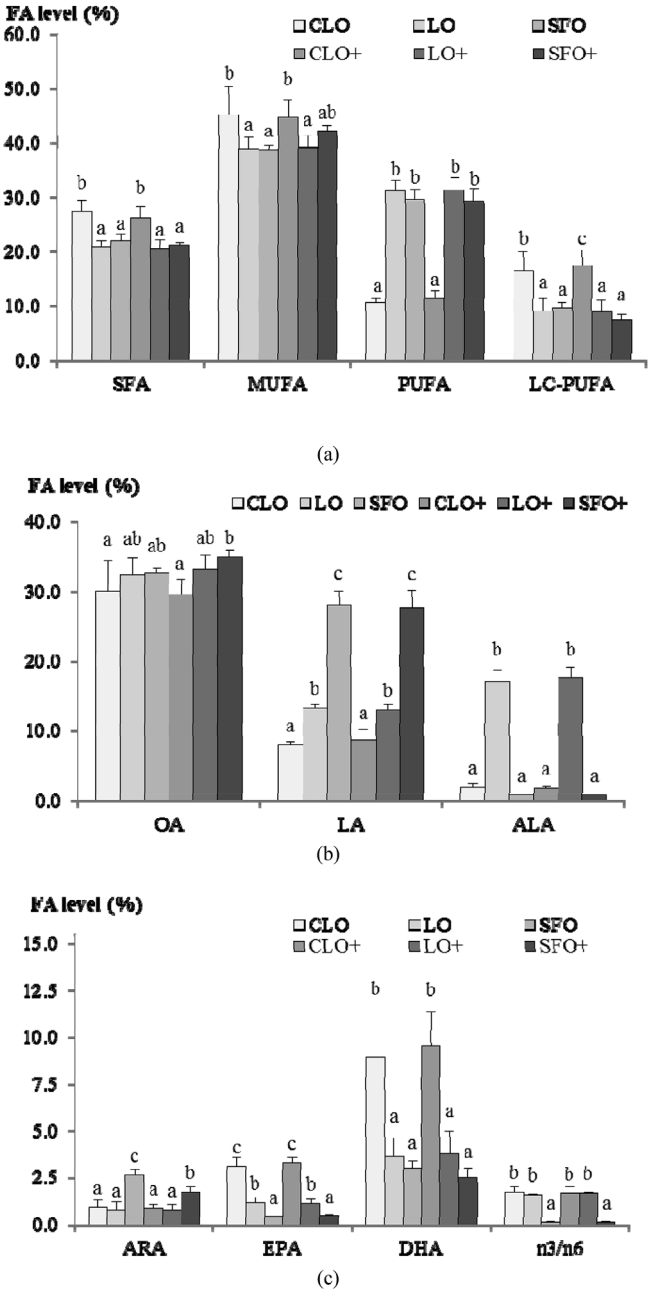

Fig. 2. Fatty acid composition (% of total identified fatty acids) of all fatty acid groups (a), selected C18 unsaturated fatty acids (b) and LC-PUFA (c) in common carp muscle after the 9-week feeding period.

diets were about 3 times higher than in the corresponding tissue of fish fed the SFO-diets. Interestingly enough, the n-3/n-6 ratios were close to 1.7 for the CLO and LO groups, while being very low (around 0.1) in the muscle of SFO-fed fish ($P < .05$, Fig. 2c).

CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: C18-polyunsaturated fatty acids, LC-PUFA: long chain polyunsaturated fatty acids, OA: oleic acid, LA: linoleic acid, ALA: α-linolenic acid, ARA: arachidonic acid, EPA: eicosapentanoic acid, DHA: docosahexanoic acid. Columns with no common letter within the same group denote significant differences ($P < .05$)

The expression of genes related to FA biosynthesis processes (*fads-6a, elovl5*) was determined in fish liver tissue (Fig. 3). The expression levels of *fads-6a* and *elovl5* genes were comparable between fish fed the two plant oil-based diets and those receiving CLO with or without β-glucan supplementation. *Fads-6a* was up-regulated in SFO-fed fish in comparison to LO-fed fish, but this difference was not observed when the feeding treatment included β-glucans. Such an interaction between SFO and β-glucan supplementation was not observed for *elovl5* expression.

CLO: cod liver oil, LO: linseed oil and SFO: sunflower oil with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. fads-6a: fatty acid desaturase delta 6; elovl5: elongase very long delta 5. Columns with no common letter within the same group denote significant differences ($P < .05$)

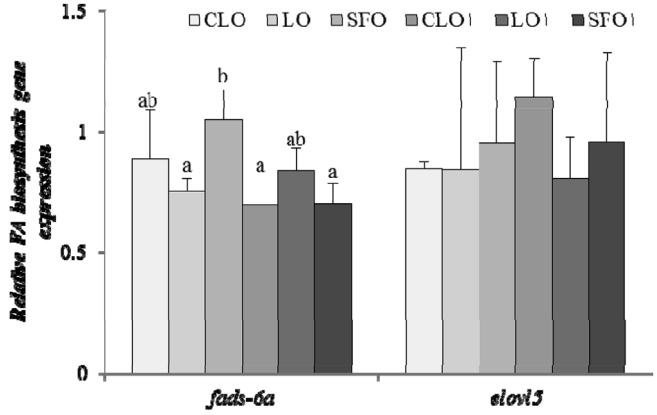

Fig. 3. Expression of genes involved in fatty acid biosynthesis in common carp.

3.3. Immune response and expression of related immune genes

After a 10-day challenge test with an *A. hydrophyla* dose of 5.01×10^8 CFU/mL, the observed mortality was lower than 50% and varied from 12.6 to 13.7% with no difference between experimental conditions either for non-supplemented fish or β-glucan treated ones.

On D63 (healthy fish), in the groups fed with diets without β-glucans, the plant oil-based diets did not negatively affect plasma lysozyme activity (Fig. 4). SFO-fed fish even displayed higher values ($P < .05$) than CLO fish. In contrast, SFO- or LO-based diets with β-glucan supplementation lowered the lysozyme activity, as fish fed SFO + or LO + displayed lower lysozyme activities than fish fed CLO +. In infected fish (D65), plant oil-based diets did not impair the lysozyme activity, which was even higher in fish fed LO than a CLO-based diet. Moreover, the lysozyme response with β-glucan supplementation was comparable between LO + and CLO + groups, but was the lowest ($P < .05$) in fish fed SFO +, indicating a negative interaction with SFO.

Values are represented by means ± SD. Ctrl: fish at D0 of feeding trial and non-injected fish with bacteria in challenge test; CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. Data were transformed in Log before statistical analysis. Values with no common letter within columns denote significant differences between diets ($P < .05$)

Values are represented by means ± SD. Ctrl: fish at D0 of feeding trial and non-injected fish with bacteria in challenge test; CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. Data were transformed in Log before statistical

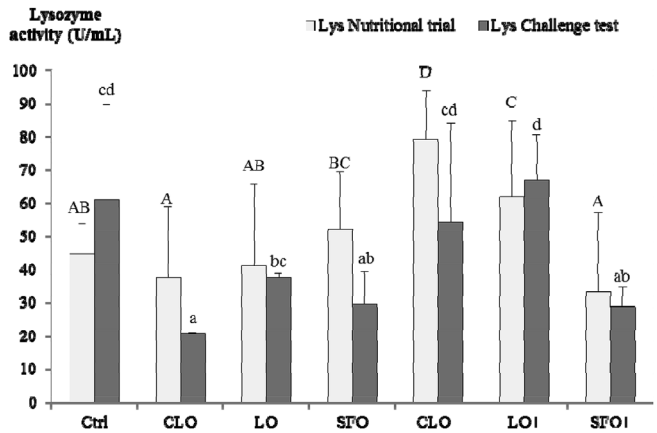

Fig. 4. Lysozyme activity in the blood plasma of common carp at the end of the nutritional trial (D63) and after two days of challenge test (D65).

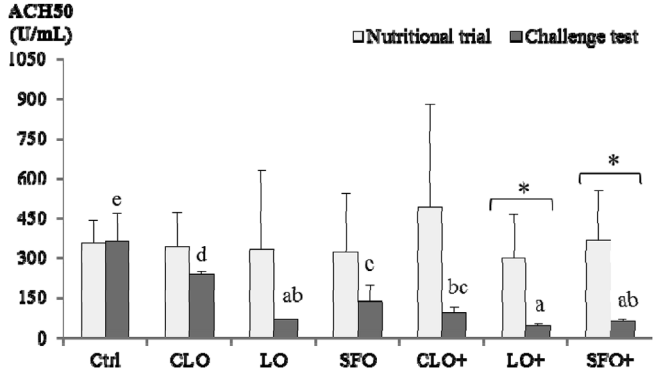

Fig. 5. Alternative complement activity (ACH50) in the blood plasma of common carp at the end of the nutritional trial (D63) and after two days of challenge test (D65).

analysis. Values with no common letter within columns denote significant differences between diets ($P < .05$). Symbol (*) denotes a significant difference within a diet group, before and after the challenge test ($P < .05$)

Regarding the results of alternative complement activity (ACH50) (Fig. 5), no negative effects of plant oil-based diets were observed without or with β-glucans as values were comparable between all experimental groups in healthy fish on D63. β-glucan supplementation did not induce any alteration in ACH50 response whatever the oil source. ACH50 values were lowered by bacterial infection in all experimental groups, especially when plant oils were combined with β-glucans as for fish fed LO + and SFO + compared to fish fed CLO + ($P < .05$).

The expression of several immune genes (*nk, lys* and *il8*) was assayed in kidney (Fig. 6). The *nk* gene expression level in SFO was higher than in CLO and LO fish, while this difference was not found in groups fed additionally with β-glucans. The dietary β-glucan supplementation enhanced the expression of *nk* in fish fed a CLO-based diet whereas any stimulation was observed for LO + and SFO + groups. Regarding *lys* gene expression, the level was comparable between groups without β-glucans, while the response to β-glucans was altered in LO + fed fish but not in SFO + ones. Concerning the expression of the *il8* gene, no negative effect of plant oils was observed with or without β-glucans and this supplementation induced *il8* up-regulation in only CLO-fed fish.

CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. nk: natural kille cell enhancing factor; lys: lysozyme; il8: interleukin 8. Columns with no common letter within the same group denote significant differences ($P < .05$)

In liver tissues, both β-glucan supplementation and dietary FA profiles significantly affected the expression of *pla* and *pge* genes, while no differences were found in *alox* gene expression (Fig. 7). Specifically,

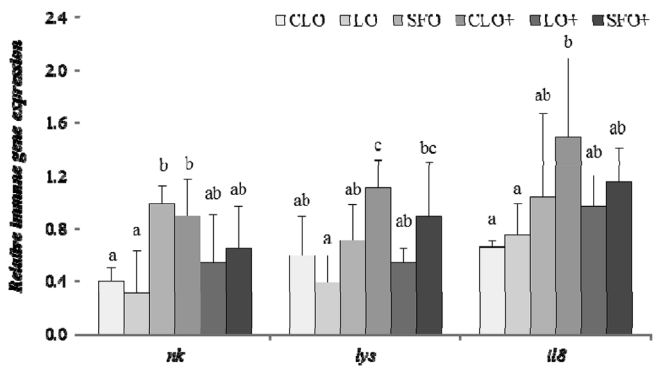

Fig. 6. Expression of related immune genes in common carp kidney.

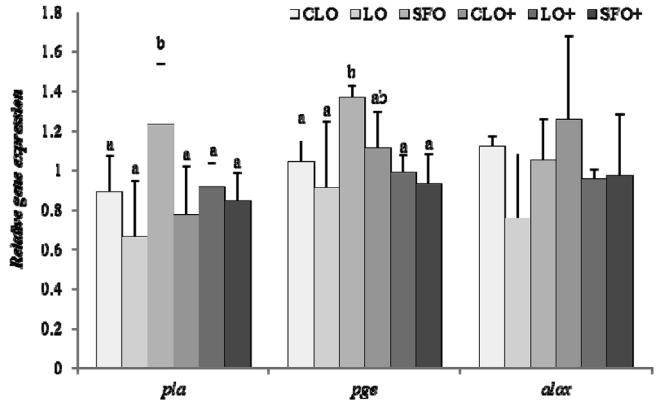

Fig. 7. Expression of genes involved in eicosanoid metabolism processes in common carp liver.

we found an up-regulation of *pla* and *pge* gene expression in the SFO group compared with CLO- and LO-fed fish without β-glucans ($P < .05$); but no modulation of the expression of these genes was observed whatever the oil source after β-glucan supplementation as the expression levels did not differ between CLO+, LO+ and SFO + groups.

CLO: cod liver oil-based diet, LO: linseed oil-based diet and SFO: sunflower oil-based diet with (CLO^+, LO^+, SFO^+) or without (CLO, LO, SFO) β-glucan supplementation. pla: phospholipase A2; pge: prostaglandin E synthase 2; alox: arachidonate 5-lipoxygenase. Columns with no common letter within the same group denote significant differences ($P < .05$).

4. Discussion

4.1. Growth parameters

No growth differences were recorded between the experimental diets. This indicates that the lower amounts of LC-PUFAs produced by common carp fed with LO or SFO diets compared to fish fed with a CLO diet did not negatively influence the fish growth performance. As reported in the introduction, a similar trend was observed in previous studies, suggesting that a total plant oil utilisation can generally be used in freshwater or omnivorous fish species. However the total replacement of FO by plant-derived oil in marine or carnivorous fish diet frequently induces a reduction of growth performance [17,19,21–24,92]. In our experiment, the SGR values (from 1.0 to 1.3%/day) were similar to those of the study in Ref. [68] (about 1.3%/day) and higher than those reported in Ref. [26] (0.4%/day) for the same species and the same developmental stage (juveniles of 40–50 g). In the current study, we used soluble fish protein concentrate as one of the protein sources. This ingredient does not contain the fish oil usually present in commercial fish meal (containing from 5 to 10% fish oil [93]). It is also interesting to note that the profile of LC-PUFAs did not affect the growth rate, as fish fed a CLO diet displayed the highest levels of EPA and DHA while fish fed SFO only produced higher levels of ARA.

We also observed that supplementation with β-glucans did not improve the husbandry parameters. Similar observations were found in previous studies with common carp where the authors used different compounds such as β-glucans [49], chitosan [94] or May chang *Litsea cubeba* leaf powder [81] as dietary immunostimulants.

4.2. Fatty acid composition of liver and muscle and related gene expression

FA profiles in common carp tissues reflected those of the respective diets. Tissues from fish fed a CLO diet were rich in EPA and DHA, whereas tissues of LO-fed fish were rich in ALA, and SFO-fed fish were rich in LA. Nonetheless, tissues from fish fed plant oil-based diets contained substantial levels of LC-PUFA, with higher levels of ARA for the SFO conditions, and higher levels of EPA in the LO conditions as compared to the SFO conditions. These two observations indicate that common carp has an active capacity for biosynthesis of LC-PUFA from the precursors contained in plant oils, enabling them to have enough essential FAs to sustain optimal growth performance. Similar findings were previously reported by several authors [26,67,68,95] on the same species, suggesting a specific ability of common carp to biosynthesise ARA from LA, and EPA from ALA. The levels of EPA and DHA in the liver and muscle of fish fed a CLO diet (0.9 and 2.8% in liver and 2.8 and 9% in muscle) were higher than those reported in Ref. [96] (0.8 and 2.4% respectively) [97], (1.16 and 5.26% respectively) and [98] (0.85 and 1.63% respectively) in the same species.

In the present study, a lower dietary ALA/LA ratio (0.03 in SFO-based diet *vs.* 0.4 in CLO and 1.8 in LO diets) is associated to a lower EPA level. Similar observations were also reported in Murray cod *Maccullochella peelii* [99] and juvenile tambaqui *Colossoma macropomum* [100]. Apart from a good ALA/LA ratio, the LO and CLO diets also induced the highest n-3/n-6 ratios in common carp muscle (around 1.7), which is higher than those previously reported in the same species in Refs. [98,101,102]. The present results indicate that these two oils support the production of high quality fish fillet for human consumption, as far as the n3/n6 ratio is concerned. Indeed, the dietary n-3/n-6 ratios are implicated in controlling markers of metabolic parameters, including insulin sensitivity, inflammation, lipid profiles and adiposity [103]. According to several authors [104–106] humans have been evolutionary adapted to a diet with a n-3/n-6 ratio close to 1. This observation supports the suitability of linseed oil as a plant-derived oil to substitute fish oil in carp feed, not only in terms of carp culture performance, but also from a human nutrition perspective.

Regarding the results of FA profiles in diets and liver, the LA (a precursor of ARA) levels in SFO diets reached about 53%, while ALA (a precursor of EPA) in LO diets reached 39%. However, the ARA levels in SFO-fed fish liver were around 1%, whereas EPA levels in LO-fed fish liver were limited to 0.3%. The lower level of anabolic conversion in the case of ALA-EPA may be linked to the lower accumulation of ALA in the tissues, as compared to LA, probably because ALA is more prone to be used as an energetic substrate as it has been reported in mammals [107–109]. In addition, a link may also be made with the higher expression level of *fads-6a* in SFO fish, suggesting higher desaturase enzyme activity in the FA biosynthesis pathway of SFO fish than LO or CLO fish. Nonetheless, the expression of *elovl5* did not differ between experimental treatments, although the LC-PUFA was influenced by dietary FA composition.

4.3. Immune status and immunomodulatory response

We observed a marked influence of dietary lipid source and β-glucan supplementation on plasma lysozyme activity ($P < .05$) (Fig. 5) at the end of the nutritional trial (D63) and after the challenge test (D65). Lysozyme is a bacteriolytic enzyme that is widely distributed throughout the body and is part of the nonspecific defense mechanisms in most animals [110]. Similar results were found in some studies with common carp fed diets containing nucleotides isolated from yeast RNA [74], chitosan [77,111], chitin [111], plant extract [81], lipopolysaccharide [49,78] or β-glucans [79,94,112]. The highest lysozyme activity was measured in CLO+ fish plasma (79 U/mL), where it was more than two times higher than the values reported in Ref. [94] (about 30 U/mL after 56 days of rearing) with dietary β-glucans at a much higher dose than in our experiment (900 mg/kg diet instead of 250 mg/kg diet in our work), or in Ref. [77] (about 40 U/mL) where the authors supplemented the diet with chitosan oligosaccharides and *Bacillus coagulans*. On the other hand, our results were several times lower than those reported in Ref. [78] where the authors used LPS as an immunostimulant. LPS is an endotoxin and it could stimulate the inflammatory response, inducing an increase in the lysozyme activity. We

also observed a higher lysozyme activity in SFO-fed fish than in CLO-fed fish at D63, but lower at D65, indicating that there was an interactional influence ($P < .05$) between β-glucan supplementation and dietary FA on this immune parameter. This could be explained by the high level of ARA in SFO-fed fish. ARA is the major precursor of highly active eicosanoids [38,40] that play a role in immune and inflammatory responses [37,38], inducing an increase of lysozyme activity in SFO fish (rich in ARA), which was higher than those measured in CLO and LO groups. Besides this, β-glucan is known to be an immunomodulatory factor as cited in the introduction. The β-glucan supplementation in diets rich in ARA (SFO+) could reduce the lysozyme activity compared to diets from the same lipid source but without β-glucans (SFO). A similar explanation can be provided for the lysozyme activity after bacterial challenge.

Alternative complement activity (ACH50), a major pathway of the innate immune response in teleost fish [113], did not show any difference on D63 between fish fed the different lipid sources, regardless of β-glucan supplementation, while significant differences were observed after bacterial infection, as well as a decrease of ACH50 in fish fed diets enriched with β-glucan. The alternative complement pathway is independent of antibodies and is activated directly by foreign microorganisms [114]. Similar results, but with high interspecific variations, were reported in large yellow croaker *Pseudosciaena crocea* [47], channel catfish *Ictalurus punctatus* [115], rainbow trout [116] and common carp [49,94]. ACH50 activity was higher in fish fed SFO- and FO-based diets than in those fed a LO-based diet. It has been shown that these fish were richer in ARA and EPA, and these FAs are precursors of the eicosanoid metabolism process, which could enhance the inflammatory response during bacterial infection. Although we did not investigate here the responses of adaptive immune biomarkers, it has been shown in several studies that the dietary supplementation with immunostimulant compounds was able to enhance some adaptive immune responses [52,53,117].

Regarding immune gene expression, we found that the effects of dietary lipid sources were only significant for *nk*, whereas dietary β-glucan supplementation significantly influenced the expression of all candidate immune genes. NK cells (knows as cytotoxic cells) are able to eliminate a range of spontaneously xenogeneic targets, traditional targets of natural killer cells in mammals [118]. NKs are innate lymphoid cells; however, they share a common progenitor with T cells and also directly contribute to adaptive immune responses, interacting with dendritic cells and triggering T cell responses [119], suggesting the influence of NK enhancing factor on the activity of innate and adaptive immune cells. According to Ref. [120], β-glucan triggers macrophages, neutrophils, monocytes, NK cells and dendritic cells. Our results could confirm this statement as fish fed a diet containing β-glucans displayed up-regulation of *nk*. On the other hand, we observed a down-regulation of *nk* expression in LO and CLO groups compared to the SFO group, this decrease could be explained by the influence of a diet rich in n-3 PUFA as previous published results in rats [121] or humans [122,123] have shown. The other candidate gene, *il8*, was the first known chemokine and pro-inflammatory factor, and plays a key role in the movement of immune effector cells to sites of infection [124,125]. Expression of *il8* has been demonstrated in various teleost species such as rainbow trout [126], common carp [127] and catfish [128] in response to infection with pathogens. In our experiment, *il8* gene expression also displayed up-regulation in the CLO+ group and this shows that this immune gene could be stimulated by β-glucans, a kind of fungal polysaccharide. A similar result was reported by Ref. [80] when they also used β-glucans in an experimental diet.

The highest expression of *pla* and *pge* genes, two key genes in the eicosanoid metabolism process, in SFO-fed fish liver was explained by the abundance of ARA in SFO-fed fish. An up-regulation of these genes could have induced the secretion of ARA from liver membrane layers of fish in the SFO group and eicosanoid metabolism activity was higher here than other groups. A similar result was published for large yellow croaker *Larmichthys crocea* [77] in testing the kidney macrophages with different ARA doses. However, the *pla* and *pge* gene expression in SFO+ was lower than SFO-fed fish, indicating the immunomodulatory effect of β-glucans in the diet, which was able to inhibit some inflammatory responses such as prostaglandin production, pain response, etc.

In conclusion, our results have shown that common carp fed plant oils are able to produce substantial amounts of LC-PUFAs for sustaining similar growth rates, immune status and disease resistance to fish fed fish oil. The differences in the capacity for production of LC-PUFA by the two plant oils were associated to differential activation of some immune pathways, which explains how the use of these plant oils did not affect the overall immunocompetence of fish challenged with bacteria. Moreover, the two tested plant oils did not induce substantial negative effects on the immunomodulatory action of carp when supplemented with β-glucans, confirming that these oils are suitable for sustaining a good immunocompetence status in common carp.

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