Plasma biochemistry, gene expression and liver histomorphology in common carp (*Cyprinus carpio*) fed with different dietary fat sources

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ABSTRACT

Demand for omega-3 long chain polyunsaturated fatty acids has become global challenge for aquaculture and different components have been used to increase nutritional value of fillets. The aim of this study was to evaluate influences of feeds on zootechnical parameters, biochemical plasma parameters, expression of lipid-dependent genes, hepatocyte histomorphologies, and fatty acid profiles in common carp fillets. We compared a control diet (CTRL), mimicking a commercial feed formulation for common carp, with three diets containing blends of vegetable oils and a DHA-rich alga (*Schizochytrium* sp.) included at 3.125% (CB1) or 1.563% (CB2), and 2.1% salmon oil (CB3). The study revealed no differences in final body weight of fish fed CB1-3 diets in comparison with significantly lower CTRL. Concentrations of all biochemical parameters in plasma increased gradually in fish fed CB1-3 diets when compared to CTRL diet, with exception of triacylglycerol levels. Expression of hepatic *fas*, *elovl-5a* and *ppara* genes increased significantly in fish fed CB1 and CB2. Additionally, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) accumulation in muscle tissue was directly proportional to the amounts supplied in the diets. Our study revealed that carp fillet profiles can be manipulated for DHA and EPA-contents using enriched diets, depending on the source of fat.

1. Introduction

The essential role of omega-3 polyunsaturated fatty acids (n-3 PUFAs) in human nutrition has been studied widely in recent years (EFSA, 2014). The n-3 PUFAs functions in neurogenesis, neurotransmission, protection against oxidative stress, and are particularly important during brain development (Innis, 2007). Moreover, they are components of cell walls, determining fluidity, elasticity and permeability, and have beneficial effects against development of various human conditions, such as inflammation and autoimmune, cardiovascular and neurodegenerative diseases (Arts and Kohler, 2009; Liu and Ma, 2014; Zhang et al., 2019). The recommended daily intakes for eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (recommended by national and international authorities – 250mg/day) is achieved by only 26% of Europeans (Sioen et al., 2017).

A good source of n-3 fatty acids in human diets is fish, specifically marine fish. Generally, EFA (essential fatty acid) contents of freshwater fish is lower, and dependent on physico-chemical parameters of their habitat, season and geographical location as well as physiology (Williams et al., 2017). Freshwater fish fillets are more susceptible to changes in dietary fatty acid (FA) profile, i.e. ingredients in new feed blends. For example, fillets from Nile tilapia (*Oreochromis niloticus*) fed increased proportions of fish oil (FO) in an experimental diet had contained more DHA and EPA compared with fish fed a control diet (Özlüer Hunt et al., 2018). A comparable observation has been described for Atlantic salmon (*Salmo salar*) (Sissener, 2018). This supports the rational of utilizing sustainable raw materials rich in PUFAs for feed production. Decreasing availability and growing costs associated with animal ingredients rich in PUFAs has forced producers to consider plant-based ingredients (e.g. rapeseed oil, sunflower oil), insects (e.g. black soldier fly larvae meal), microalgae (e.g. *Schizochytrium* sp. meal) and by-products from fish processing (e.g. salmon meal). However, new implemented ingredients are potentially hazardous, because may include a range of contaminants, like heavy metals, mycotoxins, pesticide

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Abbreviations

18SrRNA	18S ribosomal RNA
40sRNA	40S ribosomal protein S11
acox1	peroxisomal acyl-coenzyme A oxidase 1
actb	β-actin
ALA	α-linolenic acid (18:3n-3)
ARA	arachidonic acid (20:4n-6)
DGLA	dihomo-γ-linoleic acid
DHA	docosahexaenoic acid (22:6n-3)
ef-1a	elongation factor 1-alpha
EFA	essential fatty acids
elovl5	fatty acid elongase 5
EPA	eicosapentaenoic acid (20:5n-3)
FA	fatty acid
FBW	final body weight
fads6	fatty acid desaturase 6
fas	fatty acid synthase and
FCR	feed conversion ratio
FM	fish meal
FO	fish oil
FRS	Fisheries Research Station
gapdh	glyceraldehyde 3-phosphate dehydrogenase
GLA	γ-linoleic acid (GLA)
HDL	high-density lipoprotein cholesterol
HUFA	highly unsaturated fatty acid
LA	linoleic acid (18:2n-6)
LC-PUFA	total long chain polyunsaturated fatty acid
LDL	low-density lipoprotein cholesterol
MUFA	monounsaturated fatty acid
n-3 PUFA	total omega-3 polyunsaturated fatty acid
n-6 PUFA	total omega-6 polyunsaturated fatty acid
NLFA	neutral lipid fatty acid
PER	protein efficiency ratio
PLFA	polar lipid fatty acids
pparα	peroxisome proliferator-activated receptor *α*
PUFA	polyunsaturated fatty acids
rpl8	60S ribosomal protein L8
SG	specific growth rate
TAG	triacylglycerol
TC	total cholesterol
TG	total growth
ZUT	West Pomeranian University of Technology in Szczecin

residues, as well as pathogens (Van der Spiegel et al., 2013). Currently studies assessing toxicological safety of new feed ingredients are conducted. For instance, Fedorova-Dahms et al. (2011) evaluated applicability of *Schizochytrium* sp. and revealed that DHA-rich alga is safe for intended use in human consumption and feed production. In contrast to marine fish, adverse effects, linked to feeds with lower EPA and DHA, on fish performance are rarer in freshwater aquaculture, such as Nile tilapia and common carp (*Cyprinus carpio*), because these fish have active metabolic pathways that convert dietary α-linolenic acid (ALA) and linoleic acid (LA) to DHA, EPA and arachidonic acid (AA).

Common carp is one of the world's most consumed freshwater fish, cultured primarily in Europe and Asia. Globally, Cyprinids production accounts for around 40% but, in freshwater aquaculture, it is about 70% (Xu et al., 2014). Carp are reared predominantly in earthen ponds, and nutrition programmes are based on natural food supplemented with cereal grains, such as wheat, maize and barley, depending on price and local availability. The growing demand for aquaculture products, and the high phenotypic plasticity of carp, have led to intensive production models farmed in closed aquaculture systems. Such systems are based solely on artificial feed, often enriched with ingredients intended to improve the nutritional composition of products. Simply put, feeding carp with EFA-enriched feeds translate into increased FA concentrations in fillets (Csengeri et al., 2013). However, different concentrations and compositions of fat in feeds might affect fish blood biochemistry (cholesterol, triacylglycerol) (Nasir and Al-Sraji, 2013), liver histology (Poleksić et al., 2014), nutrient retention and, subsequently, compositions of their different tissues (Böhm et al., 2014) as well as gene expression in the biosynthesis of n-3 fatty acids (Ren et al., 2012). The effectiveness of FA profiling in fish might also depend on levels of lipid oxidation for internal requirements, such as energy production and storage, or membranes construction and maintenance (Sargent et al., 2002).

In the scientific literature, numerous studies report the effects of utilizing feeds containing different amounts of n-3 PUFAs on the chemical composition of fish. However, there is little information about the impact of FA from different sources (especially DHA and EPA) on the function of the organism, which – ultimately – shapes the chemical compositions and FA profiles of fillets. To determine these interactions, multidisciplinary studies are needed, considering molecular (gene activity analysis), biochemical (lipid profile in blood plasma), histological (hepatocyte morphology), and nutritional (chemical compositions and

FA profiles of fillets). Thus, the purpose of this study was to determine the influence of feeds containing different sources of fat (i.e. salmon oil, soybean and rapeseed oils and *Schizochytrium* sp. meal) on carp physiology by assessing: i) zootechnical parameters; ii) blood biochemistry (total cholesterol [TC], low-density lipoprotein cholesterol [LDL], high-density lipoprotein cholesterol [HDL], triacylglycerol [TAG] and non-HDL cholesterol); iii) expression of five lipid metabolism-related genes in liver, i.e. peroxisome proliferator-activated receptor *α* (*pparα*), peroxisomal acyl-coenzyme A oxidase 1 (*acox1*), fatty acid desaturase 6 (*fads6*), fatty acid synthase (*fas*) and fatty acid elongase 5 (*elovl5*); iv) liver histomorphology; and v) total fat contents and fatty acid compositions in carp fillets.

2. Materials and methods

2.1. Experimental diets

Four isolipidic, isonitrogenous and isoenergetic diets, three experimental (CB1, CB2, CB3) and one control (CTRL) (Table 1), were designed to evaluate the impact of lipid sources on fish physiology and fillet composition. CB1, CB2 and CB3 were enriched with selenised yeast, microalgae (*Spirulina* sp., *Chlorella* sp. and *Schizochytrium* sp.) and macroalgae (*Laminaria digitata*) meals. Soybean and rapeseed oils (1:1) were the only lipid sources in the CTRL diet while, in CB3, salmon, soybean and rapeseed oils (1.05:1:1) were used. Lipid sources in CB1 and CB2 diets were rapeseed oil and *Schizochytrium* sp., which is ca. 66% fat (Allen et al., 2019). Diets were manufactured by SPAROS Lda (Olhão, Portugal). All powder ingredients were mixed, accordingly to the target formulations, in a double-helix mixer (500L, TGC Extrusion, Roullet-Saint-Estèphe, France) and ground (less than 200 μm) in a micropulveriser hammer mill (SH1, Hosokawa-Alpine, Germany). Diets (floating pellet size 6.0 mm) were manufactured with a twin-screw extruder (BC45, Clextral, Firminy, France; screw diameter 55.5 mm). Extrusion conditions were feeder rate (77 kg/h), screw speed (247 rpm), water addition in barrel 1 (330 ml/min), temperature barrel 1 (32–34 °C) and temperature barrel 3 (111–115 °C). Extruded pellets were dried in a vibrating fluid bed dryer (DR100, TGC Extrusion, Roullet-Saint-Estèphe, France). Oils were added post-extrusion by vacuum coating (PG-10VCLAB, Dinnissen, Sevenum, Netherlands). After coating, diets were packed in bags and shipped to the West Pomeranian University of Technology in Szczecin (Poland, ZUT).

Table 1

Formulation of experimental diets for common carp trail.

Ingredients, %	CTRL	CB1	CB2	CB3
Fishmeal 60 [a]	5.000	2.500	2.500	2.500
Porcine blood meal [b]	2.000	2.000	2.000	2.000
Algae meal (*Spirulina* sp.) [c]		1.000	1.000	1.000
Algae meal (*Chlorella* sp.) [d]		1.000	1.000	1.000
Algae meal (*Schizochytrium* sp.) [e]		3.125	1.563	
Soy protein concentrate [f]	2.500	2.500	2.500	2.500
Corn gluten meal [g]	4.000	4.000	4.000	4.000
Soybean meal 44 [h]	25.000	25.000	25.000	25.000
Rapeseed meal [i]	7.000	7.000	7.000	7.000
Sunflower meal [j]	12.500	12.500	12.500	12.500
Wheat meal [k]	22.500	21.224	21.786	22.349
Wheat bran [l]	5.000	5.000	5.000	5.000
Corn meal [m]	2.500	2.500	2.500	2.500
Salmon oil [n]				2.100
Soybean oil [o]	3.000			2.000
Rapeseed oil [o]	3.000	4.100	5.100	2.000
Vitamins and minerals premix [p]	1.000	1.000	1.000	1.000
Betaine HCl [q]	0.100	0.100	0.100	0.100
Binders [r]	1.000	1.000	1.000	1.000
Macroalgae meal (*Laminaria digitata*) [s]		0.541	0.541	0.541
Antioxidant [t]	0.200	0.200	0.200	0.200
Sodium propionate [u]	0.100	0.100	0.100	0.100
Sodium phosphate [v]	2.100	2.100	2.100	2.100
Selenised yeast [w]		0.010	0.010	0.010
L-Lysine [x]	0.700	0.700	0.700	0.700
L-Tryptophan [y]	0.200	0.200	0.200	0.200
DL-Methionine [z]	0.600	0.600	0.600	0.600
Proximate composition (%)				
Crude protein	30.20	30.60	30.40	30.30
Crude fat	8.10	8.00	8.00	8.10
Crude ash	3.00	3.00	3.00	3.00
Crude fiber	5.00	5.00	5.00	5.00
Main fatty acids (%)				
$20{:}5n-3$ (EPA)	0.05	0.27	0.22	0.05
$22{:}6n-3$ (DHA)	0.09	0.62	0.38	0.12
ΣEPA + DHA	0.14	0.89	0.60	0.17

[a] CONRESA 60: 61.2% crude protein (CP), 8.4% crude fat (CF), Conserveros Reunidos S.A., Spain.

[b] Porcine blood meal: 89% CP, 1% CF, SONAC BV, The Netherlands.

[c] *Spirulina* meal: 72% CP, 1% CF, Willows Ingredients Ltd, Ireland.

[d] *Chlorella* meal: 62% CP, 9% CF, ALLMICROALGAE, Portugal.

[e] ALL-G RICH (*Schizochytrium*), Alltech Portugal.

[f] Soycomil P: 63% CP, 0.8% CF, ADM, The Netherlands.

[g] Corn gluten meal: 61% CP, 6% CF, COPAM, Portugal.

[h] Solvent extracted soybean meal: 43.8% CP, 3.3% CF, CARGILL, Spain.

[i] Defatted rapeseed meal: 32.7% CP, 4.1% CF, Ribeiro & Sousa Lda, Portugal.

[j] Defatted sunflower meal: 29.1% CP, 1.8% CF, Ribeiro & Sousa Lda, Portugal.

[k] Wheat meal: 10.2% CP, 1.2% CF, Casa Lanchinha, Portugal.

[l] Wheat bran: 14.9% CP, 4.0% CF, Cerealis Moagens S.A., Portugal.

[m] Corn meal: 8% CP, 3.7% CF, Ribeiro & Sousa Lda, Portugal.

[n] Soropêche, France.

[o] Lamotte Oils GmbH, Germany.

[p] INVIVONSA Portugal SA, Portugal: Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 500 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 7.5 mg; sodium chloride, 400 mg; excipient wheat middling's.

[q] ORFFA, The Netherlands.

[r] CELATOM FP1SL (diatomite), Angelo Coimbra S.A., Portugal.

[s] Dry *Laminaria digitata*: 5.4% CP, 0.5% CF, 3700 mg iodine/kg, Agrimer, France.

[t] VERDILOX, Kemin Europe NV, Belgium.

[u] PREMIX LDA., Portugal.

[v] Vadequimica, Spain.

[w] ALKOSEL R397: 2200 mg selenium/kg, Lallemand, France.

[x] L-Lysine HCl 99%: Ajinomoto Eurolysine SAS, France.

[y] TrypAMINO 98%, Evonik Nutrition & Care GmbH, Germany.

[z] DL-METHIONINE FOR AQUACULTURE 99%, EVONIK Nutrition & Care GmbH, Germany.

2.2. Feeding trial

Ethical approval was obtained from the ethical committee of Faculty of Food Sciences and Fisheries (ZUT in Poland). We adhered to the "Guidelines for the treatment of animals in behavioural research and teaching" published in Animal Behaviour (Anon, 2012).

The fish trial was performed at the Fisheries Research Station (FRS), Nowe Czarnowo, Poland (N: 53°120 36″ E: 14° 270 48″), in floating cages (net volume 3 m^3 each) submerged in cooling water discharged from the Dolna Odra Power Plant. One week prior to the start, 1200 carp (296 ± 10 g mean initial body weight) were selected from a pond farm located in Maliniec (NW Poland), transported to the FRS and randomly distributed (n = 100) in 12 cages for acclimatization (100 fish/cage). The 100-day trial (July–October 2018) was performed in triplicate (n = 3/diet). Feeding was by hand three times a day (at 10:00, 14:00 and 18:00 h, in equal portions) until apparent satiation (2% metabolic dose). During the experiment, the temperature of water ranged between 13.3 °C and 34.2 °C.

2.3. Measuring growth indices

In order to evaluate the dietary impact on common carp, the following growth parameters were calculated: total growth (TG), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) using the following formulas:

$$TG = \frac{\text{weight gain (g)}}{\text{initial body weight (g)}} \times 100$$

$$FCR = \frac{\text{feed consumed (g)}}{\text{weight gain (g)}}$$

$$SGR = \left[\frac{(\text{Ln final weight} - \text{Ln initial weight})}{\text{rearing days}}\right] \times 100$$

$$PER = \frac{\text{weight gain (g)}}{\text{protein consumed (g)}}$$

2.4. Samples collection

On three occasions (days 30, 60 and 100), five fish from each dietary treatment were anesthetised with 0.2 ml/l of 2-phenoxyethanol (Sigma-Aldrich, St. Louis, USA) and blood drawn from the caudal vein using a sterile 5 ml hypodermic syringe and a 23-gauge needle, transferred to 1.5 ml tube (Eppendorf, Hamburg, Germany) before being stored overnight at 4 °C to clot. Then, fish were sacrificed using a lethal dose of the 2-phenoxyethanol (2 ml/l). Liver samples (n = 5 per treatment and sampling period) were collected immediately, preserved in DNA/RNA Shield™ (Zymo Research, Irvine, USA) and stored at −80 °C until RNA extraction. Additionally, at the end of the feeding trial, liver samples (approx. 125 mm^3) were collected for histomorphological assessment; these were placed in 5 ml glass jars and covered with 10% buffered formalin solution for 5 h at room temperature.

2.5. Plasma lipid profile

After overnight clotting, blood samples were centrifuged (Centrifuge 5415 R, Eppendorf, Hamburg, Germany) at $4000 \times g$ for 10 min. Plasma was transferred to labelled Eppendorf tubes and frozen (−80 °C) until analysis. Biochemical analyses of the lipid profile indices in plasma were conducted at SPSK- 2 hospital laboratory (Pomeranian

Medical University, Szczecin, Poland). Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triacylglycerol (TAG) and non-HDL cholesterol were assessed using dedicated reagent sets for chemiluminescence (CMIA) on an ARCHITECT c8000 clinical chemistry analyser (Abbott Diagnostics, Lake Forest, USA).

2.6. Gene expression analysis

At the Department of Aquatic Bioengineering and Aquaculture (ZUT in Szczecin), samples were homogenized in 750 µl Tri Reagent® (Zymo Research, Irvine, USA) for 60 s with a Minilys ® personal homogenizer (Bertin Corp., Rockville, USA). Total RNA was extracted using Direct-zol™ RNA MiniPrep kit (Zymo Research, Irvine, USA), with 15 min DNase I treatment according to manufacturer instructions. Quantity and quality of RNA was assessed using NanoDrop 2000 (TermoFisher Scientific, Waltham, USA), all samples 260/280 ratio varied around 2.0. Reverse transcription was performed immediately after RNA extraction using a Reverse Transcription System kit (Promega, Walldorf, Germany) according to the manufacturer's instructions. Real-time PCR was performed on a QuantStudio™3 (TermoFisher Scientific, Waltham, USA) using PowerUp™ SYBR™ Green Master Mix 2x (TermoFisher Scientific, USA), 0.3 µM of each primer and 1 µl of cDNA templates in a final volume of 10 µl. The reaction was conducted using 2 min of activation at 50 °C, 2 min at 95 °C, 40 cycles of denaturation at 95 °C for 1 s and annealing/extending at 60 °C for 30 s. Melting curve analysis (60–95 °C) was conducted at the end of each PCR thermal profile and negative samples for each primers set were run to ensure the specificity of amplification. The stability of six reference genes [i.e. 18S ribosomal RNA (*18SrRNA*), 40S ribosomal protein S11 (40sRNA), 60S ribosomal protein L8 (*rpl8*), β-actin (*actb*), glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) and elongation factor 1-alpha (*ef-1a*)] was evaluated using geNorm and NormFinder algorithms (Table 2). Activities of five lipid metabolism-related genes [i.e. peroxisome proliferator-activated receptor α (*ppara*), peroxisomal acyl-coenzyme A oxidase 1 (*acox1*), fatty acid desaturase 6 (*fads6*), fatty acid synthase (*fas*) and fatty acid elongase 5 (*elovl5*)] against two reference genes (40sRNA and *rpl8*) were assessed. Relative gene expression was calculated using GeneEx (MultiD Analyzes, Sweden) software and the results normalised against expression in livers sampled at the beginning of the trial.

2.7. Liver histomorphology

Livers fragments were fixed in buffered formalin and samples dehydrated through alcohol and saturated in intermediate solutions (benzene, benzene: paraffin) before being embedded in paraffin blocks, trimmed, sectioned (4 ± 1 µm, Rotary Microtome MPS-2, Opta-Tech, Poland), stained with haematoxylin and eosin, and mounted on slides with DPX balsam (Burck, 1975). Twelve glass slides (3 fish × 4 slides) for each diet were randomly selected and examined by two members of the laboratory using an Eclipse E600 microscope (Nikon, Nikon, Japan) with 100x objective and, using NIS-Elements Basic Research software (Nikon Instruments Europe B.V, Japan), screened for hepatocyte (C) and hepatocyte nucleus (N) areas (300 measurements/parameter). Additionally, the nuclear:cytoplasm areas ratio (N/C) of hepatocytes were calculated, and the significance of observed differences was assessed for each parameter.

2.8. Fillets fatty acid composition

Samples of muscle tissue from five fish per diet were prepared as pooled homogenates (n = 5 per treatment) and all following analyses were run in triplicates. Crude lipid content was determined gravimetrically after Soxhlet lipid extraction using a Tecator Soxtec System HT

Table 2
Sequences of qPCR primers for the detection of reference and lipid metabolism related genes in common carp by real-time PCR.

Gene symbol	Sequence 5' –> 3'	Tm	Function	References
18SrRNA [a]	CCTGTCGCCGCTGAATACC	55.4 °C	ribosomal RNA gene	Huang et al. (2015)
	TCGCTTTCGTCCGTCTTGC	53.2 °C		
40sRNA [b]	CCGTGGGTGACATCGTTACA	53.8 °C	ribosomal RNA gene	Gonzalez et al. (2007)
	TCAGGACATTGAACCTCACTGTCT	55.7 °C		
rpl8 [c]	CTCCGTCTTCAAAGCCCATGT	54.4 °C	ribosomal protein coding	Bickley et al. (2009)
	TCCTTCACGATCCCCTTGATG	54.4 °C		
actb [d]	ATCCGTAAAGACCTGTATGCCA	53.0 °C	cell stability	Tang et al. (2012)
	GGGGAGCAATGATCTTGATCTTCA	55.7 °C		
gapdh [e]	AGCTCAATGGCAAGCTTACTGG	54.8 °C	cellular homeostasis	
	GTGGATACCACCTGGTCCTCTG	58.6 °C		
ef-1a [f]	GTCAAGTCCGTTGAGATGCACC	56.7 °C	protein biosynthesis	
	GGATGATGACCTGAGCATTGAAGC	57.4 °C		
ppara [g]	GGGAAAGAGCAGCACGAG	52.6 °C	lipid metabolism	Corcoran et al. (2015)
	GCGTGCTTTGGCTTTGTT	48.0 °C		
acox1 [h]	ACAGCACAGCAAGAGCAATG	51.8 °C	peroxisomal β oxidation	
	ACAGAGTGGACAGCCGTATC	53.8 °C		
fads6-a [i]	ATCGGACACCTGAAGGGAGCG	58.3 °C	fatty acid desaturation	Ren et al. (2012)
	CATGTTGAGCATGTTGACATCCG	55.3 °C		
elovl5-a [j]	GTCCTGACCATGTTCCAGACATCTTG	59.5 °C	elongation of very long-chain fatty acids	
	CTGTAAGCGGACGAGGTGTCGTC	60.3 °C		
fas [k]	TGCTGGATGCTTTGTTTGAG	49.7 °C	fatty acid synthesis	Designed based on sequence KY378913.1
	ACTACACCACCAGCGATTCC	53.8 °C		

[a] 18S ribosomal RNA.
[b] 40S ribosomal protein S11.
[c] Ribosomal protein L8.
[d] Beta actin.
[e] Glyceraldehyde 3-phosphate dehydrogenase.
[f] Eukaryotic translation elongation factor 1 alpha.
[g] Peroxisome proliferator activated receptor alpha.
[h] Acyl-CoA oxidase 1.
[i] Fatty Acid Desaturase 6.
[j] Elongation of very long chain fatty acids protein 5.
[k] Fatty acid synthase.

1043 (FOSS Analytical Co., Ltd., Hillerød, Denmark). Fatty acid profile was assessed by Polcargo (Szczecin), using gas chromatography in accordance with PN-EN ISO 12966–1:2015–01. Based on the profiles obtained, specific indicators, such as total saturated fatty acid (SFA), total monounsaturated fatty acid (MUFA), total polyunsaturated fatty acid (PUFA), total long chain polyunsaturated fatty acid (LC-PUFA), total omega-3 polyunsaturated fatty acid (n-3 PUFA), total omega-6 polyunsaturated fatty acid (n-6 PUFA) and ratio between n-3 and n-6, were calculated.

2.9. Statistical analysis

All data are shown as mean ± standard deviation unless otherwise specified. All statistical analyses were performed using Statistica 13 (StatSoft, Inc., Krakow, Poland). Normal distribution of data was assessed with Shapiro-Wilk test (Significance level $p < 0.05$). Differences between parameters were determined using the Kruskal-Wallis test followed by Tukey HSD post-hoc test. Additionally, two-way ANOVA test was applied to assess influence of blends on blood biochemistry parameters. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Growth indices

At the beginning of experiment no significant differences were found for initial body weight. At the end of the 100-day feeding trial period, there were significant differences in weights between CTRL and CB1-3 treatments (Table 3), i.e. later groups had significantly higher final body weight (FBW). No differences were found in growth response (TG or SGR) and feed utilization indices (FCR or PER), and no differences in mortality or disease were noted within or between groups.

3.2. Blood biochemistry

Two-way ANOVA showed that total and non-HDL cholesterol were affected by diet, sampling time, and there was an interaction between these factors (Table 4). HDL was not affected by any factor, while LDL was affected strongly by sampling time. TAG concentrations were time and interaction between time and diet dependent. Non-HDL concentrations associated with CB1-3 were significantly different ($p \leq 0.05$) from CTRL at the end of trial, but not one another. However, CB1-2 TC was significantly different ($p \leq 0.05$) from CRTL after 60 days and remained so at 100 days. All parameters in plasma from carp fed CB1-3 increased during the trial; the opposite was observed in CTRL fish for TC, HDL, LDL and non-HDL, but TAG increased. The increase in TAG was less in fish receiving the experimental diets (CB1-3, increase

41–72%) compared with CTRL fish (183%, $p \leq 0.05$). In fish receiving CB3, changes in TC and non-HDL were the greatest, while TAG, HDL and LDL were the lowest overall throughout the experiment.

3.3. Gene expression

Among six reference genes examined, expressions of *rpl8* and 40sRNA were the lowest and most stable across all samples and dietary treatments. Therefore, both genes were used as internal controls subsequently (Fig. 1). The expression of genes related to lipid metabolism pathways in the liver of common carp are shown in Fig. 2. Dietary levels of EPA and DHA affected significantly the expression of *fads6*, *fas*, *elovl5-a*, *pparα* and *acox1* during the 100-day feeding trial. Liver expression of *fas*, *elovl5-a* and *pparα* increased significantly in carp fed diets containing more EPA and DHA (i.e. CB1, CB2) with an opposite (i.e. down-regulation with increasing EPA and DHA) observed for *acox1*. Transcripts of *fads6-a* in the liver of carp fed diets containing more EPA and DHA were stable, while decreased in CTRL and CB3-fed fish.

3.4. Liver histomorphology

No pathological (disease-related) indicators were found in any samples. However, there were significant differences ($p \leq 0.05$) in hepatocyte morphologies amongst the treatments (Table 5). More specifically, fish fed CB1 had the smallest hepatocytes and nuclei, while the largest were noticed in livers from fish fed CB2. N/C ratios were largest for CB2 (0.204 ± 0.004) and smallest for CB3 (0.164 ± 0.004).

3.5. Fatty acid profile

Significant differences ($p \leq 0.05$) in total lipid content of fillets were found amongst the dietary treatments (Table 6). Total n-3 PUFA, total n-3 LC PUFA, total PUFA, ALA and EPA plus DHA were significantly different in fillets from fish fed CB1-3 ($p \leq 0.05$) compared with CTRL fillets. The highest ($p \leq 0.05$) concentrations of EPA and DHA, n-3/n-6 ratio (0.38 ± 0.01) were in fillets from fish fed CB1. Simultaneously, significantly ($p \leq 0.05$) lower concentrations of palmitic acid (C16:0), total MUFA, oleic acid (C18:1n9c), γ-linoleic acid (GLA), dihomo-γ-linoleic acid (DGLA) and arachidonic acid (ARA) were noted in fillets from all experimental groups (i.e. fish fed CB1-3).

4. Discussion

Demand for essential omega-3 long chain polyunsaturated fatty acids has led to utilization of various plant and animal ingredients in aquafeed production (Tocher et al., 2019). In the present study, we demonstrated a range of impacts on common carp physiology

Table 3

Growth and feed utilization indices of common carp fed diet containing different sources of fat.

	CTRL	CB1	CB2	CB3
IBW[a] (g)	300.67 ± 29.01	294.67 ± 15.37	294.67 ± 14.57	291.67 ± 20.26
FBW[b] (g)	1085.22 ± 16.42[a]	1192.64 ± 75.59[b]	1188.73 ± 49.94[b]	1217.55 ± 36.18[b]
TG[c] (%)	263.14 ± 35.05	305.81 ± 38.40	303.63 ± 12.09	319.28 ± 40.11
FCR[d]	1.52 ± 0.12	1.45 ± 0.11	1.45 ± 0.09	1.39 ± 0.11
SGR[e] (%/d)	1.29 ± 0.10	1.40 ± 0.10	1.40 ± 0.03	1.43 ± 0.10
PER[f] (g/g)	2.18 ± 0.06	2.29 ± 0.06	2.28 ± 0.14	2.38 ± 0.03

Explanations: Results represent mean ± standard deviation (n = 3). Values with different superscripts in rows indicate significant differences ($p \leq 0.05$).
[a] Initial body weight.
[b] Final body weight.
[c] Total growth.
[d] Feed conversion ratio.
[e] Specific growth rate.
[f] Protein efficiency ratio.

Table 4
Dietary influence on blood biochemical parameters of common carp.

		Total cholesterol (mg/dl)	Cholesterol HDL (mg/dl)	Cholesterol LDL (mg/dl)	Triacylglycerol (mg/dl)	Cholesterol non-HDL (mg/dl)
CTRL	30 d	144.75 ± 5.85[A a]	7.40 ± 1.34[A a]	8.60 ± 1.52[A a]	262.60 ± 39.90[A a]	142.40 ± 12.74[A a]
	60 d	137.67 ± 9.87[A a]	6.75 ± 0.96[A a]	6.20 ± 2.95[A ab]	379.25 ± 30.58[A b]	126.40 ± 9.40[A a]
	100 d	135.67 ± 27.54[A a]	5.75 ± 1.26[A a]	6.00 ± 1.00[A b]	444.75 ± 27.24[A c]	114.50 ± 19.09[A a]
CB1	30 d	173.80 ± 26.99[A a]	6.50 ± 1.29[A a]	9.67 ± 2.52[A a]	239.80 ± 29.10[A a]	167.00 ± 26.63[A a]
	60 d	189.00 ± 1.41[B a]	6.50 ± 0.71[A a]	10.00 ± 1.41[A a]	398.00 ± 45.25[A b]	183.00 ± 1.41[B a]
	100 d	212.20 ± 28.33[B a]	7.20 ± 2.17[A a]	15.80 ± 7.26[B a]	412.60 ± 76.81[A b]	205.00 ± 27.47[B a]
CB2	30 d	157.60 ± 13.43[A a]	6.67 ± 2.08[A a]	9.75 ± 1.71[A a]	218.50 ± 47.85[A a]	149.40 ± 14.45[A a]
	60 d	171.33 ± 19.55[B ab]	7.67 ± 0.58[A a]	10.33 ± 2.08[A a]	358.00 ± 82.31[A b]	163.67 ± 19.14[AB ab]
	100 d	201.60 ± 21.81[B b]	7.80 ± 1.79[A a]	17.60 ± 5.94[B a]	369.33 ± 53.61[A b]	193.80 ± 21.72[B b]
CB3	30 d	141.67 ± 26.71[A a]	7.33 ± 0.58[A a]	9.00 ± 2.07[A a]	252.60 ± 37.69[A a]	134.33 ± 16.65[A a]
	60 d	157.00 ± 7.07[AB ab]	7.50 ± 1.91[A a]	9.40 ± 5.03[A a]	330.50 ± 62.34[Aab]	141.00 ± 18.03[A ab]
	100 d	194.60 ± 28.26[AB b]	7.80 ± 1.30[A a]	17.40 ± 6.35[B a]	357.00 ± 60.32[A b]	187.00 ± 28.38[B b]
Significance	Diet	**	n.s.	n.s.	n.s.	**
	Time	**	n.s.	**	**	**
	DxT	**	n.s.	n.s.	*	**

Explanations: Results represent mean ± standard deviation (n = 5). Values with different superscripts in columns indicate significant differences (p ≤ 0.05). Uppercase superscripts indicate differences between diets, lowercase superscripts indicate differences between sampling points for each diet. * – p ≤ 0.05; ** – p ≤ 0.01; n.s. – differences not significant; D – diet; T – time (sampling point).
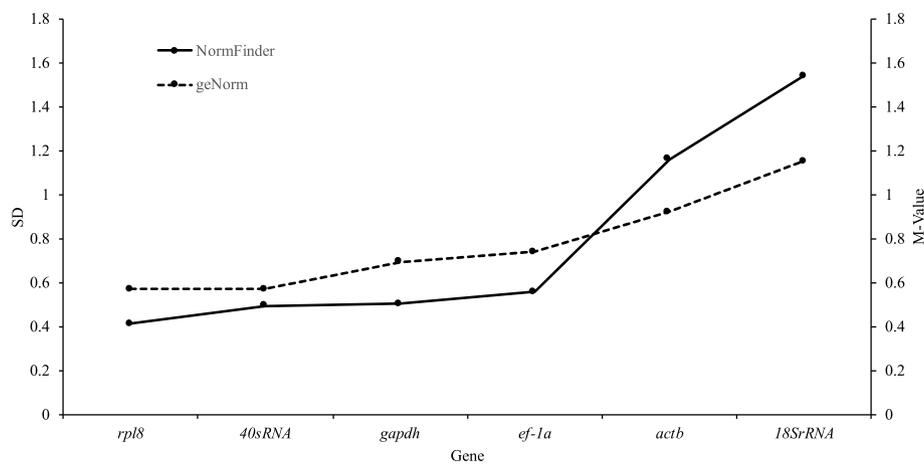

Fig. 1. Stability assessment of six reference genes across all liver samples of common carp under different dietary treatments, using two different algorithms: NormFinder (SD) and geNorm (M-Value) provided in GeneEx software.

associated with feed blends containing various sustainable sources of fat. Zootechnical parameters revealed that carp fed CB1-3 were heavier (FBW) compared with CTRL fish. This difference in FBW was presumably the result of including DHA-enrich algae and salmon oil in test diets, as formulated diets were isoenergetic. Comparable results have been found for rainbow trout (*Oncorhynchus mykiss*), salmon (*Salmo salar*) and whiteleg shrimp (*Litopenaeus vannamei*), the growth of which were higher when fed DHA-rich diets (Bell et al., 2001, 2003, Araújo et al., 2019). However, the differences in FBW for CB1-3 might also arise from addition of selenized yeast in the blends, since organic selenium is more bioavailable than the inorganic form and might affect positively fish health and performance (Berntssen et al., 2018).

Biochemical parameters revealed significantly higher TC concentrations in plasma from fish fed CB1 (containing 3.125% *Schizochytrium* sp.), which contained the most EPA and DHA. Our results are consistent with those obtained for European seabass (*Dicentrarchus labrax*); TC was elevated in fish fed more FAs (Richard et al., 2006a). Some studies have suggested there were no effects of EPA/DHA on Atlantic salmon TC (Jin et al., 2017) whilst others demonstrated TC decreased in blunt snout bream (*Megalobrama amblycephala*, Lu et al., 2013) and rainbow trout (Richard et al., 2006b) fed diets with higher concentrations of n-3 LC-PUFA. In our study, high plasma TC could have influenced CB1-3 FBW. In some fish species, such as rainbow trout and Japanese flounder (*Paralichthys olivaceus*), higher TC improved significantly feed intakes and weight gain (Twibell and

Wilson, 2004; Deng et al., 2010). However, in most aquaculture species, TC depends on feed compositions including the source of dietary protein (Romarheim et al., 2006). In most cases, increased TC (likewise TAG) reflects increased internal lipid transport (Du et al., 2005). During the experiment, we also observed increased HDL, LDL and non-HDL in fish receiving CB1-3 as well as a decrease in these indices in the CTRL fish. Deng et al. (2010) observed changes in blood lipoprotein concentrations (HDL, LDL, VLDL, IDL, intermediate density lipoproteins), revealing that fish protein-based diets increased lipoprotein, while plant protein-based diets decreased them. These responses and interactions are specific to species, dietary treatments, and sex (Deng et al., 2010). For carp in particular, this is the first report demonstrating that non-HDL concentrations depend on feed compositions. Therefore, further studies are needed to identify the sources of variation and the optimal diet for fillet composition.

Blood TAG concentrations showed no significant differences amongst dietary treatments, which might be attributed to the isolipidic (8%) feed blends. TAG in blood or liver is a derivative of fat in feeds (Lu et al., 2013). Feeding blunt snout bream with control (5% fat) and high-fat (15% fat) diets showed that hepatic TAG was higher in fish fed the high-fat diet. In contrast, plasma TAG was higher in the control group (5%), suggesting lipid transport from the liver is impacted by high-fat diets (Lu et al., 2013). TAG metabolism also depends on qualitative fat composition in the feed. Results from nutritional trials showed positive (Lemaire et al., 1991; Kjær et al., 2008) and negative (Jordal et al.,
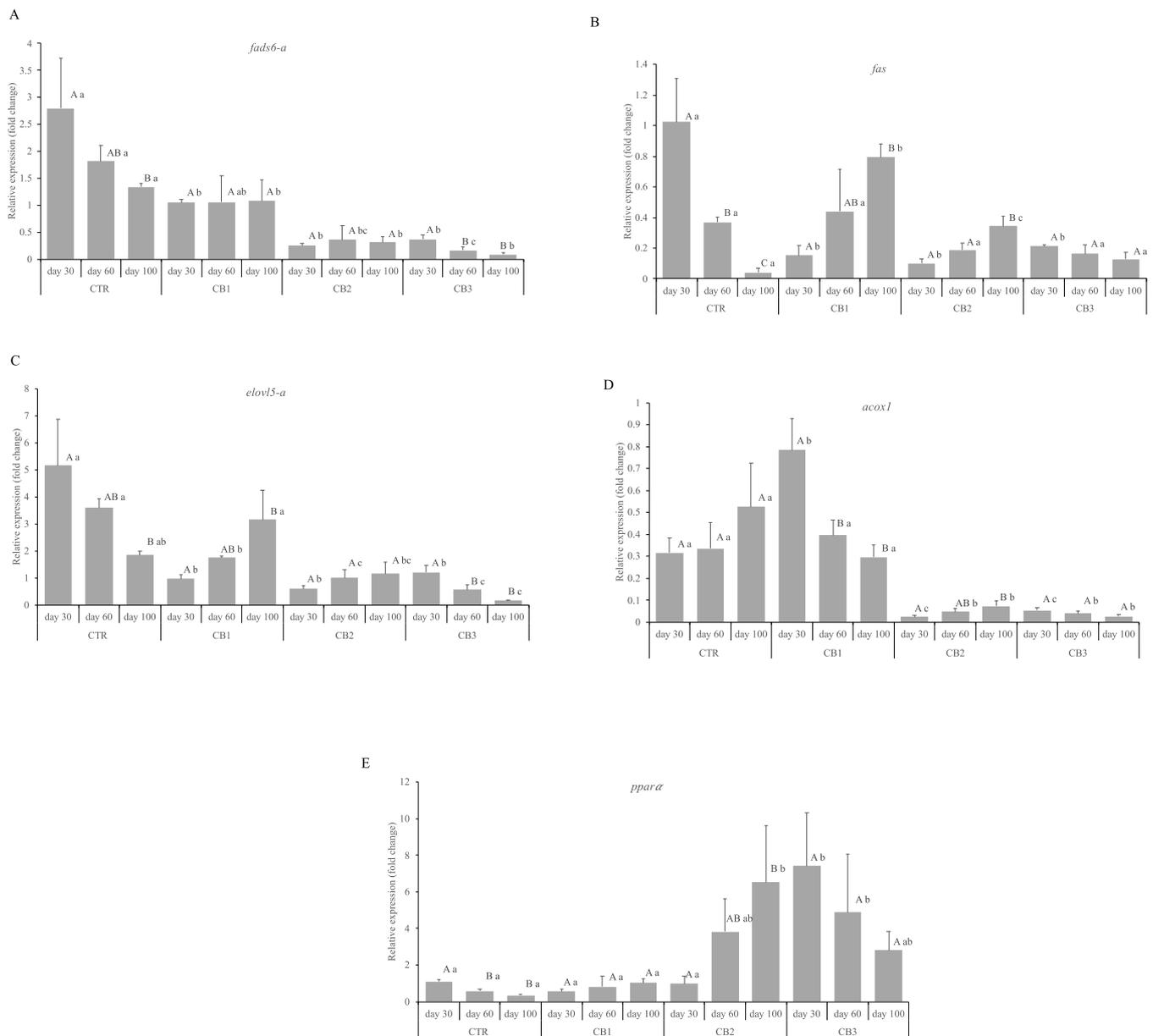
Fig. 2. Relative expression of 5 lipid metabolism related genes (A - fatty acid desaturase 6; B - fatty acid synthase; C - fatty acid elongase 5; D - peroxisomal acyl-coenzyme A oxidase 1; E − peroxisome proliferator-activated receptor α) in liver of common carp under different dietary treatments at 3 sampling points. Values are mean ± SD (n = 5). Different superscripts indicate significant difference (p ≤ 0.05), small superscripts between sampling points in diet, and large superscripts between different diets at each sampling point.

2007; Leaver et al., 2008) correlations between increased n-3 PUFA feed content and reduced plasma TAG in European seabass and Atlantic salmon. In our study, plasma TAG was similar across all dietary treatments at all time points, despite differences in feed compositions. Similar results were observed in Atlantic salmon fed with a diet where FO was replaced by plant oils with the same fish meal (FM) contents

(Jordal et al., 2007). During our trial, plasma TAG increased, probably in relation to increased secretion of TAG-rich VLDL (very low-density lipoprotein) particles from the liver in the post-absorptive phase, which is regulated by dietary FA availability and composition (Torstensen et al., 2011). The greatest increase (183%) was in CTRL fish, which received the diet with the most plant ingredients. Torstensen et al.

Table 5
Liver histological parameters of common carp fed with different sources of fat.

	CTRL	CB1	CB2	CB3
Hepatocyte area (µm^2)	123.37 ± 7.10[ab]	93.19 ± 30.21[a]	154.82 ± 13.02[b]	120.26 ± 8.27[ab]
Hepatocyte nucleus area (µm^2)	20.68 ± 1.28[a]	17.08 ± 4.73[a]	31.00 ± 2.43[b]	18.81 ± 1.53[a]
N/C[1]	0.175 ± 0.007[a]	0.197 ± 0.013[b]	0.204 ± 0.004[b]	0.164 ± 0.004[a]

Explanations: Results represent mean ± standard deviation (n = 3). Values with different superscripts in rows indicate significant differences (p ≤ 0.05). [1] Hepatocyte nucleus area/hepatocyte area.

Table 6
Muscle fatty acid composition (% of total fatty acids) of common carp fed blends with different sources of fat.

	CTRL	CB1	CB2	CB3
Crude fat (%)	2.98 ± 0.36^a	3.68 ± 0.52^{ab}	3.07 ± 0.13^a	4.51 ± 0.16^b
C16:0 (PA)	16.19 ± 0.07^d	14.90 ± 0.03^b	14.82 ± 0.06^a	15.23 ± 0.08^c
C18:0 (SA)	3.70 ± 0.01^b	3.45 ± 0.03^a	3.67 ± 0.04^b	3.70 ± 0.01^b
Total SFA [a]	24.55 ± 1.79	24.34 ± 2.14	24.22 ± 2.08	24.07 ± 1.97
C18:1n9c (OA)	43.58 ± 0.02^d	40.31 ± 0.23^b	38.96 ± 0.86^a	42.95 ± 0.07^c
Total MUFA [b]	53.95 ± 0.02^c	51.98 ± 0.21^b	50.17 ± 0.84^a	52.68 ± 0.05^b
C18:3n3 (ALA)	1.55 ± 0.02^a	2.18 ± 0.01^c	2.05 ± 0.02^b	2.08 ± 0.01^b
C20:5n3 (EPA)	0.17 ± 0.01^a	0.84 ± 0.01^c	0.81 ± 0.03^c	0.50 ± 0.00^b
C22:6n3 (DHA)	0.91 ± 0.02^a	3.33 ± 0.07^d	2.97 ± 0.19^c	1.80 ± 0.03^b
Total n-3 PUFA [c]	2.68 ± 0.02^a	6.48 ± 0.07^d	5.96 ± 0.24^c	4.49 ± 0.03^b
Total n-3 LC PUFA [d]	1.12 ± 0.01^a	4.27 ± 0.07^d	3.88 ± 0.21^c	2.38 ± 0.03^b
C18:2n6c (LA)	15.47 ± 0.04^a	15.26 ± 0.09^a	16.97 ± 0.22^c	16.49 ± 0.03^b
C18:3n6 (GLA)	0.69 ± 0.00^d	0.31 ± 0.01^a	0.39 ± 0.01^b	0.48 ± 0.00^c
C20:3n6 (DGLA)	0.73 ± 0.00^c	0.54 ± 0.03^a	0.66 ± 0.04^b	0.62 ± 0.01^b
C20:4n6 (AA)	1.56 ± 0.02^c	0.81 ± 0.03^a	1.08 ± 0.10^b	0.98 ± 0.01^b
Total n-6 PUFA [e]	18.45 ± 0.03^b	16.90 ± 0.14^a	19.10 ± 0.36^c	18.57 ± 0.02^b
Total PUFA [f]	22.10 ± 0.04^a	24.09 ± 0.22^b	25.90 ± 0.63^c	23.81 ± 0.01^b
EPA + DHA	1.08 ± 0.02^a	4.17 ± 0.07^d	3.78 ± 0.22^c	2.30 ± 0.03^b
n-3/n-6	0.15 ± 0.01^a	0.38 ± 0.01^d	0.31 ± 0.01^c	0.24 ± 0.01^b

Explanations: Results represent mean $\pm$ standard deviation (n = 3). Values with different superscripts in rows indicate significant differences (p $\leq$ 0.05). Some fatty acids in trace amount such as 12:0, 14:0, 16:0, 17:0, 20:0, 22:0, 24:0, 16:1, 18:1n9t, 20:1n $-$ 9, 22:1n $-$ 9, 22:1n-11, 16:2n $-$ 6, 18:4n $-$ 3, 20:3n $-$ 3 and 20:4n $-$ 3 were not listed.

[a] Saturated fatty acids.
[b] Monounsaturated fatty acids.
[c] Omega-3 polyunsaturated fatty acids.
[d] Omega-3 long-chain (C_{20-24}) polyunsaturated fatty acids.
[e] Omega-6 polyunsaturated fatty acids.
[f] Polyunsaturated fatty acids.

(2011) found significantly and consistently increased plasma TAG in fish fed a diet containing high-plant protein and vegetable oils, demonstrating an interaction between dietary lipids and proteins with lipid metabolic consequences. Increased TAG concentrations were also associated with lower water temperatures (Torstensen et al., 2011), in line with the decrease observed in our study (28.9 °C - 23rd July to 14.6 °C - 30th October). Data for the effects of dietary fat intakes and quality on serum indices were inconclusive, meaning further studies are required to better understand the regulation of indices related to lipid metabolism in common carp.

As a freshwater aquaculture species, carp can synthesise HUFA, and nutritional trials with *Cyprinus carpio* var. Jian showed the efficiency of HUFA biosynthesis depended mainly on the quality and quantity of FAs in feeds (Ren et al., 2012). Ren et al. (2012) also demonstrated greater expression of genes involved in HUFA biosynthesis, specifically *fads6-a* and *elovl5-a*, when the fish were fed LA and ALA-fortified diets compared to EPA/DHA rich diet. In our experiment, *fads6-a* expression in fish fed CB1 and CB2 (highest EPA/DHA concentrations) did not differ, which confirms a mechanism inhibiting *fads6-a* activity by dietary HUFA, as described by Tocher (2003) and Zheng et al. (2005). Moreover, constant (CB1 and CB2) or decreasing (CTRL and CB3) *fads6-a* activities were observed during our 100-day trial that might be associated with lower 18:3n-3/18:2n-6 ratios or excess LA or ALA (Izquierdo et al., 2008; Li et al., 2008). It is well known that the *fads6* enzyme in fish has greater affinity with n-3 than n-6 FAs. However, substrates can also compete specifically with one another (Vagner and Santigosa, 2011).

Mechanisms behind *fads6-a* activity regulation require further studies, however, could explain greater expression observed for CTRL and CB1 fed fish in comparison to those receiving CB2 and CB3. Interestingly, expression of *elovl5-a*, except in CB1 fish, was similar to *fads6-a*. Both genes are involved in HUFA biosynthesis, and increased *elovl5-a* expression in CB1 fish is most likely linked to increased proportions of ALA, EPA and DHA in the feed. As shown for Atlantic salmon (Miller et al., 2008) and common carp var. Jian. (Ren et al., 2012), HUFA most likely reduced *elovl5-a* activity through reduced

promoter activity (Zheng et al., 2009). Ren et al. (2012) showed that expression of *elovl5-a* and *elovl5-b* were consistent, which is contrary to our findings, which suggest increased *elovl5-a* activity in relation to CB1-3 feed compositions.

In CTRL and CB3 fish, FM and salmon oil HUFA down-regulated *elovl5-a*. In turn, HUFA, derived from the thraustochytrid microalga *Schizochytrium* sp. (CB1 and CB2), together with other algae meal components, stimulated expression of this gene. Differences in *fads6-a* and *elovl5-a* activities might result from the fact that many *elovl5* elongase genes in fish species have been reported to prefer C_{18} and C_{20} PUFAs as substrates, with residual conversion toward C_{22} substrates (Monroig et al., 2012; Ren et al., 2012). Thus, reduced efficiency of elongation, 22:5n-3 to 24:5n-3, which is the substrate for Δ6D, would stabilize hepatic *fads6-a* expression in CB1 carp, as demonstrated in this study. Comparisons of *elovl5-a* and *fas* activities also support these dietary effects on fat metabolism, as described above.

The activity of *fas* gene increased in CB1 and CB2 fed fish (highest EPA/DHA) and increased feed FAs triggered a dose-dependent increase in hepatic *fas* mRNA. However, results for Atlantic salmon (Morais et al., 2011) and mammals (Davidson, 2006) indicated an inhibitory effect of HUFA on hepatic *fas* activity, leading to hypotriglyceridemia resulting from lipogenesis and enhanced fatty acid oxidation. In our study, *fas* expression was dependent on HUFA concentrations, as shown in large yellow croaker (*Larmichthys crocea*), where FM-enriched feed was associated with increased *fas* activity (Qiu et al., 2017). In contrast, Zhu (2005) found that expression of *fas* in grass carp (*Ctenopharyngodon idella*) muscle and liver was inhibited significantly by lard, and soybean and fish oils in the diet. Levels of *fas* activity also correlate with plasma TAG concentrations (Semenkovich, 1997), because encoded FAS enzyme catalyses synthesis of long chain FAs, mainly through acetyl coenzyme A and malonyl coenzyme A. We observed a link between *fas* activity and TAG in relation to the CB1 diet, which contained the most EPA/DHA. Analyses also revealed differences in *fas* expression were quality dependent, as the diets were isolipidic (8% in all variants).

Expression of hepatic *ppar-α* increased over time in CB1 and CB2 fed carp. Lu et al. (2014) observed that feeding blunt snout bream with a

high-fat diet decreased *ppar-α* expression and inhibited fat β-oxidation and steatosis. Higher hepatic *ppar-α* activities in CB1 and CB2 fed fish could be due to increased EPA and DHA concentrations in the feeds and/or additional SFAs and MUFAs, which are utilized preferentially during the β-oxidation (Kjær et al., 2008; Lu et al., 2014). Moreover, EPA induces mitochondrial proliferation and reduces intracellular lipid (Kjær et al., 2008). Hepatic *ppar-α* activity decreased in CTRL and CB3 fish, which might be explained by the increased content of plant-derived components (Ye et al., 2019) that down-regulated lipid metabolism-related gene expression, including carnitine palmitoyltransferase 1 (*cpt1*), apolipoprotein AI (*apo-aI*) and lipoprotein lipase (*lpl*). Further research is required to assess the mechanisms underlying *ppar-α* expression amongst the dietary groups in this study.

Activity of *acox1*, which determines β-oxidation capacity, was also dependent on qualitative fat compositions in the feeds. However, the dynamics of this gene's activities were different from those of *ppar-α* across all diets. Decreased *acox1* expression was noted in CB1 fish (the highest EPA and DHA content), while in CTRL fish (the lowest EPA and DHA content) *acox1* activity did not change. Previous studies have shown that feeds with higher proportions of n-3 FAs increased *acox1* activity in rainbow trout (Figueiredo-Silva et al., 2012) but had no effect on Atlantic salmon (Caballero-Solares et al., 2018). Literature for lipid metabolism in common carp is sparse and, thus, further studies are needed to determine the mechanisms of action.

Dietary fat concentrations, as well as composition of the lipids, in feeds influence ectopic lipid accumulation in farmed fish tissues (liver, abdominal adipose tissue), which is often reflected in hepatocyte histomorphology (Caballero et al., 1999; Yan et al., 2015). Isolipidic feeds used in the experiment were not associated with any pathological (disease-related) changes in hepatocytes structure, suggesting the amounts of dietary lipid or energy provided did not exceed the capacity of hepatocytes to oxidise FAs. Differences in hepatocyte and/or nucleus size observed in our study concerned carp fed elevated amounts of EFA (CB1-2). However, a more informative indicator, elaborating on the dynamics of changes occurring in hepatocytes, was the N/C ratio (i.e. hepatocyte nucleus area to hepatocyte area). The highest ratio was found for CB1 and CB2 fed fish, indicating intensive metabolism, as confirmed by increased expression of *fas* and *elovl5-a*. Ye et al. (2019) showed that lower activities of genes involved in hepatic fat metabolism resulted in higher occurrences of hepatocyte vacuolisation and nuclear atrophy, disappearance or reduction, which are signs of nuclear pycnosis. Ostaszewska et al. (2005) showed the highest metabolic activity of rainbow trout hepatocytes in fish fed the control diet without extracted soybean meal or soybean protein concentrate.

Ultimately, the use of CB1 and CB2 feeds was reflected in carp fillet FA profiles. A similar relationship, between feed and fillet composition, has been shown for zebrafish (*Danio rerio*) muscle, where DHA increased as a result of a feed containing 50% of commercial ingredients and 50% freeze-dried marine microalga *Schizochytrium* sp. (Byreddy et al., 2019). Schultz et al. (2018) showed that common carp can almost triple n-3 PUFA contents after consuming a finishing diet containing FO for only 30 days. Schultz et al. (2018) demonstrated that FA profiles in finishing feeds were the most accurate predictors of dorsal fillet composition, specifically total lipid content, regardless of the source (e.g. natural pond zooplankton, terrestrial or marine), although carp fillets contained more lipids when supplied with marine feeds. In our study, the highest fat content (4.51%) was observed in in fillets from CB3 fed fish, the only diet containing oil extracted from wild salmon by-products. Ahlgren et al. (1994) showed that SFA and MUFA (but not PUFA) concentrations were correlated with muscle fat contents, mainly because increasing total lipids in fish muscle is associated with storage lipids (TAG) rather than structural lipids (e.g. phospholipids). Moreover, amounts of phospholipids are influenced primarily by taxa-specific cell membrane requirements (Gause and Trushenski, 2013; Böhm et al., 2014) and farming conditions, e.g. temperature (Vagner and Santigosa, 2011, and references therein). Therefore, results described

by Steffens and Wirth (2007) are potentially interesting, as they noted higher n-3 and n-6 FA concentrations in fillets from carp reared in extensive systems and fed natural feeds.

5. Conclusion

The present study demonstrates that different sources of dietary lipids can influence zootechnical parameters, regulate metabolic pathways at biochemical and transcriptional levels, and affect liver histology and muscle FA profiles in common carp. Considering the information available currently, in the near future, carp could contribute more as a source of n-3 PUFA in human nutrition. Carp has huge potential for sustainable aquaculture and, as identified in our study, the high phenotypic plasticity of carp can be exploited for fillets with beneficial nutrient compositions without impacting FBW. Understanding how dietary FA supplies are absorbed, retained and metabolised in common carp, and how FA profiles can be manipulated efficiently, requires further work but could unlock the full potential of an important fish farmed worldwide.

CRediT authorship contribution statement

P. Eljasik: Writing - review & editing, Investigation, Visualization. **R. Panicz:** Conceptualization, Writing - review & editing, Investigation. **M. Sobczak:** Writing - review & editing, Supervision, Formal analysis. **J. Sadowski:** Resources. **V. Barbosa:** Writing - review & editing. **A. Marques:** Writing - review & editing. **J. Dias:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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