Dietary Phospholipid-Supplementation Affects Blood Metrics, Reproductive Indices, and Biochemical Parameters of Female Shark Catfish, *Pangasius nasutus*

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ABSTRACT

Dietary phospholipids are known to enhance fish reproductive performance, but their effects on female shark catfish (*Pangasius nasutus*) remain underexplored. This study investigated the impact of dietary phospholipid supplementation on growth, reproductive performance, and biochemical parameters of female *P. nasutus*. The experiment used four cages (4×4×3 m) in an earthen pond, each housing 15 fish with an initial average weight of 643±0.34 g and length of 43.3±0.12 cm. Fish were fed a commercial diet supplemented with 0% (control), 1.5%, 2%, or 2.5% phospholipids for 90 days. Results indicated that phospholipid supplementation significantly improved the gonadosomatic index, oocyte development, and steroid hormone levels. The 2.5% phospholipid group exhibited the highest vitellogenin and 17β-estradiol levels, corresponding to advanced oocyte maturation. Hematological parameters, blood biochemistry, and fatty acid composition were unaffected, and no significant improvements in somatic growth or nutrient utilization were observed. The highest supplementation level (2.5%) yielded the greatest increases in the GSI, vitellogenin, and 17β-estradiol levels, resulting in the most developed oocytes. These findings demonstrate that dietary phospholipid supplementation, particularly at 2.5%, effectively enhances broodstock quality and reproductive potential in female *P. nasutus*.

Keywords: 17-estradiol, Fatty acids, Oocytes development, Soybean lecithin, Vitellogenin

INTRODUCTION

Among members of the family Pangasiidae, *Pangasius nasutus* is an emerging species with aquaculture potential. However, fish farmers still rely heavily on wild-caught broodstock for seed production (Hassan *et al*., 2011). This practice leads to several issues, including variability in broodstock quality, unsustainability, and the risk of natural stock depletion beyond recovery (Iswanto and Tahapari, 2011). Therefore, there is an urgent need for foundational studies aimed at producing high-quality hatchery-bred broodstocks, particularly female broodstock, to support large-scale fish production (Azani *et al*., 2022). These studies may include optimizing broodstock diets to enhance seed production quality.

The quality and nutrient composition of broodstock diets are well known to significantly influence reproductive performance and the quality of offspring (Tercero *et al*., 2015; Yıldız *et al*.,

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2020; Zuo *et al*., 2022). Among dietary components, lipids are particularly important as they serve not only as a source of energy but also play critical roles in metabolism, growth, and development (Gibbs *et al*., 2015; Yoo *et al*., 2022; Torsabo *et al*., 2022). The fatty acid composition of lipids has been identified as a key dietary factor in determining broodstock reproductive success and offspring survival (Izquierdo *et al*., 2001). Importantly, higher dietary lipid inclusion has been demonstrated to exert a protein-saving effect in fish. However, excess lipids can lead to oxidation if poorly utilized (Guo *et al*., 2019). In addition, excessive lipid accumulation can result in nutritional metabolic liver disease, negatively impacting fish health (Huang *et al*., 2022). Thus, it is crucial to optimise lipid inclusion in fish diets or employ dietary treatments with lipid-lowering effects.

Phospholipids, phosphorus-containing lipids with several structural and functional roles (Turchini *et al*., 2022), represent one such dietary treatment. The lipid-lowering effects of phospholipids were first reported in mammals (Buang *et al*., 2005) and later demonstrated in fish species, including hybrid snakehead (*Channa argus* × *Channa maculate*) (Feng *et al*., 2017) and large yellow croaker (*Larimichthys crocea*) (Lin *et al*., 2018). The study by Buang *et al*. (2005) also reported that dietary phospholipids inhibit fatty acid synthesis and alleviate hepatic lipid deposition via fatty acid oxidation. However, the effect of phospholipid supplementation on fish can vary significantly between species. For instance, no enhancement in hepatic lipid deposition was observed in starry sturgeon (*Acipenser stellatus*) (Uyan *et al*., 2009), channel catfish (*Ictalurus punctatus*) (Sink and Lochmann, 2014), or amberjack (*Seriola, dumerili*) (Jafari *et al*., 2021). Despite this variability, dietary phospholipids supplementation has been shown to enhance growth performance, maintain physiological balance, and reduce stress in fish (Zhao *et al*., 2013; Huang *et al*., 2021; Torsabo *et al*., 2022; 2023).

Given the limited research on *P. nasutus*, this study aims to evaluate the effects of dietary phospholipid supplementation on its growth, reproductive performance, and selected biochemical parameters.

MATERIALS AND METHODS

Experimental design

Female *P. nasutus* broodstocks were purchased from Three Ocean Fish Pond & Trading Sdn. Bhd., located in Selangor, Malaysia, and transferred to the experimental station within the same facility. Prior to the experiment, the fish underwent a three-week acclimation period to adapt to a basal diet. During this phase, they were fed twice daily to satiation with a commercial feed TP 2 (Charoen Pokphand Foods, Malaysia) containing 32% protein and 4% lipid.

After acclimation, the fish were randomly assigned to one of four treatment groups: a control group (0% phospholipid) and phospholipid-treated groups (1.5%, 2.0%, and 2.5%). Each group was stocked with 15 fish in one of 4 cages, measuring 4×4×3 m, which were installed in an open earthen pond (91.44×48.8 m). The broodstock had an average initial weight of 643.00±0.34 g and an average total length of 43.30±0.12 cm. During the experimental period, the fish were fed to satiation with the experimental diets twice daily, at 9 a.m. and 5 p.m. Water quality parameters were maintained within acceptable ranges throughout the study, with a water temperature of 27.83 °C and dissolved oxygen levels at 6.7 $mg \cdot L^{-1}$.

Diets preparation/coating

A commercial 4 mm fish feed containing 32% protein and 4% lipid served as the base for the experimental diets. The treatment diets were top-coated with varying concentrations of soy phospholipids (Sigma-Aldrich, 11145): 0% (control), 1.5%, 2.0%, and 2.5%, resulting in crude lipid levels of 5.62%, 6.32%, and 6.98%, respectively. To facilitate the coating process, phospholipid granules were mixed with water in a beaker and heated in a water bath. The resulting liquid was sprayed evenly onto the feed using a compressed air sprayer while the feed was mixed with a planetary mixer (model B20-A food machinery, China). Agar (Sigma-Aldrich, Germany) was used as a gelling agent at a 2% concentration. It was first dissolved in 50 mL of hot water (45 °C) and then sprayed onto the coated feed in the mixer to ensure uniform

distribution. After coating, the diets were dried overnight in an oven at 35 °C to reduce moisture content. Once dried, the diets were packed, sealed in plastic bags, and stored at 20 °C until used.

The proximate composition of the diets was analyzed using standardized methods established by the Association of Official Analytical Chemists (AOAC, 2006), while the fatty acid content was determined following the procedure described by Abdulkadir and Tsuchiya (2008). Phospholipid content in the diet was measured using the Phospholipid Assay Kit (MAK122, Sigma Aldrich) according to the manufacturer's instructions. Tables 1 and 2 summarize the proximate and fatty acid compositions of the experimental diets, respectively.

Table 1. Proximate composition of the experimental diets (% dry weight of feed).

Parameter	PL 0	PL 1.5	PL 2.0	PL 2.5
Lipid	4.23	5.62	6.32	6.98
Protein	32.22	35.80	36.53	34.38
Ash	4.34	4.61	5.43	5.61
Moisture	4.53	3.55	4.31	4.56
Phospholipids	1.07	1.62	2.32	2.98

Note: PL 0 = control (0% phospholipids); PL 1.5 (1.5% phospholipids); PL 2.0 (2% phospholipids); PL 2.5 (2.5% phospholipids).

Table 2. Fatty acid composition (% of total FAME) of the experimental diets.

Treatment	PL 0	PL 1.5	PL 2.0	PL 2.5
C14:0	3.01	3.01	2.42	2.67
C16:0	1.77	1.77	1.70	1.80
C18:0	1.30	1.30	1.74	1.91
C20:0	0.55	0.44	0.56	0.57
C23:0	2.47	2.07	1.11	1.15
C18:1n-9	41.92	42.78	44.46	45.26
C20:1n-9	3.32	3.11	3.11	3.11
20:4n-6	1.57	1.57	1.12	1.22
20:5n-3	0.17	0.17	2.08	0.13
C18:2n-6	19.34	18.16	15.92	19.44
C20:2n-9	3.45	4.23	5.77	4.45
C18:3n-3	13.41	12.41	11.76	11.98
C18:3n-6	2.79	3.98	4.21	4.08
C22:6n-3	1.25	1.25	4.00	0.58
n-3	14.66	13.66	15.76	12.51
n-6	22.13	22.14	20.13	23.52
n-3/n-6	1.50	1.62	1.28	1.88
∑ SFA	9.13	8.59	7.53	8.10
∑ MUFA	45.24	44.57	47.57	48.37
∑ PUFA	41.98	41.86	44.86	41.88

Note: PL 0 = control (0% phospholipids); PL 1.5 (1.5% phospholipids); PL 2.0 (2% phospholipids); PL 2.5 (2.5% phospholipids).

Sample collection

After 90 days feeding period, five fish from each enclosure were selected for analysis. The fish were transferred from their breeding enclosures to smaller tanks, where crushed ice was introduced to immobilize them. Blood samples (3.5 mL) were collected from the vein at the base of the spine using 21–gauge needle and a 5 mL disposable syringe. The blood was placed into ethylenediamine tetraacetic acid (EDTA) tubes for hematological tests and plain BD vacutainers for biochemical analyses. For the measurement of 17β-estradiol and vitellogenin levels, 1.5 mL of blood was centrifuged at 3,500 rpm for 10 min, and the resulting plasma was frozen at -80 °C. Following blood collection, the fish were euthanized, and samples of the liver, muscles, and gonads were collected. Portions of the liver and gonad samples were preserved in 10% buffered formalin for 24 h and then transferred to 70% ethanol for histological examination. Additionally, samples of the liver, gonads, and muscles were frozen at -80 °C, freeze-dried, and ground for fatty acid profile and biochemical composition analysis.

Growth performance and biological indices

Data on growth performance and biological indices were collected at the end of the trial. Specimens were dissected to remove and weigh their gonads, liver, coelomic fat, and viscera. The gonadosomatic index (GSI), hepatosomatic index (HSI), viscerosomatic index (VSI), coelomic fat index (CFI), and condition factor (K) were calculated using standard formulas.

Specific growth rate (SGR %)

$$= 100 \times \frac{\text{(Ln Final Weight-Ln Initial Weight)}}{\text{Total time of the experiment}}$$

Feed conversion ratio (FCR)

$$= \frac{\text{Feed provided (dry weight)}}{\text{Weight gain (Wet weight)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Weight gain (WG)} = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}}$$

$$\text{GSI} = 100 \times \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}}$$

$$\text{HSI} = 100 \times \frac{\text{Liver weight (g)}}{\text{Body weight (g)}}$$

$$\text{VSI} = 100 \times \frac{\text{Visceral weight (g)}}{\text{Body weight (g)}}$$

$$\text{CFI} = 100 \times \frac{\text{Coelomic fat weight (g)}}{\text{Body weight (g)}}$$

$$\text{K} = 100 \left(\frac{\text{W}}{\text{L}^3}\right)$$

The body weight of fish is denoted by W in grams while the overall length of fish is denoted by L in centimeters.

Biochemical analysis

The analysis of crude lipid, crude protein, moisture, and ash followed the methods outlined by the Association of Official Agricultural Chemists (AOAC, 2006). Moisture content was determined by drying the sample in an oven at 105 °C for 24 h until a consistent weight was achieved and then calculating the difference between the wet and dried weights. Ash content was determined using a muffle furnace at 550 °C for 8 h. The crude protein level was determined using the Kjeldahl method by measuring nitrogen and multiplying it by 6.25. Crude lipid content was analysed using a Soxhlet extractor using petroleum ether at a boiling range of 60–80 °C.

Hematology and blood biochemistry

Hematological parameters were analysed from whole blood samples stabilized with EDTA using an IDEXX ProCyte Dx analyser (IDEXX Laboratories, Inc., ME, USA). Examined parameters included red blood cell count, hematocrit, hemoglobin levels, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, white blood cell count,

neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count, platelet distribution width, mean platelet volume, and plateletcrit. Blood biochemistry parameters, including glucose, creatinine, total protein, albumin, globulin, alanine transaminase, and alkaline phosphatase, were determined using an IDEXX Catalyst One analyser (IDEXX Laboratories, Inc., ME, USA). The biuret technique was employed, excluding haemolysed samples to avoid artificially elevated readings (Oddsdóttir *et al*., 2023).

Determination of reproductive hormone profiles

Blood samples for reproductive hormone profile analysis were collected from five fish per treatment using 5 mL disposable syringes with 21-gauge needles. Blood samples were drawn from the caudal circulation at the study's conclusion and stored in ethylene EDTA vacutainer tubes. Plasma was separated by centrifugation at 3,500 rpm for 10 min, after which 2 mL aliquots were transferred into tubes and stored at -80 °C. Commercial enzyme-linked immunosorbent assay (ELISA) kits (ELISA) kits (Cayman Chemicals, USA) were used to analyses reproductive hormones. Blood plasma was thawed, diluted (1:500 and 1:1000), and tested in triplicate following the manufacturer's instructions. Absorbance for vitellogenin and 17β-estradiol was read at 492 nm and 414 nm, respectively, using a SpectraMax iD5 multi-mode microplate reader (San Jose, California, USA).

Oocytes diameter determination

At the end of the feeding trial, five fish from each experimental group were weighed and dissected. Ovarian samples (midway through the ovary) were collected, preserved in 10% buffered formalin for 72 h, and then transferred to 70% ethanol. Samples were processed using standard histological techniques (Korkmaz *et al*., 2020). Tissue sections (5 µm thick) were mounted on glass slides and stained with hematoxylin and eosin (H & E). After staining, the slides were dried and examined under a light microscope. However, the section quality was insufficient for precise identification of developmental stages. Consequently, only rough identification of oocyte stages was performed to facilitate diameter measurements. To assess oocyte size frequency distribution, 100 oocytes per specimen were measured using ImageJ software following the method by Abduh *et al*. (2021). Oocyte diameters were categorized by developmental stage: Stage 1 (10–30 µm), Stage 2 (30–150 µm), Stage 3 (150–300 µm), Stage 4 (300–600 µm), Stage 5 (600–800 µm), and Stage 6 (800–1000 µm).

Fecundity

A total of three ovaries per treatment was used to estimate fecundity. After measuring the total gonad weight, fecundity was determined by gravimetric sampling. A subsample was weighed, cleaned to separate the eggs from the ovarian tissue, and dried on blotting paper. The total number of eggs was calculated using the equation by Yeldan and Avsar (2000).

$$F = nG/g$$

where F represents fecundity (eggs·ind^{-1}), n represents the number of eggs in the subsample, G represents the overall weight of the ovaries, and g represents the weight of the subsample in the same units.

Fatty acid analysis of liver, gonad, and muscle

Liver, gonad, and muscle samples from *P. nasutus* were collected and subjected to a 72 h freeze-drying process. The samples were then finely ground and stored at -40 °C until analysis. A one-step method described by Abdulkadir and Tsuchiya (2008) was employed to combine the extraction and esterification steps into a single tube for Fatty Acid Methyl Ester (FAME) extraction. After extraction, the FAMEs were transferred into clean sample vials and analysed using a gas chromatograph (GC 14-B Shimadzu) equipped with a flame ionization detector (GC-FID). For FAME analysis, an Agilent Technologies, Inc. gas chromatograph was used. Fatty acids were identified by comparing the relative retention times of the FAMEs with those of a known standard.

Fatty acid concentrations (CFA, $mg \cdot g^{-1}$ of dry sample) were calculated by comparing the peak area of fatty acids in the sample with the peak area of the internal standard using the formula:

$$CFA = A_S/A_{IS} \times C_{IS}/W_S$$

Where:

A_S = Peak area of fatty acid in the sample in the chromatograph;
A_{IS} = Peak area of the internal standard in the chromatograph;
C_{IS} = Concentration of the internal standard (mg);
W_S = Weight of the sample (g).

Statistical analysis

The collected data were presented as means ±standard deviation. The data were tested for normal distribution using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test for equal variance. One-way analysis of variance (ANOVA) was performed using SPSS 22.0 to analyse the data. Differences between means were evaluated using a post-hoc Tukey's test with statistical significance set at $p<0.05$. To assess the extent of oocyte dependence on treatments, Fisher's test was used. Significant differences among the counted oocytes within each developmental stage were determined using the Marascuilo procedure. Second-order polynomial regression analysis was used to evaluate the complex, non-linear relationship between phospholipid-supplemented diets and their effects on key blood parameters.

RESULTS

Growth and biological indices

The growth and biochemical indicators of female *P. nasutus* fed varying levels of phospholipid-coated diets are shown in Table 3. After 90 days of the trial, survival rates of the experimental fish ranged between 93.00±0.03% and 100%, with no significant differences ($p = 0.547$). Overall, phospholipid supplementations did not affect growth parameters (BW, TL, SGR, and WG), condition factor, or FCR ($p = 0.090–0.878$). Similarly, indices such as HSI, VSI, and CFI were not influenced by the supplementation ($p = 0.378–0.974$). However, the GSI was significantly higher ($p = 0.005$) in the group fed the PL 2.5 diet, reaching 5.12±1.35%, which was notably higher than the control and lower supplementation groups.

Table 3. Growth and biological indices of *Pangasius nasutus* fed phospholipid-supplemented diets for 90 days.

Treatment	PL 0	PL 1.5	PL 2.0	PL 2.5	p-value
BW (g)	760±29.2	860±79.7	800±59.2	959±69.5	0.166
TL (cm)	43.82±0.66	45.22±1.05	43.94±1.01	46.69±0.5	0.090
SGR (%)	0.43±0.08	0.18±0.04	0.30±0.10	0.23±0.08	0.161
WG	117±69.5	217±29.2	157±79.7	316±59.2	0.166
K	0.90±0.01	0.92±0.03	0.94±0.03	0.94±0.06	0.878
FCR	1.80±0.22	1.70±0.12	1.62±0.26	1.55±0.28	0.876
GSI	$1.34±0.46^b$	$1.18±0.42^b$	$1.69±0.19^b$	$5.12±1.35^a$	0.005
HSI	1.13±0.11	1.22±0.07	1.10±0.05	1.00±0.13	0.457
VSI	1.45±0.14	1.49±0.12	1.48±0.08	1.42±0.17	0.984
CFI	4.52±0.55	4.46±0.63	5.18±0.86	3.20±1.03	0.378
Survival (%)	100	93	96	100	-

Note: Mean±SD in the same row superscripted with different lowercase letters are significantly different ($p<0.05$).

Hematological and blood biochemistry indices

Table 4 presents the hematological parameters of *P. nasutus* fed diets supplemented with phospholipids. The RBC count significantly increased with phospholipid supplementation, with the highest counts recorded in the PL 2.5 (2.34± $0.12\times10^{12}\cdot L^{-1}$) and PL 2.0 ($2.03\pm0.01\times10^{12}\cdot L^{-1}$) groups compared to PL 0 and PL 1.5 ($p = 0.001$). A similar trend was observed for HCT, where PL 2.5 (29.67±1.68%) and PL 2.0 (27.93±0.18%) had significantly higher HCT values than PL 0 and PL 1.5 ($p = 0.002$). Hemoglobin levels were significantly higher in the PL 2.5 (9.27±0.48 $g\cdot dL^{-1}$) and PL 2 (7.37±0.09 $g\cdot dL^{-1}$) groups compared to PL 0 and PL 1.5 ($p = 0.005$). The WBC count also varied significantly, with the PL 2.0 group exhibiting the highest count ($58.97\pm1.12\times10^{9}\cdot L^{-1}$), followed by PL 2.0., PL 1.5, and PL 0 ($p = 0.001$).

A second-order polynomial regression analysis (Figure 1) reveals varying effects of phospholipid diets on blood metrics. Haematocrit (HCT) and hemoglobin (HGB) show strong correlations, with dietary factors accounting for 66.88% and 91.58% of their variability, respectively. Red blood cell (RBC) variability, however, is weakly associated with diet at 13.02%. White blood cells (WBC) and platelets (PLT) show moderate associations, with r^2 values of 46.55% and 54.47%. Red cell distribution width (RDW) demonstrates the strongest relationship, with 95.24% of its variation influenced by phospholipid supplementation.

The blood biochemistry of *P. nasutus* fed phospholipid-supplemented diets for 90 days is shown in Table 5. Plasma glucose (GLU) was highest in PL 2.5 ($p = 0.000$). Total protein (TP)

Table 4. Hematological Indices of *Pangasius nasutus* fed phospholipid-supplemented diets for 90 days.

Treatment	PL 0	PL 1.5	PL 2.0	PL 2.5	p-value
RBC ($10^{12}\cdot L^{-1}$)	1.79±0.25^{b}	1.01±0.08^{c}	2.03±0.01^{a}	2.34±0.12^{a}	**0.001**
HCT (%)	25.13±0.53^{b}	13.03±1.01^{c}	27.93±0.18^{a}	29.67±1.68^{a}	**0.002**
HGB ($g\cdot dL^{-1}$)	7.10±1.11^{b}	4.20±0.59^{c}	7.37±0.09^{b}	9.27±0.48^{a}	**0.005**
MCV (fL)	140.03±0.56^{a}	129.4±0.5^{c}	137.37±0.46^{b}	126.67±0.74^{d}	**0.000**
MCH (pg)	39.37±0.86	41.33±2.68	36.23±0.35	39.57±0.12	0.162
MCHC ($g\cdot dL^{-1}$)	28.13±0.52^{c}	31.93±1.99^{a}	26.37±0.14^{b}	31.27±0.18^{a}	**0.016**
RDW (%)	9.27±0.03^{c}	12.43±0.14^{a}	12.83±0.09^{a}	10.97±0.12^{b}	**0.000**
WBC ($10^{9}\cdot L^{-1}$)	21.35±0.46^{d}	27.06±5.77^{c}	58.97±1.12^{a}	39.09±1.16^{b}	**0.000**
NEU ($10^{9}\cdot L^{-1}$)	12.40±0.81^{d}	17.37±3.78^{c}	38.41±1.65^{a}	23.41±1.41^{b}	**0.000**
LYM ($10^{9}\cdot L^{-1}$)	8.16±0.41^{b}	8.53±1.87^{b}	17.32±1.08^{a}	14.82±1.27^{a}	**0.002**
MONO ($10^{9}\cdot L^{-1}$)	0.46±0.2^{b}	0.90±0.21^{b}	2.98±0.05^{a}	0.64±0.01^{b}	**0.000**
EOS ($10^{9}\cdot L^{-1}$)	0.09±0.02	0.16±0.01	0.13±0.02	0.14±0.05	0.523
BASO ($10^{9}\cdot L^{-1}$)	0.06±0.02	0.10±0.05	0.12±0.02	0.07±0.02	0.440
PLT ($K\cdot\mu L^{-1}$)	4.67±0.67	2.67±0.88	6.33±0.88	6.00±1.00	0.062
MPV (fL)	19.33±1.11^{b}	13.4±0.31^{c}	23.93±0.24^{a}	23.50±0.55^{a}	**0.000**
PDW (fL)	10.73±1.54	7.47±0.41	8.53±0.73	10.37±2.07	0.340
PCT (%)	0.010±0.00	0.003±0.00	0.013±0.00	0.017±0.00	0.055

Note: Mean±SD in the same row superscripted with different lowercase letters are significantly different ($p<0.05$).

was numerically highest in PL 2.5 but significantly lower in PL 1.5. Albumin (ALB) and globulin (GLOB) were significantly higher in PL 2.5 compared to PL 1.5, while the albumin-to-globulin ratio (ALB/GLOB) significantly reduced in fish received phospholipid supplement except for the PL 2.5, which showed no significant differences ($p = 0.037$) with the control. Alanine aminotransferase (ALT) was significantly elevated in PL 2.5 ($p = 0.002$). Regression analysis (Figure 2) revealed varying impacts of phospholipid diets, with r^2 values for TP, ALB, and GLOB at 72.39%, 84.03%, and 87.74%, respectively, and for GLU and ALT at 94.76% and 83.97%, indicating strong influences of dietary phospholipids on these parameters.

Vitellogenin and Estradiol levels

Figures 3a and 3b show the plasma levels of vitellogenin (VTG) and 17β estradiol (E2) in female *P. nasutus*. Plasma VTG levels increased from PL 1.5 to PL 2.5, with PL 2.5 recording a significantly high VTG concentration (10.69±0.45 $mg \cdot mL^{-1}$). Adding phospholipids raised the levels of 17β estradiol (E2) in the plasma, and PL 2.5 had a significantly ($p<0.05$) high E2 level (51.86±3.01 $pg \cdot mL^{-1}$). At the same time, there was no significant difference between treatments PL 1.5 and PL 2.0, while the control (PL 0) had a significantly lower concentration compared to all the phospholipid-supplemented groups.

Table 5. Blood biochemistry indices of *Pangasius nasutus* fed phospholipid-supplemented diets for 90 days.

Treatment	PL 0	PL 1.5	PL 2.0	PL 2.5	p-value
GLU ($mmol \cdot L^{-1}$)	3.94±0.02^b	3.94±0.01^b	3.86±0.01^b	5.64±0.01^a	**0.000**
UREA ($mmol \cdot L^{-1}$)	1.55±0.55	0.90±0.00	2.00±0.00	1.20±0.00	0.161
TP ($g \cdot L^{-1}$)	116.00±4.00^a	42.00±0.00^b	111.00±2.00^a	120.00±0.00^a	**0.000**
ALB ($g \cdot L^{-1}$)	48.50±0.05^b	18.00±0.00^c	46.50±0.05^b	60.00±0.00^a	**0.000**
GLOB ($g \cdot L^{-1}$)	63.00±1.00^b	24.00±0.00^c	65.50±2.05^b	81.00±3.00^a	**0.000**
ALB/GLOB	0.78±0.01^a	0.70±0.00^b	0.70±0.00^b	0.74±0.03^b	**0.037**
ALT ($U \cdot L^{-1}$)	27.19±0.15^c	28.02±0.02^c	93.54±0.49^b	128.50±1.50^a	**0.002**

Note: Means±SD in the same row superscripted with different lowercase letters are significantly different ($p<0.05$).

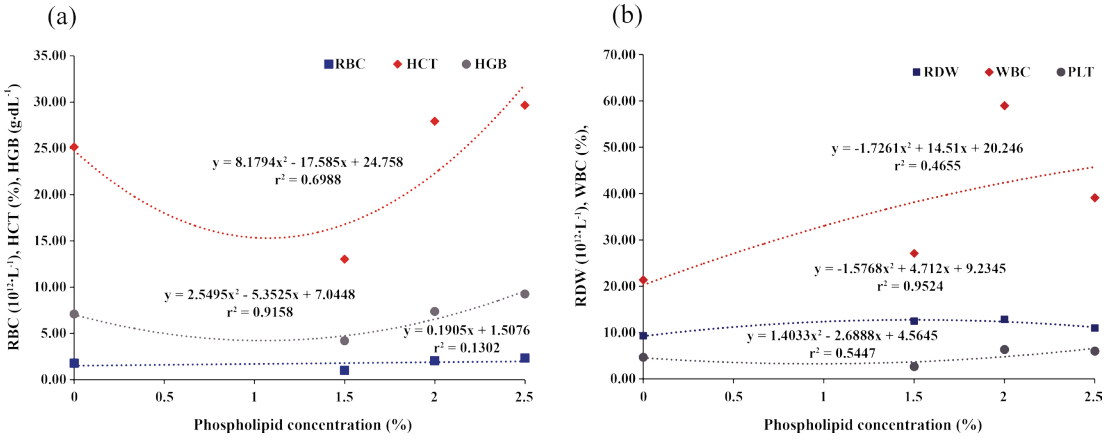

Figure 1. Second-order polynomial regression of some mean haematological parameters vs phospholipid supplementation levels.

Oocyte size-frequency distribution and fecundity

Figure 4 shows the oocyte size-frequency distribution of the oocyte diameters of *P. nasutus* fed different levels of phospholipid-supplemented diet. The results showed that the oocyte count depended on the treatments administered ($p<0.05$). The oocyte size-frequency distribution shows that frequencies of stages 1 (10–30 µm) oocytes showed minimal differences among treatments, with a significantly lower count observed in PL 1.5. The frequency of stage 2 (30–150 µm) oocytes in PL 0 remained significantly high (125), whereas PL 2.5 had 74; no significant difference was observed between PL 1.5 and PL 2.0. A decrease in the countof stage 3 (150–300 µm) oocytes was observed in PL 0 (51), while PL 2.0 demonstrated a significant increase (135). Notably, PL 2.5 recorded the highest number of stages 6 (800–1,000 µm) oocytes among all treatment groups.

The absolute fecundity (Figure 5) varied between treatments. Fish fed PL 2.5 had a significantly higher absolute fecundity (29,291± 9.23 eggs·ind^{-1}) than fish fed PL 1.5 (15,546±9.54 eggs·ind^{-1}) or the control group (PL 0). However, there was no statistically significant difference in absolute fecundity between fish fed PL 2.5 and PL 2.0 (28,851±10.32 eggs·ind^{-1}).

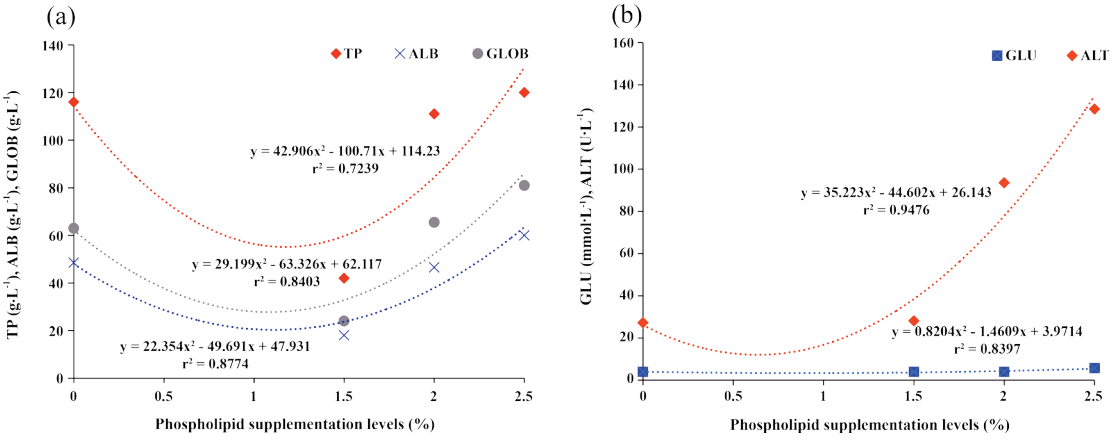

Figure 2. Second-order polynomial regression of some mean blood biochemistry metrics vs phospholipid supplementation levels.

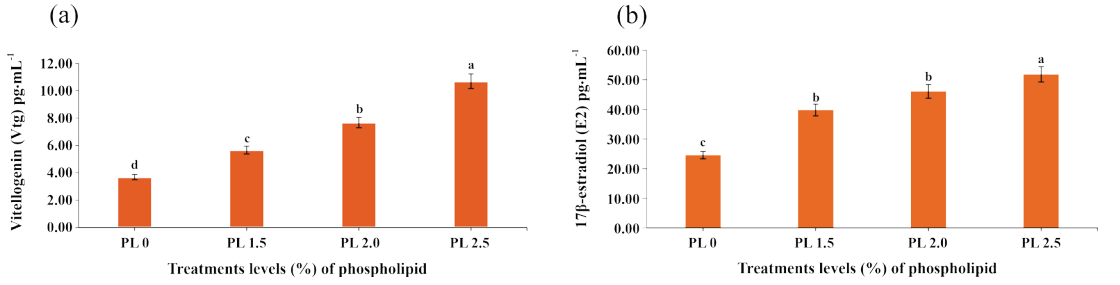

Figure 3. Plasma Vitellogenin (VTG) (a), and Plasma 17β-estradiol (E2) (b) levels of female *Pangasius nasutus* fed varying phospholipid-supplemented diets for 90 days. Bars represent mean values and error bars represent standard deviation (SD). Different lowercase letters above bars indicate significant differences ($p<0.05$) between means.

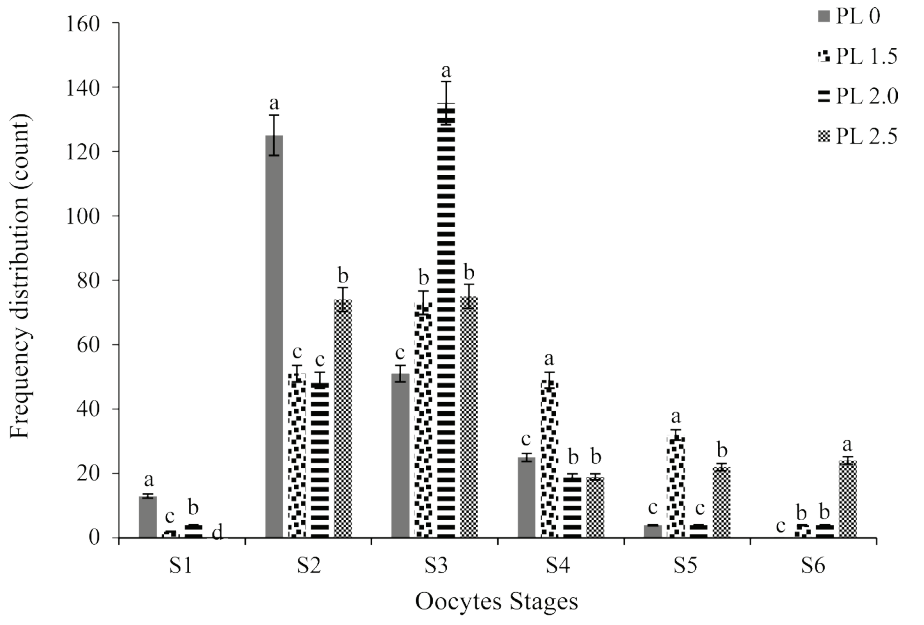

Figure 4. Frequency distribution of different oocyte stages (from oocytes diameter) of female *Pangasius nasutus* fed varying levels of phospholipid-supplemented diets for 90 days. S1–S6 (stage 1–stage 6). Bars represent mean values and error bars represent standard deviation (SD). Different lowercase letters above bars indicate significant differences ($p<0.05$) between means of each oocyte stage.

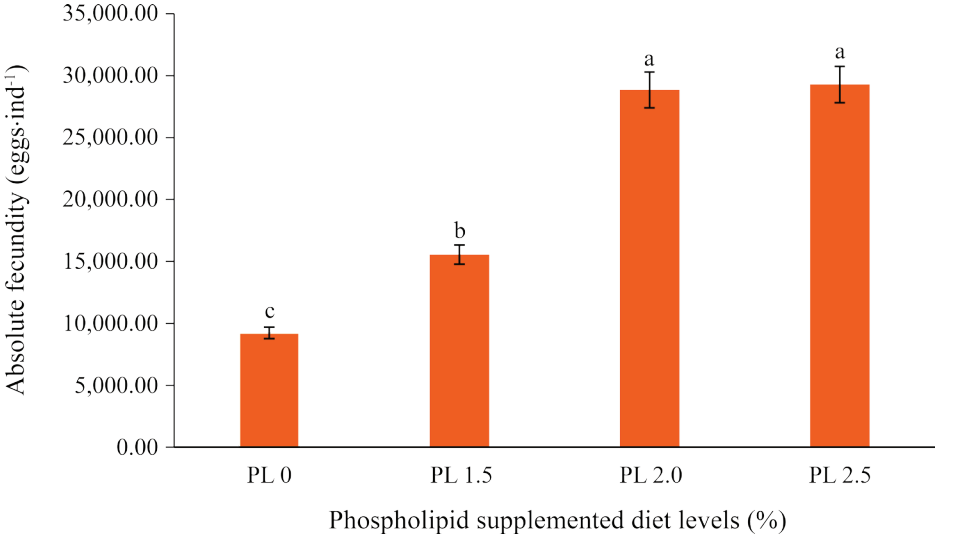

Figure 5. Absolute fecundity of female *Pangasius nasutus* fed varying levels of phospholipid-supplemented diets for 90 days. Bars represent mean values and error bars represent standard deviation (SD). Different lowercase letters above bars indicate significant differences ($p<0.05$) between means.

DISCUSSION

Growth, biological, hematological, and blood biochemistry

Adding phospholipids to the diet did not significantly affect the body weight or overall length of the female broodfish. Phospholipids are known to improve growth performance in various fish species during early life stages (Kiron, 2012), as observed in large yellow croaker (*Larimichthys crocea*) (Feng *et al*., 2017), gilthead seabream (*Sparus aurata*) (Kokou *et al*., 2021), Atlantic salmon (*Salmo salar*) (Taylor *et al*., 2015; Jaxion-Harm, 2021), and hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lancolatus* ♂) (Huang *et al*., 2022). However, the absence of growth improvement in the present study may be attributed to the reallocation of energy in adult fish towards reproductive activities rather than somatic growth.

The condition factor (K) is an important indicator of fish health and body condition, reflecting energy reserves and overall well-being (Jisr *et al*., 2018; Hammock *et al*., 2022). In this study, no substantial variations in K were observed across treatments, thus indicating that phospholipid supplementation did not affect the overall condition of *P. nasutus*. This finding aligns with the results of Turchini *et al*. (2009). Similarly, indices such as the HSI, VSI, and CFI were not significantly different among treatments. HSI represents the relative size and condition of the liver, while the VSI reflects the size and condition of the viscera, and the CFI indicates lipid reserves (Lee *et al*., 2002; Dong *et al*., 2014; Rizzo and Bazzoli, 2020). The comparable values of these indicators across treatments suggest that phospholipid supplementation did not alter resource allocation between the liver, visceral tissues, and other metabolic processes. These findings are consistent with previous studies emphasizing the role of phospholipids in supporting reproductive processes, such as those conducted on zebrafish fed a microdiet supplemented with phospholipids (Martins *et al*., 2020a).

The feed conversion ratio (FCR) of the diet containing 2.5% phospholipids was numerically the lowest among treatments, although differences between the 1.5%, 2%, and control diets were not statistically significant. While a lower FCR typically reflects improved feed efficiency, the lack of significant differences suggests that phospholipid supplementation at the tested levels did not substantially affect nutrient utilization. Reports have linked phospholipid supplementation to improved nutrient utilization in other fish species. For instance, diets supplemented with phospholipids enhanced FCR in largemouth bass larvae, zebrafish (Tocher, 2003; Martins *et al*., 2020a; Wang *et al*., 2022), and channel catfish (*Ictalurus punctatus*), where a 4% phospholipid diet resulted in better FCR (1.34±0.12) compared to 2% and control diet (Sink and Lochmann, 2014). Similarly, hybrid snakehead fed diets with varying phospholipid levels (8.5–50.8 $g \cdot kg^{-1}$) exhibited differing FCRs, with the lowest phospholipid level yielding the highest FCR (Lin *et al*., 2018).

Phospholipid supplementation at 2.5% significantly increased RBC, HCT, HGB, WBC, LYM, MPV, and PCT in female *P. nasutus*. These changes enhance oxygen transport, immune potential, and platelet function. Increases in RBC, HCT, and HGB suggest improved erythropoiesis and hemoglobin synthesis, potentially boosting aerobic performance and hypoxia tolerance. Elevated WBC and LYM levels indicate enhanced leukocyte production and adaptive immune responses, while higher MPV and PCT reflect increased platelet size and number, improving clotting and wound healing. These findings align with studies by Witeska *et al*. (2016; 2022) and a review by Fazio (2019), which highlighted the role of phospholipids in modulating fish hematological parameters. Similar results were reported for Senegalese sole (*Solea senegalensis*) (Salas-Leiton *et al*., 2018), rainbow trout (*Oncorhynchus mykiss* fingerlings (Zargar *et al*., 2019), as well as in striped catfish (*Pangasianodon hypophthalmus*) (Torsabo *et al*., 2023).

The highest phospholipid level (2.5%) also increased blood GLU, TP, ALB, GLOB, ALT, and ALKP levels, indicating altered metabolic and liver functions. This finding supports those of Hadarabi *et al*. (2011), who reported increased glucose levels with 6% dietary phospholipids in sturgeon fish (*Huso huso*), although ALT, AST, ALP, and LDH levels remained unaffected. Additionally, De Santis *et al*. (2015) demonstrated that dietary phospholipids influenced biochemical and morphological parameters in Atlantic salmon fry. Polynomial regression analysis (r^2 values) further confirmed the strong to moderate contributions of phospholipids to hematological and biochemical parameters, reinforcing their importance in enhancing fish health and physiological performance.

Gonadal development, and fecundity

GSI is an important indicator of gonadal development and maturity (Flores *et al*., 2019; Petersen and Warner, 2002). A significant correlation exists between GSI and the percentage of mature (post-vitellogenic) oocytes, as observed in Indian threadfin (*Alectis indicus*) (Khamcharoen *et al*., 2023). In this study, GSI was significantly higher in the groups fed phospholipid-supplemented diets, particularly in the PL 2.5 treatment, compared to the control group. This finding suggests a stimulatory effect of dietary phospholipids on gonadal development and their potential to enhance reproductive performance. The GSI for the PL 2.5 group (5.12±1.35%) exceeded the values reported for wild female *P. nasutus* (3.81%) during the onset of the spawning season (Torsabo *et al*., 2024) and fell within the range of 1.97–4.42% for hybrid catfish broodstock (*Pangasius larnaudii* × *P. hypophthalmus*) (Sattang *et al*., 2021), and 4.24–11.37% for sexually mature *P. hypophthalmus* (Datta *et al*., 2018). Variation in GSI among fish at a gonad stage may be attributed to rearing conditions, sampling times, and species-specific characteristics. These results align with earlier studies emphasizing the role of phospholipids in supporting fish reproduction (Tocher, 2015).

Histological analysis further confirmed the asynchronous reproductive pattern of *P. nasutus*, characterized by the presence of all oocyte developmental stages (Lubzens *et al*., 2010), though certain stages were dominant depending on the dietary treatment. Fish fed the control diet exhibited a higher proportion of Stage 1 oocytes, while those fed PL 2.5 displayed a dominance of Stage 6 oocytes, indicative of ovulation readiness were dominant in fish fed PL 2.5 diet with few numbers of stage 1 oocytes (Figure 5). Stage 5 oocytes, representing final maturation stages, were also significantly more abundant in the PL 2.5 group. These findings corroborate the GSI results, highlighting the role of phospholipid-supplemented diets in advancing oocyte maturation and promoting ovulation.

PLs in broodstock diets are crucial for adequate gametogenesis and embryogenesis (Fraher *et al*., 2016; Martins *et al*., 2020b). The abundance of matured oocytes in phospholipid-supplemented groups can likely be attributed to the bioavailability of polyunsaturated fatty acids (PUFAs), which are the major constituents of phospholipids (Martins *et al*., 2020b). PUFAs such as n-3 and n-6 are commonly found in the hydrophobic tails of phospholipids, where they play essential roles in cellular structure, signalling, and various physiological processes in organisms (Martins *et al*., 2020a). Additionally, PUFAs contribute to the modulatory effects of prostaglandin synthesis and steroidogenesis (Wathes *et al*., 2007).

Although *P. nasutus*, as a freshwater species, possesses the ability to biosynthesis PUFAs such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from α-linolenic acid (LNA, 18:3n-3), as well as arachidonic acid (ARA, 20:4n-6) from linoleic acid (LA, 18:2n-6) de novo. However, PL biosynthesis depends on desaturase and elongase enzymes, which are highly influenced by the developmental stages of the organism (Alimuddin *et al*., 2005). A PUFA-rich diet eliminates the need for de novo biosynthesis, thereby reducing metabolic effort and enhancing

biological efficiency (Martins *et al*., 2020a). Our findings suggest that PL uptake from supplemented diets in *P. nasutus* decreases endogenous PUFA biosynthesis, consequently reducing metabolic effort and improving reproductive performance.

Phospholipid-supplemented diet significantly influences the absolute fecundity of female *P. nasutus*. Specifically, the PL 2.5 group exhibited a significantly higher number of matured oocytes, corresponding to increased fecundity. This effect is likely attributed to the abundance of PUFAs in phospholipids, which enhance reproductive performance in teleost fish (Izquierdo *et al*., 2001; 2015). Phospholipid supplementation has also been reported to increase levels of 17β-estradiol and 11-ketotestosterone in striped catfish, hormones essential for reproduction and secondary sexual characteristic development (Torsabo *et al*., 2023).

Vitellogenin (VTG) and 17β-estradiol (E2) concentrations

In this study, a progressive increase in plasma VTG and E2 concentrations was observed in *P. nasutus* fed phospholipid-supplemented diets, with the highest levels recorded in the PL 2.5 group. This finding indicates a direct relationship between dietary phospholipid supplementation and reproductive activity, as VTG and E2 are critical markers of gonadal development in fish. Phospholipids likely play dual roles by facilitating nutrient transport and modulating metabolic pathways (Jaxion-Harm, 2021).

Phospholipids have been more extensively utilized in diets to enhance reproductive performance in crustaceans compared to finfish (Wee *et al*., 2023). Phospholipids, due to their amphipathic nature, allowing them to form lipoprotein complexes and effectively transport lipophilic molecules such as fatty acids, sterols, and vitamins across cell membranes (Wang *et al*., 2013). This property enhances the uptake and distribution of essential nutrients required for E2 synthesis and VTG production. Estradiol, synthesized in the ovaries, regulates oocyte development and vitellogenesis, wherein VTG is produced in the liver and subsequently transported to the ovaries to support yolk formation (Lubzens *et al*., 2010).

The observed increases in VTG and E2 in this study align with the physiological demands of gonadal development, as phospholipid-supplemented groups exhibited a greater abundance of vitellogenic and mature oocytes compared to the control group. These findings are consistent with previous studies demonstrating the role of dietary lipids in enhancing the reproductive performance of fish. For instance, Song *et al*. (2019) reported that dietary phospholipids enhance ovarian development by stimulating E2 production and VTG synthesis in marine fish. Moreover, phospholipids are crucial for the incorporation and transport of long-chain polyunsaturated fatty acids (LC-PUFAs), which are essential for steroidogenesis and maintainance of membrane fluidity in developing oocytes (Lin *et al*., 2020).

The role of phospholipids in nutrient transport may also extend to their interaction with other critical nutrients. Fatty acids and sterols serve as precursors for hormone synthesis and membrane biogenesis, while vitamins such as E and A are essential for antioxidant protection and gene expression during oogenesis (NRC, 2011). By facilitating the incorporation of these components into gonadal tissues, phospholipids likely contribute to the observed increases in VTG and E2, further supporting enhanced reproductive activity.

Biochemical and fatty acids composition of liver, gonad, and muscle

Phospholipid supplementation significantly influenced the biochemical composition of liver, gonad, and muscle tissues in female *P. nasutus* as shown in Supplementary Tables 1-4 (Supplementary Table 1-4). In the liver, increased protein and lipid content was observed, particularly at the 2.5% supplementation level (Supplementary Table 1), indicating enhanced protein metabolism and lipid storage. These findings are consistent with studies on other fish species, including Atlantic salmon

(*Salmo salar*) (Ibarz *et al*., 2023), channel catfish (*Ictalurus punctatus*) (Liu *et al*., 2020), and other experimental animals (Cohn *et al*., 2008).

In the gonads, elevated protein and lipid content, particularly in the PL 2.5 group (Supplementary Table 1), supported reproductive processes, consistent with previous research linking dietary phospholipids to improved reproduction and egg quality in fish (Nandi *et al*., 2007; Wathes *et al*., 2007; Schlotz *et a*l., 2012; Sarih *et al*., 2020). Muscle tissue also showed significant changes, including increased protein, lipid, ash, and moisture content (Supplementary Table 1). The increase in muscle protein indicates improved growth and fillet quality, while elevated lipid levels suggest enhanced energy storage. Similar responses in muscle tissue have been reported in other fish species (Bargui *et al*., 2021; Kizmaz, 2021).

Fatty acid composition varied across tissues, reflecting tissue-specific metabolic effects. The liver showed increased saturated fatty acids (SFAs), with the highest levels of monounsaturated fatty acid (MUFA) and PUFAs observed in the PL 1.5 and PL 2.0 groups, respectively (Supplementary Table 2). Gonads showed the highest PUFA levels in the PL 2.5 group (Supplementary Table 3)., similarly, muscle tissue exhibited peak PUFA levels in the PL 2.5 group and MUFA in the PL 2.0 group (Supplementary Table 4). These changes align with studies on lipid metabolism in European catfish (*Silurus glanis* L) (Egessa *et al*., 2024) and the reproductive roles of n-3 and n-6 PUFAs (Izquierdo *et al*., 2001; Carboni *et al*., 2013). Phospholipid supplementation influenced specific fatty acids, such as C18:1n-9, C18:3n-6, and C20:3n-3, reflecting differential regulation of MUFA and PUFA pathways. Similar trends in fatty acid transformation and metabolic roles have been reported (Tan *et al*., 2022; Wee *et al*., 2023). This study highlights the organ-dependent synthesis, incorporation, and distribution of fatty acids, with implications for metabolic processes and nutritional quality. Further research is needed to elucidate the underlying mechanisms, such as enzymatic modulation, driving these observed effects.

CONCLUSIONS

This study demonstrates the multifaceted benefits of dietary phospholipid supplementation in *P. nasutus*. Significant improvements were observed in GSI, oocyte development and maturation, and elevated VTG and E2, particularly at 2.5% phospholipid supplementation. Biochemical composition analyses revealed enhanced protein metabolism and lipid storage in liver and gonad tissues, alongside improved reproductive activity. No adverse effects were observed in *P. nasutus* broodstock health, as indicated by stable blood metrics. However, somatic growth and nutrient utilization were not significantly influenced, likely due to energy prioritization for reproduction. Fatty acid analysis confirmed the role of dietary phospholipids in lipid metabolism, energy utilization, and cellular functions. Overall, these findings emphasize the multifaceted benefits of dietary phospholipid supplementation in enhancing physiological processes in *P. nasutus*, while also identifying areas for further investigation into underlying mechanisms and aquaculture applications.

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LITERATURE CITED

Abduh, M.Y., I.C.C Koh, A.B. Abol-Munafi, N.H. Norazmi-Lokman and N. Mat Noordin. 2021. Effects of dietary fish oil and corn oil on gonadosomatic and hepatosomatic index, gonadal histology, 17β-oestradiol level and fatty acids profile of mahseer (*Tor tambroides*) broodstock in captivity. **Aquaculture Nutrition** 27(5): 1448−1459. DOI: 10.1111/anu.13282.

Abdulkadir, S. and M. Tsuchiya. 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. **Journal of Experimental Marine Biology and Ecology** 354(1): 1−8. DOI: 10.1016/j.jembe.2007.08.024.

Alimuddin, Y.G., V. Kiron, S. Satoh and T. Takeuchi. 2005. Enhancement of EPA and DHA biosynthesis by over-expression of masu salmon Δ6-desaturase-like gene in zebrafish. **Transgenic Research** 14(2): 159–165. DOI: 10.1007/s11248-004-7435-7.

Association of Official Analytical Chemists (AOAC). 2006. **Official Methods of Analysis of AOAC International,** 18th ed. AOAC International, Gaithersburg, USA. 2000 pp.

Azani, N., A.B. Abol-Munafi, H.J. Liew, S. Kamarudin, A. Arshad, M.M. Hassan, A.H.M. Kamal and N.W. Rasdi. 2022. Different dietary effects on growth and reproduction of freshwater zooplankton *Ceriodaphnia cornuta* (Sars, 1885) and its potential use in *Pangasius nasutus* larval rearing. **International Aquatic Research** 14(3): 193−202. DOI: 10.22034/IAR.2022.1955847.1261.

Baras, E., R. Hafsaridewi, J. Slembrouck, A. Priyadi, Y. Moreau, L. Pouyaud and M. Legendre. 2010. Why is cannibalism so rare among cultured larvae and juveniles of *Pangasius djambal*? Morphological, behavioural and energetic answers. **Aquaculture** 305(1–4): 42−51. DOI: 10.1016/j.aquaculture.2010.04.004.

Bargui, R., A. Solgadi, B. Prost, M. Chester, A. Ferreiro, J. Piquereau and M. Moulin. 2021. Phospholipids: identification and implication in muscle pathophysiology. **International Journal of Molecular Sciences** 22(15): 8176. DOI: 10.3390/ijms22158176.

Buang, Y., Y.M. Wang, J.Y. Cha, K. Nagao and T. Yanagita. 2005. Dietary phosphatidylcholine alleviates fatty liver induced by orotic acid. **Nutrition** 21(7−8): 867−873. DOI: 10.1016/j.nut.2004.11.019.

Carboni, S., A.D. Hughes, T. Atack, D.R. Tocher and H. Migaud. 2013. Fatty acid profiles during gametogenesis in sea urchin (*Paracentrotus lividus*): Effects of dietary inputs on gonad, egg and embryo profiles. **Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology** 164(2): 376−382. DOI: 10.1016/j.cbpa.2012.11.010.

Cohn, J.S., E. Wat, A. Kamili and S. Tandy. 2008. Dietary phospholipids, hepatic lipid metabolism and cardiovascular disease. **Current Opinion in Lipidology** 19(3): 257−262. DOI: 10.1097/MOL.0b013e3282ffaf96.

Cui, Z., C. Liu, W. Rao, P. Chen, K. Lei, K. Mai and W. Zhang. 2023. Dietary phospholipids improve growth performance and change the lipid composition and volatile flavor compound profiles in the muscle of abalone *Haliotis discus hannai* by affecting the glycerophospholipid metabolism. **Aquaculture Reports** 30: 101567. DOI: 10.1016/j.aqrep.2023.101567.

Datta, S.N., A. Singh and G. Jassal. 2018. Study of gonadal development in striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) during breeding season in Punjab, India. **Journal of Experimental Zoology India** 21(1): 185–189.

De Santis, C., J.F. Taylor, L. Martinez-Rubio, S. Boltana and D.R. Tocher. 2015. Influence of development and dietary phospholipid content and composition on intestinal transcriptome of Atlantic salmon (*Salmo salar*). **PLoSOne** 10(10): e0140964. DOI: 10.1371/journal.pone.0140964.

Dong, G.F., Q. Zou, H. Wang, F. Huang, X.C. Liu, L. Chen, C.Y. Yang and Y.O. Yang. 2014. Conjugated linoleic acid differentially modulates growth, tissue lipid deposition, and gene expression involved in the lipid metabolism of grass carp. **Aquaculture** 432: 181−191. DOI: 10.1016/j.aquaculture.2014.05.008.

Fazio, F. 2019. Fish hematology analysis as an important tool of aquaculture: A review. **Aquaculture** 500: 237−242. DOI: 10.1016/j.aquaculture.2018.10.030.

Feng, S., Z. Cai, R. Zuo, K. Mai and Q. Ai. 2017. Effects of dietary phospholipids on growth performance and expression of key genes involved in phosphatidylcholine metabolism in larval and juvenile large yellow croaker, *Larimichthys crocea*. **Aquaculture** 469: 59−66. DOI: 10.1016/j.aquaculture.2016.12.002.

Flores, A., R. Wiff, K. Ganias and C.T. Marshall. 2019. Accuracy of gonadosomatic index in maturity classification and estimation of maturity ogive. **Fisheries Research** 210: 50−62. DOI: 10.1016/j.fishres.2018.10.009.

Fraher, D., A. Sanigorski, N.A. Mellett, P.J. Meikle, A.J. Sinclair and Y. Gibert. 2016. Zebrafish embryonic lipidomic analysis reveals that the yolk cell is metabolically active in processing lipid. **Cell Reports** 14(6): 1317−1329. DOI: 10.1016/j.celrep.2016.01.016.

Egessa, R., A. Szűcs, L. Ardó, J. Biró, E. Lengyel-Kónya, V. Banjac and Z.J. Sándor. 2024. Dietary inclusion of insect oil: Impact on growth, nutrient utilisation, lipid metabolism, antioxidant and immune-related responses in European catfish (*Silurus glanis* L). **Aquaculture** 592: 741213. DOI: 10.1016/j.aquaculture.2024.741213.

Gibbs, V.K., L.E. Heflin, W.T. Jones, M.L. Powell, A.L. Lawrence, R. Makowsky and S.A. Watts. 2015. Optimizing dietary levels of menhaden and soybean oils and soybean lecithin for pre-gonadal somatic growth in juveniles of the sea urchin *Lytechinus variegatus*. **Aquaculture** 446: 198–205. DOI: 10.1016/j.aquaculture.2015.05.013.

Guo, Q., L. Liu and B.J. Barkla. 2019. Membrane lipid remodeling in response to salinity. **International Journal of Molecular Sciences** 20(17): 4264. DOI: 10.3390/ijms20174264.

Gustiano, R., G.G. Teugels and L. Pouyaud. 2003. Revision of the *Pangasius kunyit* catfish complex, with description of two new species from South-East Asia (*Siluriformes; Pangasiidae*). **Journal of Natural History** 37(3): 357−376. DOI: 10.1080/713834687.

Hadarabi, M.E., C.R. Saad, S.A. Harmin, M.K.A. Satar and A.A. Kenari. 2011. Effects of phospholipids in the diet on biochemical factors of sturgeon fish (*Huso huso*) juveniles. **African Journal of Biotechnology** 10(42): 8511−8516. DOI: 10.5897/AJB10.1788.

Hammock, B.G., R. Hartman, R.A. Dahlgren, *et al*. 2022. Patterns and predictors of condition indices in a critically endangered fish. **Hydrobiologia** 849(3): 675–695. DOI: 10.1007/s10750-021-04738-z.

Hassan, A., M.A. Ambak and A.P.A. Samad. 2011. Crossbreeding of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Pangasius nasutus* (Bleeker, 1863) and their larval development. **Journal of Sustainability Science and Management** 6(1): 28−35.

Huang, Y., J. Xu, Z. Sheng, N. Chen and S. Li. 2021. Integrated response of growth performance, fatty acid composition, antioxidant responses and lipid metabolism to dietary phospholipids in hybrid grouper (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂) larvae. **Aquaculture** 541: 736728. DOI: 10.1016/j.aquaculture.2021.736728.

Huang, Y., J. Xu, Z. Sheng, R. Xie, H. Zhang, N. Chen and S. Li. 2022. Effects of dietary phospholipids on growth performance, fatty acid composition, and expression of lipid metabolism related genes of juvenile hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lancolatus*♂). **Aquaculture Reports** 22: 100993. DOI: 10.1016/j.aqrep.2021.100993.

Ibarz, A., I. Sanahuja, W.G. Nuez-Ortín, L. Martínez-Rubio and L. Fernández-Alacid. 2023. Physiological benefits of dietary lysophospholipid supplementation in a marine fish model: deep analyses of modes of action. **Animals** 13(8): 1381. DOI: 10.3390/ani13081381.

Iswanto, B. and E. Tahapan Ari. 2011. Embryogenesis and development of catfish larvae hybridized between female Siamese catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) with male *jambal* catfish (*Pangasius djambal* Bleeker, 1846) and male catfish nasutus (*Pangasius nasutus* Bleeker, 1863). **Journal of Aquaculture Research** 6(2): 169−186. DOI: 10.15578/jra.6.2.2011.169-186.

Izquierdo, M.S., H. Fernandez-Palacios and A.G.J. Tacon. 2001. Effect of broodstock nutrition on reproductive performance of fish. **Aquaculture** 197(1–4): 25–42. DOI: 10.1016/S0044-8486(01)00581-6.

Izquierdo, M.S., S. Turkmen, D. Montero, M.J. Zamorano, J.M. Afonso V. Karalazos and H. Fernández-Palacios. 2015. Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. **Aquaculture** 449: 18–26. DOI: 10.1016/j.aquaculture.2015.03.032.

Jafari, F., F. Noori, N. Agh, A. Estevez, A. Ghasemi, C. Alcaraz and E. Gisbert. 2021. Phospholipids improve the performance, physiological, antioxidative responses and, lpl and igf1 gene expressions in juvenile stellate sturgeon (*Acipenser stellatus*). **Aquaculture** 541: 736809. DOI: 10.1016/j.aquaculture.2021.736809.

Jaxion-Harm, J. 2021. Effects of dietary phospholipids on early-stage Atlantic Salmon (*Salmo salar*) performance: A comparison among phospholipid sources. **Aquaculture** 544: 737055. DOI: 10.1016/j.aquaculture.2021.737055.

Jisr, N., G. Younes, C. Sukhn and M.H. El-Dakdouki. 2018. Length-weight relationships and relative condition factor of fish inhabiting the marine area of the Eastern Mediterranean city, Tripoli-Lebanon. **The Egyptian Journal of Aquatic Research** 44(4): 299–305. DOI: 10.1016/j.ejar.2018.11.004.

Kiron, V. 2012. Fish immune system and its nutritional modulation for preventive health care. **Animal Feed Science and Technology** 173(1−2): 111−133. DOI: 10.1016/j.anifeedsci.2011.12.015.

Kizmaz, V. 2021. Seasonal Variation of fatty acid composition of phospholipid subclasses in muscle tissue in male, *Alburnus Mussulensis*. **Journal on Mathematic, Engineering and Natural Sciences** (EJONS) 5(17): 32−43. DOI: 10.38063/ejons.373.

Khamcharoen, M., K. Panwanitdumrong, P. Chaivichoo, T. Leamkom, T. Jutagate, K. Payooha and U. Na-Nakorn. 2023. Reproductive parameters of Indian threadfin *Alectis indicus* in Andaman Sea, Thailand. **Journal of Fisheries and Environment** 47: 85–98.

Kokou, F., A. Vasilaki C. Nikoloudaki, A.B. Sari, V. Karalazos and E. Fountoulaki. 2021. Growth performance and fatty acid tissue profile in gilthead seabream juveniles fed with different phospholipid sources supplemented in low-fish meal diets. **Aquaculture** 544: 737052. DOI: 10.1016/j.aquaculture.2021.737052.

Korkmaz, C., Ö. Ay, A.E. Dönmez, B. Demirbağ and C. Erdem. 2020. Influence of lead on reproductive physiology and gonad and liver histology of female *Cyprinus carpio*. **Thalassas: An International Journal of Marine Sciences** 36(2): 597−606. DOI: 10.1007/s41208-020-00232-w.

Lazard, J., P. Cacot J. Slembrouck and M. Legendre. 2009. The fish farming of the *Pangasiidae*. **Cahiers Agricultures** 18(2−3): 164−173. DOI: 10.1684/agr.2009.0284.

Lee, S.M., I.G Jeon and J.Y. Lee. 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). **Aquaculture** 211(1–4): 227–239. DOI: 10.1016/S0044-8486(01)00880-8.

Lin, S.M., F.J. Li, B. Yuangsoi and S. Doolgindachbaporn. 2018. Effect of dietary phospholipid levels on growth, lipid metabolism, and antioxidative status of juvenile hybrid snakehead (*Channa argus* ×*Channa maculata*). **Fish Physiology and Biochemistry** 44(1): 401–410. DOI: 10.1007/S10695-017-0443-3.

Lin, Z., F. Han, J. Lu, J. Guo, C. Qi, C. Wang and L. Chen. 2020. Influence of dietary phospholipid on growth performance, body composition, antioxidant capacity and lipid metabolism of Chinese mitten crab, *Eriocheir sinensis*. **Aquaculture** 516: 734653. DOI: 10.1016/j.aquaculture.2019.734653.

Liu, G., S. Ma, F. Chen, W. Gao W. Zhang and K. Mai. 2020. Effects of dietary lysolecithin on growth performance, feed utilization, intestinal morphology and metabolic responses of channel catfish (*Ictalurus punctatus*). **Aquaculture Nutrition** 26(2): 456−465. DOI: 10.1111/anu.13008.

Lubzens, E., G. Young, J. Bobe and J. Cerdà. 2010. Oogenesis in teleosts: how fish eggs are formed. **General and Comparative Endocrinology** 165(3): 367−389. DOI: 10.1016/j.ygcen.2009.05.022.

Martins, G., P. Diogo, T. Santos, E. Cabrita, W. Pinto, J. Dias and P.J. Gavaia. 2020b. Microdiet formulation with phospholipid modulate zebrafish skeletal development and reproduction. **Zebrafish** 17(1): 27−37. DOI: 10.1089/zeb.2019.1794.

Martins, G.P., B.S.M. Mazini, M.A.F. Campos, D.S. Oliveira and I.G. Guimarães. 2020a. Effect of replacing fish meal protein by crystalline amino acid and soy protein concentrate on growth, feed utilization, and metabolism of *tambaqui Colossoma macropomum* juveniles. **Journal of the World Aquaculture Society** 51(5): 1250–1269. DOI: 10.1111/jwas.12688.

Mendivil, C.O. 2021. Dietary fish, fish nutrients, and immune function: A review. **Frontiers in Nutrition** 7: 617652. DOI: 10.3389/fnut.2020.617652.

Lubzens, E., G. Young, J. Bobe and J. Cerdà. 2010. Oogenesis in teleosts: how fish eggs are formed. **General and Comparative Endocrinology** 165(3): 367−389. DOI: 10.1016/j.ygcen.2009.05.022.

Martins, G., P. Diogo, T. Santos, E. Cabrita, W. Pinto, J. Dias and P.J. Gavaia. 2020b. Microdiet formulation with phospholipid modulate zebrafish skeletal development and reproduction. **Zebrafish** 17(1): 27−37. DOI: 10.1089/zeb.2019.1794.

Martins, G.P., B.S.M. Mazini, M.A.F. Campos, D.S. Oliveira and I.G. Guimarães. 2020a. Effect of replacing fish meal protein by crystalline amino acid and soy protein concentrate on growth, feed utilization, and metabolism of *tambaqui Colossoma macropomum* juveniles. **Journal of the World Aquaculture Society** 51(5): 1250–1269. DOI: 10.1111/jwas.12688.

Mendivil, C.O. 2021. Dietary fish, fish nutrients, and immune function: A review. **Frontiers in Nutrition** 7: 617652. DOI: 10.3389/fnut.2020.617652.

Nandi, S., P. Routray, S.D. Gupta, S.C. Rath, S. Dasgupta, P.K. Meher and P.K. Mukhopadhyay. 2007. Reproductive performance of carp, *Catla catla* (Ham.), reared on a formulated diet with PUFA supplementation. **Journal of Applied Ichthyology** 23(6): 684−691. DOI: 10.1111/j.1439-0426.2007.00874.x.

National Research Council (NRC). 2011. **Nutrient Requirements of Fish and Shrimp.** National Academies Press, Washington, D.C., USA. 392 pp.

Oddsdóttir, C., H.K. Jónsdóttir and E. Sturludóttir. 2023. Haematological reference intervals for pregnant Icelandic mares on pasture. **Acta Veterinaria Scandinavica** 65: 57. DOI: 10.1186/s13028-023-00721-x.

Okomoda, V.T., I.C.C. Koh, A. Hassan, T. Amornsakun and M.S. Shahreza. 2018. Performance and characteristics of the progenies from the reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822). **Aquaculture** 489: 96–104. DOI: 10.1016/j.aquaculture.2018.02.011.

Petersen, C.W. and R.R. Warner. 2002. The ecological context of reproductive behavior. **Coral Reef Fishes** 103–118. DOI: 10.1016/B978-012615185-5/50007-4.

Rahman, M.A., M.R.K. Manon, M.R. Ullah, A. Alam, M.J. Islam, M.A. Siddik and M.M. Iqbal. 2023. Biometric indices, growth pattern, and physiological status of captive-reared indigenous yellowtail brood catfish, *Pangasius pangasius* (Hamilton, 1822). **Environmental Science and Pollution Research** 30(35): 83388−83400. DOI: 10.1007/s11356-023-28328-9.

Rizzo, E. and N. Bazzoli. 2020. **Reproduction and embryogenesis.** In: Biology and Physiology of Freshwater Neotropical Fish (eds. B. Baldisserotto, E.C. Urbinati and J.E.P. Cyrino), pp. 287−313. Academic Press. Cambridge, Massachusetts, United States. DOI: 10.1016/B978-0-12-815872-2.00013-0.

Salas-Leiton, E., M. Conde-Sieira, N. Pelusio, A. Marques, M.R.G. Maia, J.L. Soengas and L.M.P. Valente. 2018. Dietary protein/carbohydrate ratio in low-lipid diets for Senegalese sole (*Solea senegalensis*, Kaup 1858) juveniles. Influence on growth performance, nutrient utilization and flesh quality. **Aquaculture Nutrition** 24(1): 131−142. DOI: 10.1111/anu.12541.

Sarih, S., A. Djellata, H. Fernández-Palacios, R. Ginés, R. Fontanillas, G. Rosenlund M. Izquierdo and J. Roo. 2020. Adequate n-3 LC-PUFA levels in broodstock diets optimize reproductive performance in GnRH injected greater amberjack (*Seriola dumerili*) equaling to spontaneously spawning broodstock. **Aquaculture** 520: 735007. DOI: 10.1016/j.aquaculture.2020.735007.

Sattang, S., D. Amornlerdpison, S. Tongsiri, D. Palić and K. Mengumphan. 2021. Effect of freshwater fish oil feed supplementation on the reproductive condition and production parameters of hybrid catfish (*Pangasius larnaudii* × *Pangasianodon hypophthalmus*, Sauvage, 1878) broodstock. **Aquaculture Reports** 20: 100598. DOI: 10.1016/J.AQREP.2021.100598.

Schlotz, N., J.G. Sørensen and D. Martin-Creuzburg. 2012. The potential of dietary polyunsaturated fatty acids to modulate eicosanoid synthesis and reproduction in *Daphnia magna*: a gene expression approach. **Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology** 162(4): 449−454. DOI: 10.1016/j.cbpa.2012.05.004.

Sebastião, F.A., D. Nomura, R. Sakabe and F. Pilarski. 2011. Hematology and productive performance of Nile tilapia (*Oreochromis niloticus*) naturally infected with *Flavobacterium columnare*. **Brazilian Journal of Microbiology** 42: 282−289. DOI: 10.1590/S1517-83822011000100036.

Sink, T.D. and R.T. Lochmann. 2014. The effects of soybean lecithin supplementation to a practical diet formulation on juvenile channel catfish, *Ictalurus punctatus*: growth, survival, hematology, innate immune activity, and lipid biochemistry. **Journal of the World Aquaculture Society** 45(2): 163–172. DOI: 10.1111/jwas.12108.

Song, D., B. Shi, L. Ding, M. Jin, P. Sun, L. Jiao and Q. Zhou. 2019. Regulation of dietary phospholipids on growth performance, antioxidant activities, phospholipid metabolism and vitellogenesis in prereproductive phase of female swimming crabs, *Portunus trituberculatus*. **Aquaculture** 511: 734230. DOI: 10.1016/j.aquaculture.2019.734230.

Szlinder-Richert, J., Z. Usydus, M. Wyszyński and M. Adamczyk. 2010. Variation in fat content and fatty-acid composition of the Baltic herring *Clupea harengus membras*. **Journal of Fish Biology** 77(3): 585−599. DOI: 10.1111/j.1095-8649.2010.02696.x.

Tan, P., P. Zhang, L. Zhang, W. Zhu, L. Wang, R. Chen, Q. Zhu and D. Xu. 2022. Effects of soybean lecithin on growth performance, intestine morphology, and liver tissue metabolism in rock bream (*Oplegnathus fasciatus*) larvae. **Frontiers in Marine Science** 9: 942259. DOI: 10.3389/fmars.2022.942259.

Taylor, J.F., L. Martinez-Rubio, J. del Pozo, J.M. Walton, A.E. Tinch, H. Migaud and D.R. Tocher. 2015. Influence of dietary phospholipid on early development and performance of Atlantic salmon (*Salmo salar*). **Aquaculture** 448: 262−272. DOI: 10.1016/J.aquaculture.2015.06.012.

Tercero, J.F., C. Rosas, M. Mascaró, G. Poot, P. Domingues, E. Noreña, C. Caamal-Monsreal, C. Pascual, J. Estefanell and P. Gallardo. 2015. Effects of parental diets supplemented with different lipid sources on *Octopus maya* embryo and hatching quality. **Aquaculture** 448: 234−242. DOI: 10.1016/j.aquaculture.2015.05.023.

Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. **Reviews in Fisheries Science** 11(2): 107−184. DOI: 10.1080/713610925.

Tocher, D.R. 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. **Aquaculture** 449: 94–107. DOI: 10.1016/j.aquaculture.2015.01.010.

Torsabo, D., S.D. Ishak, N.M. Noordin, I.C.C. Koh, M.Y. Abduh, B.T. Iber, M.K. Kuah and A.B. Munafi. 2022. Enhancing reproductive performance of freshwater finfish species through dietary lipids. **Aquaculture Nutrition** 2022: 7138012 DOI: 10.1155/2022/7138012.

Torsabo, D., B.T. Iber, N. Idris, V.T. Okomoda, I.C.C. Koh, M.Y. Abduh, N.M. Noordin and A.B. Abol-Munafi. 2023. Phospholipid-supplemented diet impacts on growth, blood metrics, reproductive indices, and fatty acid profiles of *Pangasianodon hypopthalmus*. **Aquaculture Reports** 33: 101802. DOI: 10.1016/j.aqrep.2023.101802.

Torsabo, D., G.A. Ataguba, B.T. Iber, N.M. Noordin, N. Idris, I.C.C. Koh and A.B. Abol-Munafi. 2024. Comparative investigation of biological indices and biochemical compounds of reproductive indicators in male and female *Pangasius nasutus*. **Biodiversitas Journal of Biological Diversity** 25(9): 3105–3115 DOI: 10.13057/biodiv/d250932.

Turchini, G.M., B.E. Torstensen and W.K. Ng. 2009. Fish oil replacement in finfish nutrition. **Reviews in Aquaculture** 1(1): 10−57. DOI: 10.1111/j.1753-5131.2008.01001.x.

Turchini, G.M., D.S. Francis, Z.Y. Du, R.E. Olsen, E. Ringø and D.R. Tocher. 2022. **The lipids.** In: Fish Nutrition (eds. R.W. Hardy and S.J. Kaushik), pp. 303–467. Academic Press. Cambridge, Massachusetts, USA. DOI: 10.1016/B978-0-12-819587-1.00003-3.

Uyan, O., S. Koshio, M. Ishikawa, S. Yokoyama, S. Uyan, T. Ren and L.H.H. Hernandez. 2009. The influence of dietary phospholipid level on the performances of juvenile amberjack, *Seriola dumerili*, fed non-fishmeal diets. **Aquaculture Nutrition** 15(5): 550–557. DOI: 10.1111/J.1365-2095.2008.00621.X.

Wang, J., H. Zhang, X. Chen, Y. Chen and Q. Bao. 2012. Selection of potential probiotic lactobacilli for cholesterol-lowering properties and their effect on cholesterol metabolism in rats fed a high-lipid diet. **Journal of Dairy Science** 95(4): 1645−1654. DOI: 10.3168/jds.2011-4768.

Wang, L., D. Zuo, W. Lv, J. Li, Q. Wang and Y. Zhao. 2013. Effects of dietary soybean lecithin on gonadal development and vitellogenin mRNA expression in the female redclaw crayfish *Cherax quadricarinatus* (von M artens) at first maturation. **Aquaculture Research** 44(8): 1167−1176. DOI: 10.1111/j.1365-2109.2012.03128.x.

Wang, Z., E. Karrar, Y. Wang R. Liu, M. Chang and X. Wang. 2022. The bioactive of four dietary sources phospholipids on heavy metal-induced skeletal muscle injury in zebrafish: A comparison of phospholipid profiles. **Food Bioscience** 47: 101630. DOI: 10.1016/j.fbio.2022.101630.

Wathes, D.C., D.R.E. Abayasekara and R.J. Aitken. 2007. Polyunsaturated fatty acids in male and female reproduction. **Biology of Reproduction** 77(2): 190−201. DOI: 10.1095/biolreprod.107.060558.

Wee, W., G. Téllez-Isaías, Z. Abdul Kari, R. Cheadoloh, M.A. Kabir, K. Mat, S.A. Mohamad Sukri, M.M. Rahman, N.D. Rusli and L.S. Wei. 2023. The roles of soybean lecithin in aquafeed: a crucial need and update. **Frontiers in Veterinary Science** 10: 1188659. DOI: 10.3389/fvets.2023.1188659.

Witeska, M., E. Kondera, K. Ługowska and B. Bojarski. 2022. Hematological methods in fish–Not only for beginners. **Aquaculture** 547: 737498. DOI: 10.1016/j.aquaculture.2021.737498.

Witeska, M., K. Lugowska and E. Kondera. 2016. Reference values of hematological parameters for juvenile *Cyprinus carpio*. **Bulletin of the European Association of Fish Pathologists** 36(4): 169−180.

Yang, Z., C. Guo, S. Xie, Y. Zhang, T. Zhu, W. Zhao, J. Luo, M. Jin and Q. Zhou. 2022. Interactive effects of dietary cholesterol and phospholipids on growth and metabolism of juvenile swimming crab, *Portunus trituberculatus*. **Animal Feed Science and Technology** 294: 115484. DOI: 10.1016/j.anifeedsci.2022.115484.

Yeldan, H. and D. Avşar. 2000. A preliminary study on the reproduction of the rabbitfish (*Siganus rivulatus* (Forsskal, 1775)) in the northeastern Mediterranean. **Turkish Journal of Zoology** 24(2): 173−182.

Yıldız, M., S. Ofori-Mensah, M. Arslan, A. Ekici, G. Yamaner, M.A. Baltacı, S. Tacer and F. Korkmarz. 2020. Effects of different dietary oils on egg quality and reproductive performance in rainbow trout *Oncorhynchus mykiss*. **Animal Reproduction Science** 221: 106545. DOI: 10.1016/j.anireprosci.2020.106545.

Yoo, G.Y., I.S. Park and S. Lee. 2022. Effects of graded dietary lipid levels on growth performance, fatty acid profile, and hematological characteristics of hybrid pufferfish (*Takifugu obscurusx T. rubripes*) juveniles. **Aquaculture Reports** 24: 101120. DOI: 10.1016/j.aqrep.2022.101120.

Zargar, A., Z. Rahimi-Afzal, E. Soltani, A. Taheri Mirghaed, H.A. Ebrahimzadeh-Mousavi, M. Soltani and P. Yuosefi. 2019. Growth performance, immune response and disease resistance of rainbow trout (*Oncorhynchus mykiss*) fed Thymus vulgaris essential oils. **Aquaculture Research** 50(11): 3097−3106. DOI: 10.1111/are.14243.

Zhao, J., Q. Ai, K. Mai, R. Zuo and Y. Luo. 2013. Effects of dietary phospholipids on survival, growth, digestive enzymes and stress resistance of large yellow croaker, *Larmichthys crocea* larvae. **Aquaculture** 410: 122−128. DOI: 10.1016/j.aquaculture.2013.05.018.

Zuo, R., Y. Ning, W. Di, Y. Heqiu, J. Song, J. Ding and Y. Chang. 2022. Effects of dietary lipid sources on the growth, gonad development, fatty acid composition and spawning performance of broodstock, and early larvae quality of sea urchin (*Strongylocentrotus intermedius*). **Frontiers in Marine Science** 9: 927116. DOI: 10.3389/fmars.2022.927116.