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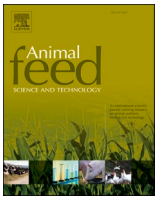

Improving lipid utilization and growth through lecithin inclusion in diets for giant grouper (*Epinephelus lanceolatus*)

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ABSTRACT

Giant grouper (*Epinephelus lanceolatus*) is a culturally and economically significant farmed fish species in Asia and an emerging aquaculture species in Australia. However, the historical reliance on 'trash fish' as feed has hindered the development of species-specific formulated diets. Preliminary findings suggest that methionine and choline influence lipid metabolism, highlighting the need to re-evaluate dietary lipid levels for optimal growth. Building on these findings, this study examined how dietary lipid levels and phospholipid (lecithin) inclusion interact to influence lipid metabolism and growth performance in *E. lanceolatus*. Six isoproteic diets (44.47 ± 0.05 % protein) were formulated with 10 % or 15 % lipid and 0, 0.5 %, or 1 % lecithin inclusion. The results showed that increasing lipid content from 10 % to 15 %, combined with 1 % lecithin inclusion, enhanced weight gain by 12.14 %. This growth-promoting effect was associated with elevated circulating triglycerides and altered cholesterol and glucose levels, suggesting improved lipid transport and utilization. In contract, no growth improvement was observed with 0 % or 0.5 % lecithin inclusion. These findings demonstrate the critical role of lecithin in optimizing lipid metabolism and growth performance in juvenile *E. lanceolatus*, providing a foundation for sustainable, species-specific diet formulations.

1. Introduction

Giant grouper (*E. lanceolatus*) is a fast-growing and disease-resistant species, making it a prime candidate for aquaculture (Zhi, 2005; Hasan, 2012). It is widely farmed across Asia as both a discrete species and as a paternal contributor to hybrid grouper (Rimmer and Glamuzina, 2019). However, the heavy reliance on 'trash fish' as a primary feed source poses sustainability challenges due to inconsistent supply and increasing demand from this rapidly expanding industry (Rimmer and Glamuzina, 2019; Dennis, 2021; Bunlipatanon et al., 2014; Nankervis et al., 2022). Transitioning to formulated diets tailored to species-specific requirements is essential to optimize growth, improve feed efficiency, and maintain fish health (Williams, 2009; Rimmer et al., 2004).

Despite its prominence in aquaculture, significant knowledge gaps remain regarding the nutritional requirements of *E. lanceolatus*. While its fast growth rate makes it economically attractive, the species exhibits limited ability to efficiently utilize dietary lipids for optimal growth. In species capable of effective lipid utilization, dietary lipids enhance energy density, sparing protein for growth rather than energy metabolism (Craig et al., 2017; Chandler and White, 2017; Tseng and Hwang, 2008). Conversely, poor lipid

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metabolism can result in adverse health effects, predominantly in the liver (Hansen et al., 2020; Espe et al., 2010; Cai et al., 2017; Obeid and Herrmann, 2009). Enlarged fatty livers are a common issue in grouper nutrition (Nankervis et al., 2022) which is often associated with elevated dietary fat or poor fat metabolism.

The process of lipid metabolism is inherently complex. In the aqueous environment of the digestive tract, lipids form globules that must be emulsified by bile salts and phospholipids to enable absorption in the intestine. This natural emulsification process has been identified as a key limiting factor in lipid metabolism (Kumar et al., 2012). For efficient transport to peripheral tissues, dietary lipids rely on phospholipids for their incorporation into lipoproteins and chylomicrons. Phospholipids play a crucial role in chylomicron assembly and secretion, ensuring efficient lipid absorption and transport from the intestine to other tissues (Tocher et al., 2008; Xiao and Lewis, 2012). Studies have shown that phospholipid deficiency can lead to lipid accumulation in intestinal enterocytes, impairing lipid metabolism and negatively impacting growth (Fontagne et al., 1998; Liu et al., 2002; Bai et al., 2022).

While the role of phospholipids in lipid metabolism is well established, the specific contribution of lecithin in *E. lanceolatus* remains unclear. Clarifying lecithin's role in lipid emulsification, chylomicron formation, and hepatic lipid accumulation is crucial for addressing metabolic constraints in this species.

It is hypothesized that *E. lanceolatus* demonstrates attenuated growth and feed efficiency on diets exceeding ~10 % lipid due to these metabolic constraints. Our preliminary findings suggest that optimizing dietary methionine and choline levels can enhance lipid metabolism in *E. lanceolatus* (Butler et al., 2025). Building on this, we propose that supplementing diets with intact phospholipids, such as lecithin, could further improve lipid emulsification and transport, potentially mitigating metabolic limitations at higher lipid levels. Specifically, we hypothesize that lecithin supplementation in high-lipid diets will reduce hepatic stress, enhance lipid utilization efficiency, and improve overall growth performance in *E. lanceolatus*.

Lecithin, a bio-emulsifier rich in phospholipids, has been shown to improve digestibility and promote growth in various aquaculture species (Kumar et al., 2012; El-Sayed et al., 2021; Poston, 1990, 1991). Its cost-effectiveness compared to alternative emulsifiers makes it particularly relevant for aquafeed formulations. However, its impact on grouper nutrition remains largely unexplored.

Therefore, this study investigates the role of dietary lecithin in modulating lipid metabolism in *E. lanceolatus*. Specifically, we assess

Table 1
Formulation and composition of raw materials in experimental diets, including low (10 %) and high (15 %) lipid levels and increasing lecithin inclusions, crude protein and lipid contents, ash, and moisture content.

Ingredients	Experimental Diets					
	Diet 1 (10 % Lipid, 0 %Lecithin)	Diet 2 (10 % Lipid, 0.5 % Lecithin)	Diet 3 (10 % Lipid, 1 % Lecithin)	Diet 4 (15 % Lipid, 0 % Lecithin)	Diet 5 (15 % Lipid, 0.5 % Lecithin)	Diet 6 (15 % Lipid, 1 % Lecithin)
Fish meal[a]	20	20	20	20	20	20
Wheat gluten meal[g]	15	15	15	15	15	15
Lupin seed meal[a]	5	5	4.5	4.5	4.5	4.5
Soy Protein Concentrate[a]	22	22	22	22	22	22
Blood meal[a]	5.5	5.5	5.5	5.5	5.5	5.5
Canola oil[d]	3	3	3	8.15	8.15	8.15
Fish oil[a]	3.52	3.52	3.52	3.52	3.52	3.52
Wheat (whole)[b]	23.68	23.18	23.18	19.03	18.53	18.03
Vitamin Premix[e]	0.1	0.1	0.1	0.1	0.1	0.1
L-Lysine[a]	0.67	0.67	0.67	0.67	0.67	0.67
DL-Methionine[c]	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride 70 %[h]	0.69	0.69	0.69	0.69	0.69	0.69
Sunflower lecithin[i]	0	0.5	1	0	0.5	1
Mineral Premix[f]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin E−50[j]	0.02	0.02	0.02	0.02	0.02	0.02
Yttrium oxide[k]	0.1	0.1	0.1	0.1	0.1	0.1
Proximate Composition (Dry matter basis except moisture)						
Crude Protein	44.73	44.44	44.49	44.40	44.41	44.39
Crude Lipid	12.23	12.19	12.44	17.23	17.27	17.87
Ash	8.8	8.8	8.6	8.8	8.6	8.6
Moisture Content	6.46	5.71	7.80	7.17	7.80	7.70

[a] Skretting, Australia, Cambride Tasmania
[b] Feed 2 Go, Twonsville, Australia
[c] Evonik, Essen, Germany
[d] Gold Sunset, Davies Collison Cave Pty Ltd, NSW, Australia
[e] Composition (g/kg unless otherwise stated): Biotin, 1; Folic Acid, 5; Niacin, 45; Pantothenic acid, 10; Pyridoxine, 10; Riboflavin, 20; Thiamin, 10; Vitamin B12, 0.05; Vitamin C, 150; Vitamin A, 3000 IU/g; Vitamin D, 2400 IU/kg; Vitamin K (menadione), 10; Inositol, 250; Antioxidant, 15.
[f] Composition (g/kg): Magnesium, 59.4; Copper, 1; Iron, 8; Manganese, 5; Selenium, 0.02; Zinc, 20; Iodine, 0.8; Cobalt, 0.1; Ash, 700; Moisture, 20
[g] Manildra, Gladesvale, NSW, Australia
[h] Jujia Biotechnology, China
[i] Now Foods, Bloomingdale, USA
[j] Rovimix E-50, DSM, Heerlen, Netherlands
[k] Sigma-Aldrich, Burlington, USA

whether lecithin inclusion improves lipid utilization and enhances growth in juvenile *E. lanceolatus*. Additionally, we examine whether high-lipid diets supplemented with lecithin reduce hepatic lipid accumulation, thereby alleviating metabolic stress associated with excessive dietary lipid intake.

2. Materials and methods

2.1. Sample collection and experimental design

Juvenile *E. lanceolatus* (mean weight 232.12 ± 2.82 g) were obtained from the Company One hatchery in Cairns, Queensland. Upon arrival, the fish were transferred to a 2000 L holding tank at James Cook University's Marine and Aquaculture Research Facilities Unit (MARFU) in Townsville. They were acclimated and fed a commercial marine fish feed (Nova FF, Skretting, Cambridge, Tasmania) for 4 months prior to the start of the feeding trial. Feed was withheld for 24 h before fish were weighed and stocked into experimental tanks.

Twelve fish were randomly allocated to each of 18 experimental 500 L tanks within a recirculating aquaculture system (RAS). Each dietary treatment was replicated in triplicate, with each tank being hand-fed the six formulated diets once daily to satiation for 36 days. Uneaten feed was re-collected from the tank outlet, dried to a constant weight, and used to correct feed intake.

The flow rate was maintained at 800–900 L/min for each tank. Water quality was monitored daily and kept within the following optimal ranges for giant grouper: temperature 27 ± 1 °C, salinity 31.72 ± 1.42 ppt, dissolved oxygen > 6 mg/L, total ammonia-nitrogen ≤ 0.25 ppm, nitrate-nitrogen ≤ 0.25 ppm, and pH at 8 ± 0.5.

2.2. Diet formulation and analysis

Six isoproteic diets (44.47 ± 0.05 %) were formulated with three lecithin inclusion levels (0, 0.5, and 1 %) at each of two lipid inclusion levels (10 % and 15 %) (Table 1).

The dry raw materials were individually weighed to the nearest 0.1 g before being ground through a SR 300 Rotor Beater Mill (Retsch, Haan, Germany) using a 750 μm screen. The ingredients were then mixed for 10 minutes with a A200 planetary mixer (Hobart, Troy, Ohio USA) before adding approximately 25 % water by mass. The complete mixture was then extruded to form the pelleted feed with a 35 mm single screw extruder (Telford Smith Engineering, Dandenong, Victoria, Australia). The pellets were then placed in a TD-700F Thermocline Scientific dehydrating oven at 100 °C until reaching a moisture content of 7.10 ± 0.35, as determined by a MX-50 moisture analyser (A&D, Adelaide, Australia). Lecithin was added to the oils and mixed before evenly coating the dry pellet kernels in a cement mixer. Finished feeds were then stored at −18 °C until used.

Each diet was analysed for ash content using a Digital Muffle Furnace (Daihan Scientific, Korea). Moisture content was determined using a moisture analyser (MS-70, A&D Company, Japan). Yttrium was measured using inductively coupled plasma mass spectrometry (ICP-MS). Protein content was analysed using the Kjeldahl method (N × 6.25) with a KjelFlex K-365 system (Buchi, Flawil, Switzerland). Crude lipid content was determined using a Fat Extractor E-500 (Buchi, Switzerland), following the AOAC method (1995).

2.3. Sampling and fish handling procedures

The timing of the last feeding event before sampling was adjusted for each tank to ensure that sampling was completed 19–21 h postprandially, minimizing any potential postprandial trends in data collection. All fish were euthanized with an overdose of Aqui-S® (400 mg/L) before weighing to the nearest 0.01 g and measuring to the nearest mm.

Blood was drawn from the caudal vein of six randomly selected fish, using a 1 mL syringe tipped with a 19 g × 1 ½ gauge needle (Terumo Corporation, Philippines) pre-flushed with a 10 % ethylenediaminetetraacetic acid (EDTA) solution. The blood samples were then centrifuged for 5 minutes (FC5306 Frontier centrifuge, Ohaus, Parsippany, New Jersey, USA). Plasma was extracted using a micropipette, transferred to a 1.5 mL microcentrifuge tube, and stored at –18°C until biochemical analysis.

All fish were dissected, and their livers were removed and weighed to the nearest 0.01 g. Ten fish livers from each tank were pooled for proximate analysis and stored at –18 °C before being transferred to a –80 °C freezer for 24 h, followed by freeze-drying. Crude lipid content was determined using a Fat Extractor E-500 (Buchi, Switzerland), following the methods of AOAC (1995).

Faecal matter was stripped from the hindgut of all fish post-dissection, pooled per tank, and stored at –18°C before being transferred to a –80°C freezer for 24 h, then freeze-dried. Yttrium content in the freeze-dried faecal samples was analysed using ICP-MS.

2.4. Response variables and calculations

The following equations were used to calculate the response variables for *E. lanceolatus* in this experiment:

$$\textit{Survival Rate (SR; \%)} = \left\{ \frac{\textit{No.individuals at the}\mathbf{start}\textit{of the experiment}}{\textit{No.of individuals at the}\mathbf{end}\textit{of the experiment}} \right\} x\ 100$$

$$\textit{Food Conversion Ratio (FCR)} = \frac{\textit{Total Feed Consumed}(g)}{\textit{Total Weight Gained}(g)}$$

$$\textit{Specific Growth Rate (SGR; \% / day)} = \left\{ \frac{\textit{In Final Weight}(g) - \textit{In Initial Weight}(g)}{\textit{Experimental Period}(days)} \right\} \times 100$$

$$\textit{Hepato-somatic Index (HSI; \%)} = \left\{ \frac{\textit{Liver Weight}(g)}{\textit{Body Weight}(g)} \right\} \times 100$$

$$\textit{Feed intake (g)} = \frac{\textit{Total feed consumed}(g)}{\textit{Number of fish per tank}}$$

$$\textit{Feed Intake \% Body Weight /day} = \left\{ \frac{(\textit{Feed Intake}(g)/(\textit{Final Weight}(g) + \textit{Initial Weight}(g))/\ 2)}{\textit{Experimental Period}(days)} \right\} \times 100$$

$$\textit{Condition Factor (K)} = \frac{(100 \times \textit{Weight}(g))}{\textit{Length}(cm)^3}$$

2.5. Statistical Analysis

Data exploration and statistical analysis were conducted using R and RStudio Desktop (version 2022.07.0 + 548).

All variables were first tested for normality using the Shapiro-Wilk test, followed by Levene's test for homogeneity of variance. If the assumptions of normality and equal variance were met, a two-way ANOVA was performed to assess significant differences ($P < 0.05$), followed by Tukey's post hoc test.

If the assumptions for ANOVA were not met, a non-parametric Kruskal-Wallis test was conducted, followed by Wilcoxon's signed-rank test to determine significant differences ($P < 0.05$)

3. Results

The survival rate of *E. lanceolatus* was unaffected by dietary treatments. While no significant differences were found between diets for weight gain (WG) or specific growth rate (SGR) using ANOVA, the highest mean SGR was observed in the diet containing 1 % lecithin and 15 % lipid inclusion (Table 2, Fig. 1). A *t*-test revealed a significant ($P < 0.05$) increase in SGR by 8.14 % and WG by 12.14 % for this diet compared to the equivalent diet with 1 % lecithin and 10 % lipid (Table 2). Additionally, a significant increase ($P < 0.05$) in WG by 17.41 % was observed when comparing the diet with no lecithin to the diet with 1 % lecithin at 15 % lipids. No significant differences in WG or SGR were detected with increasing lipid levels at 0 % or 0.5 % lecithin inclusion.

Feed intake (FI) was influenced by both the dietary lipid content and the interaction between lecithin and lipid inclusion ($P < 0.05$, Table 2). The increase in lipid content in the diets with 1 % lecithin led to a 14.61 % increase in FI. Diets with 10 % lipid inclusion showed a slight decrease in FI with increasing lecithin, whereas diets with 15 % lipid inclusion exhibited a 12.22 % increase in FI. The feed intake (% body weight per day) also responded significantly ($P < 0.05$) to the increase in lipid content, with a 10.46 % difference observed in diets formulated with 1 % lecithin (Fig. 2). Similarly, diets formulated with 10 % lipid inclusion showed a slight decrease

Table 2
Biometric performance of giant grouper (*E. lanceolatus*) at each treatment group of low (10 %) and high (15 %) lipid levels with increasing lecithin inclusion. Data presented as mean diet values including ± standard error, significance indicates by * when $P < 0.05$, and * * when $P < 0.01$. Sig.: Significance. ns: not significant.

Biometric parameter	Diet 1 (10 % Lipid, 0 % Lecithin)	Diet 2 (10 % Lipid, 0.5 Lecithin)	Diet 3 (10 % Lipid, 1 % Lecithin)	Diet 4 (15 % Lipid, 0 % Lecithin)	Diet 5 (15 % Lipid, 0.5 % Lecithin)	Diet 6 (15 % Lipid, 1 % Lecithin)	Sig.		
							Interaction (Lecithin × lipid)	Lecithin	Lipid
Initial Weight (g)	231.3 ± 0.96	232.21 ± 2.47	229.93 ± 1.72	231.34 ± 2.24	234.70 ± 0.72	233.21 ± 1.20	ns	ns	ns
Initial Length (cm)	220.52 ± 0.13	219.33 ± 0.05	222.3 ± 0.05	219.58 ± 0.14	220.86 ± 0.02	222.36 ± 0.06	ns	ns	ns
Finial Weight (g)	386.01 ± 11.71	392.23 ± 2.84	382.63 ± 2.50	374.9 ± 5.74	397.08 ± 9.80	407.01 ± 2.27	ns	ns	ns
Final Length (mm)	254.03 ± 1.53	254.25 ± 0.77	252.53 ± 1.56	250.32 ± 0.48	255.39 ± 0.36	256.67 ± 0.89	ns	ns	ns
Weight Gain (WG)	154.68 ± 10.84	160.02 ± 1.95	152.7 ± 1.40	143.55 ± 7.74	162.38 ± 10.47	173.79 ± 3.09	ns	ns	ns
Specific Growth Rate (SGR%)	1.13 ± 0.06	1.16 ± 0.02	1.13 ± 0.01	1.08 ± 0.05	1.17 ± 0.06	1.24 ± 0.02	ns	ns	ns
Feed Intake (g)	156.72 ± 5.05	158.36 ± 3.70	151.45 ± 3.18	155.69 ± 6.36	165.62 ± 5.88	177.35 ± 2.91	0.039 *	ns	0.016 *
Feed Intake % Body Weight d^{-1}	1.41 ± 0.02	1.41 ± 0.02	1.37 ± 0.04	1.43 ± 0.05	1.46 ± 0.04	1.54 ± 0.03	ns	ns	0.019 *
Feed Conversion Ratio (FCR)	1.02 ± 0.04	0.99 ± 0.03	0.99 ± 0.02	1.09 ± 0.02	1.02 ± 0.04	1.02 ± 0.03	ns	ns	Ns
Condition Factor (K)	2.34 ± 0.05	2.38 ± 0.02	2.37 ± 0.03	2.38 ± 0.02	2.37 ± 0.06	2.4 ± 0.01	ns	ns	Ns
Hepatosomatic Index (HIS %)	7.33 ± 0.08	7.69 ± 0.23	7.66 ± 0.15	6.67 ± 0.13	6.26 ± 0.10	6.64 ± 0.08	0.049 *	ns	8.64e−07 * *

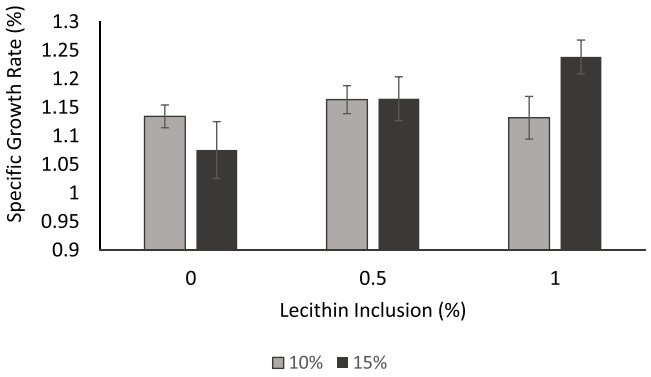

Fig. 1. Bar chart of the Specific Growth Rate (SGR) of juvenile giant grouper (*E. lanceolatus*) in each treatment group, containing low (10 %) and high (15 %) lipid levels with increasing lecithin inclusion. Bars show the means fitted with standard error and different letters indicate significant differences ($P < 0.05$).

in FI % body weight per day by 2.86 %, whereas diets with 15 % lipid inclusion showed a 7.80 % increase in FI % body weight per day.

No noticeable trend was observed in the hepatosomatic index (HSI). However, an interaction between increasing lipid content and lecithin inclusion led to a significant decrease in HSI ($P < 0.05$, Table 2).

The feed conversion ratio (FCR) was unaffected by dietary treatment ($P > 0.05$). However, when diets formulated with 15 % lipids and 1 % lecithin were tested, circulating triglyceride (TAG) levels increased by 23.69 % ($P < 0.05$) (Table 3). Plasma glucose levels were consistently higher in the low-lipid diets compared to the high-lipid diets ($P < 0.05$, Fig. 3). In the low-lipid diets, mean glucose values decreased by 13.35 % with increasing lecithin inclusion, whereas in the high-lipid diets, glucose increased by 21.79 % with lecithin inclusion (Table 3).

Both high-density lipoproteins (HDL) and alanine transaminase (ALT) levels were higher in the high-lipid diets. Mean values for both HDL and ALT decreased with increasing lecithin inclusion. Specifically, increasing lecithin to 1 % in high-lipid diets decreased ALT by 50.37 % and HDL by 11.13 %. No significant responses were observed in lactate dehydrogenase (LDH), aspartate aminotransferase (AST), low-density lipoproteins (LDL), or albumin (ALB) ($P > 0.05$, Tables 3, 4).

Liver lipid content analysis showed no significant effects from lipid or lecithin inclusion independently ($P > 0.05$). However, a statistical interaction was observed, indicating that the effect of lecithin inclusion depends on the lipid content of the diets in this experiment ($P < 0.05$, Table 5).

4. Discussion

In the present study, dietary inclusion of lecithin enhanced the growth rates of *E. lanceolatus* when fed higher lipid feeds. Increasing lipid levels has previously been shown to enhance energy yields and growth in other fish species (Gómez-Requeni et al., 2013). However, this approach poses a risk of excessive fat accumulation if lipids are not properly metabolized (Kumar et al., 2012). Lecithin, a phospholipid, plays a crucial role in the formation of lipoproteins and chylomicrons, which are essential for transporting lipids from the intestine to the liver, and from the liver to other tissues (Kumar et al., 2012; El-Sayed et al., 2021; El-Naggar et al., 2021; Feingold and Grunfeld, 2015). This study effectively demonstrated that the addition of lecithin facilitates the efficient utilization of increased dietary lipids for energy in juvenile *E. lanceolatus*.

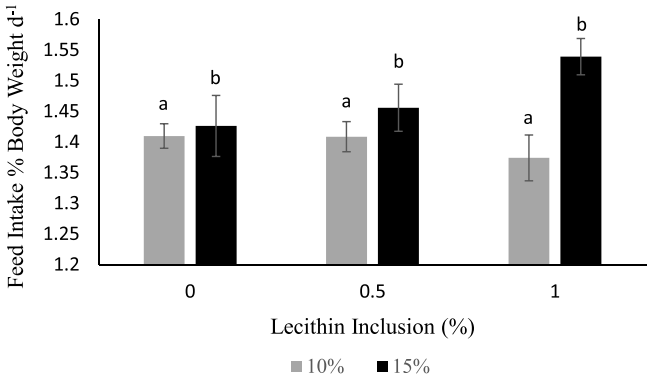

Fig. 2. Mean (± s.e.) Feed Intake (% body weight per day) of juvenile giant grouper (*E. lanceolatus*) fed diets containing low (10 %) and high (15 %) lipid levels. Means with different superscripts ($^{a\text{-}b}$) are significantly different (One way ANOVA $P < 0.05$).

Table 3
Plasma biochemistry data of juvenile giant grouper (*E. lanceolatus*) at each treatment group containing low (10 %) and high (15 %) lipid levels with increasing lecithin inclusion. Data presented as mean diet values including ± standard error, significance (sig.) indicates by different lettering.

Diets	Plasma biochemistry							
	GLUC (mmol/L)	Sig.	TAG (mmol/L)	Sig.	ALT (U/L)	ALB (g/dL)	AST (U/L)	LDH (U/L)
Diet 1 (10 % lipid, 0 % lecithin)	8.32 ± 2.24	B	1.27 ± 0.09	AB	1869.4 ± 591.93	7.14 ± 0.44	75.4 ± 17.70	2021.76 ± 712.38
Diet 2 (10 % lipid, 0.5 % lecithin)	8.32 ± 1.02	B	1.25 ± 0.16	AB	1677.47 ± 131.91	6.9 ± 0.31	136.2 ± 27.27	3485.81 ± 125.37
Diet 3 (10 % lipid, 1 % lecithin)	6.96 ± 0.47	B	1.17 ± 0.07	AB	1827.27 ± 263.92	6.62 ± 0.27	226.4 ± 129.07	2595.17 ± 920.41
Diet 4 (15 % lipid, 0 % lecithin)	4.72 ± 0.32	A	1.16 ± 0.04	AB	4418.47 ± 140.67	7.28 ± 0.16	144.2 ± 20.85	2570.31 ± 188.53
Diet 5 (15 % lipid, 0.5 % lecithin)	5.82 ± 0.73	AB	1.35 ± 0.08	AB	3791.2 ± 1018.86	7.51 ± 0.66	374.33 ± 258.93	2839.55 ± 692.99
Diet 6 (15 % lipid, 1 % lecithin)	6.03 ± 0.91	AB	1.52 ± 0.06	B	2193.27 ± 1316.89	7.05 ± 0.35	216.13 ± 54.10	2545.48 ± 266.69

GLUC: glucose, TAG: triglycerides, ALT: alanine transaminase, ALB: albumin, AST: aspartate transaminase, and LDH: lactate dehydrogenase

Although hepatic health was a concern, given that poorly formulated feeds have been associated with fat deposition in the liver (Nankervis et al., 2022; Espe et al., 2020; Lu et al., 2013; Reynaldy, 2019), the results of this study showed a decrease in HSI for fish fed high-lipid diets. This decrease likely stemmed from the lower starch content in these diets, as starch was replaced by lipids. The study demonstrated that lecithin's role in lipid metabolism was critical, as it promoted efficient mobilisation of lipids and prevented liver damage, in contrast to the lipid accumulation seen in high-fat diets without lecithin supplementation.

Increased lipid content is often reported to decrease FI in many fish species (Gélineau et al., 2001; Li et al., 2019), but this was not observed in the present study. The decreased FI in low-lipid diets suggests that nutrient requirements were met more effectively, emphasizing the role of lecithin as an emulsifying agent in improving feed formulation. Conversely, there was a significant increase in FI with high-lipid diets supplemented with lecithin, corresponding to the enhanced growth rates observed. This suggests that higher energy demands were likely driving the increased feed consumption in these treatments, further supporting the beneficial role of lecithin in optimizing growth performance.

Transaminase enzymes, such as alanine transaminase (ALT), are naturally present in plasma, but their levels rise significantly when there is organ damage, particularly in the liver (Liu et al., 2021; Peres et al., 2014). In this study, plasma ALT levels were elevated in fish fed 15 % lipid diets compared to those fed 10 % lipid diets, although this effect was mitigated by lecithin inclusion. In our previous studies, ALT levels up to 2500 U/L have been considered within the normal range, while levels exceeding 4000 U/L have been linked to liver cell damage. The higher ALT levels in the plasma of fish fed high-lipid diets without lecithin supplementation likely reflect liver damage due to steatosis (Espe et al., 2020; Sun et al., 2016). The absence of a significant trend in ALT values for the low-lipid diets may suggest that these lipid levels were sufficient to provoke a stress response in the fish, though not to the extent observed with higher lipid levels.

The increased mobility of lipids through lecithin supplementation was evident in the higher circulating triglyceride (TAG) levels observed in fish fed high-lipid diets. These results align with previous studies showing that lecithin supplementation enhances liver lipid mobilisation (El-Sayed et al., 2021; Sivaramakrishnan et al., 2021). Although there were concerns that high lipid diets would lead to insufficient lipid metabolism and result in fat accumulation, the significant decrease in HSI and the increase in circulating TAG levels indicate that lecithin successfully facilitated the transportation of lipids from the liver to the bloodstream, thereby preventing excess lipid deposition in the liver.

The increase in plasma glucose levels associated with high-fat diets has been observed in other species, including hybrid grouper, and is often linked to improved regulation of glycolipid metabolism and increased carbohydrate utilization (Cheng et al., 2006; Kikuchi et al., 2009; Wang et al., 2022; Qian et al., 2021). In this study, the consistently higher plasma glucose levels in fish fed low-lipid diets likely resulted from greater reliance on carbohydrate metabolism for energy. As lipid levels increased, the reliance on carbohydrate metabolism decreased, leading to lower plasma glucose levels. However, glucose levels increased in the high-lipid diets as energy demands rose with enhanced growth.

This study demonstrates that the inclusion of 1 % lecithin enhances the ability of *E. lanceolatus* to utilize dietary lipids, increasing lipid circulation and presumably supplying more energy to growing tissues. The results suggest that dietary lipid levels can be safely increased to 15 % without negative effects on the fish, as lecithin helps optimize lipid utilization. These findings provide valuable insights for improving dietary formulations for *E. lanceolatus*, with potential benefits for growth and energy utilization

While this study provides valuable insights into the role of lecithin in optimizing lipid utilization in *E. lanceolatus*, certain limitations should be acknowledged. Firstly, the study was conducted under controlled laboratory conditions, which may not fully replicate the complexities of commercial aquaculture environments. Differences in water quality, feeding practices, and environmental stressors could influence the observed outcomes. Secondly, the experimental period was limited to juvenile fish, and the long-term effects of high-lipid diets with lecithin supplementation on overall health and performance remain unexplored. Further research is required to assess whether similar benefits persist through later growth stages. Additionally, while biochemical markers such as ALT and triglycerides were used to evaluate liver health and lipid metabolism, histological assessments were not conducted. Future studies

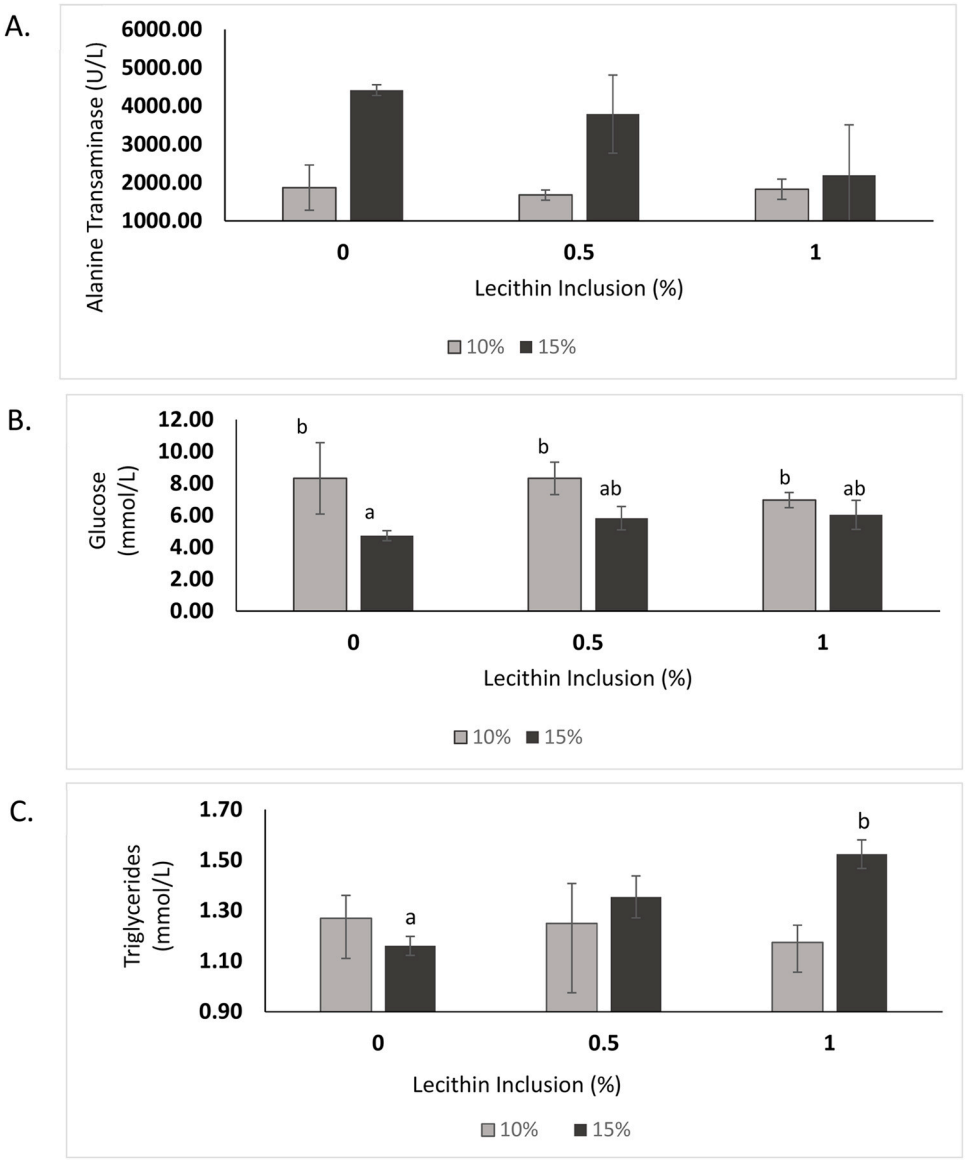

Fig. 3. Plasma biochemistry data for juvenile giant grouper (*E. lanceolatus*) at each treatment group containing low (10 %) and high (15 %) lipid levels with increasing lecithin inclusion on A: alanine aminotransferase (ALT), B: Glucose (GLUC), C: Triglycerides (TAG). Bars represent the means with standard errors, and different letters indicate significant differences ($P < 0.05$).

Table 4
Plasma biochemistry data of juvenile giant grouper (*E. lanceolatus)* at each treatment group containing low (10 %) and high (15 %) levels of lipids with increasing lecithin. Data presented as mean diet values including ± standard error, significance indicates by * when $P < 0.05$. ns: not significant.

Plasma biochemistry	Diet 1 (10 % lipid, 0 % lecithin)	Diet 2 (10 % lipid, 0.5 % lecithin)	Diet 3 (10 % lipid, 1 % lecithin)	Diet 4 (15 % lipid, 0 % lecithin)	Diet 5 (15 % lipid, 0.5 % lecithin)	Diet 6 (15 % lipid, 1 % lecithin)	Sig.		
							Interaction (Lecithin × Lipids)	Lecithin	Lipids
LDL (mmol/L)	0.83 ± 0.07	0.68 ± 0.05	0.76 ± 0.05	0.77 ± 0.08	0.69 ± 0.08	0.79 ± 0.06	ns	ns	ns
CHOL (mmol/L)	2.59 ± 0.16	2.21 ± 0.07	2.26 ± 0.10	2.74 ± 0.07	2.55 ± 0.12	2.59 ± 0.07	ns	0.05 *	0.01 *
HDL (mmol/L)	1.61 ± 0.12	1.39 ± 0.02	1.4 ± 0.07	1.7 ± 0.09	1.65 ± 0.07	1.51 ± 0.12	ns	ns	0.03 *

CHOL: cholesterol, LDL; low density lipoproteins, and HDL; high-density lipoproteins

Table 5
Liver lipid content analysis data of juvenile giant grouper (*E. lanceolatus*) at each treatment group containing low (10 %) and high (15 %) lipid levels with increasing lecithin inclusion.

Diet	Diet 1 (10 % lipid, 0 % lecithin)	Diet 2 (10 % lipid, 0.5 % lecithin)	Diet 3 (10 % lipid, 1 % lecithin)	Diet 4 (15 % lipid, 0 % lecithin)	Diet 5 (15 % lipid, 0.5 % lecithin)	Diet 6 (15 % lipid, 1 % lecithin)
Liver Lipid Content (%)	14.5 ± 0.66	18.6 ± 1.27	17.10 ± 0.92	19.43 ± 1.94	17.63 ± 0.38	17.53 ± 0.19

should incorporate detailed liver histopathology to provide a more comprehensive understanding of lipid deposition and hepatocellular changes.

Ethical approval

This study was conducted with approval from the James Cook University Animal Ethics Committee (A2713).

CRediT authorship contribution statement

GB conducted the experiment, collected the data, analysed the results, and wrote the first draft. LN supervised the student, reviewed the draft, acquired funding, and provided resources. CLC also supervised the student and reviewed the draft. SKD reviewed, edited, analysed and finalised the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Leo Nankervis reports financial support was provided by Australian Centre for International Agricultural Research. Reports a relationship with that includes:. Has patent pending to. If there are other authors, they 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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Data Availability

Data will be provided upon request.

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