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Partial Replacement of Fish Oil in Tra Catfish (*Pangasianodon hypophthalmus*) Diet with Different Levels of LYSOFORTE™ Dry

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Abstract

L.V. Bindhu., C. Sugumar, and Maria Glenda D. Esperida. 2016. Partial Replacement of Fish Oil in Tra Catfish (*Pangasianodon hypophthalmus*) Diet with Different Levels of LYSOFORTE™ Dry. *Aquacultura Indonesiana. 17 (1): 12-19*. Phospholipids are known to play a significant role in lipid transport in crustaceans. LYSOFORTE™ is a bio-emulsifier enriched with lysophospholipids used in animal feeds to improve the digestion and absorption of fat. Objective of the current study was to evaluate the growth promoting effect and fish oil sparing effect of LYSOFORTE™ Dry in Tra Catfish (*Pangasianodon hypophthalmus*) diets. This study comprised four treatment groups: positive control diet (T1) with 1% fish oil, negative control diet (with 1% less fish oil) (T2), negative control diet + LYSOFORTE™ Dry (0.05%) (T3) and negative control diet + LYSOFORTE™ Dry (0.10%) (T4). Each treatment group comprised 3 replicates with 50 juveniles (mean body weight, 20 g ± 0.1) /replicate. Negative control treatments with LYSOFORTE at 0.05% and 0.10% improved the average body weight gain by 16.08 g and 25.74 g (P>0.05) with a corresponding FCR improvement of 1 point and 11 points respectively. Specific growth rate improved by 0.18%/day (0.05% LYSOFORTE™ Dry) and 0.28%/day (0.1% LYSOFORTE™ Dry). Survival percentage also improved in the negative control diets supplemented with LYSOFORTE™ Dry by 6.67%. Protein efficiency ratio increased by 0.22 (T3) and 0.11 (T4) whereas protein retention improved by 4.89% (T3) and 3.63% (T4) with respect to the negative control diet (T2). Lipid retention increased with the use of LYSOFORTE™ Dry: 27% for T3 and 37% for T4 (P<0.05). Moreover, fish fed with negative control diet with 0.10% of LYSOFORTE™ Dry showed on par performance with those fed on the positive control diet. The results suggested the potential of formulating cat fish diets with less fish oil using LYSOFORTE™ Dry

Keywords: Aquaculture; Bio-emulsifier; Fish oil; Growth rate; LYSOFORTE™ Dry; Protein retention; Tra Catfish

Introduction

The striped catfish or tra cat fish (*Pangasianodon hypophthalmus*), farming sector has emerged as an icon of aquaculture development globally and currently accounts for the production of over one million tonnes with an export income exceeding US$ 1 billion. Tra fish culture industry has been facing several challenges such as disease outbreaks, environmental pollution and high investment cost for farming the species. Above all, increasing cost of feed ingredients is one of the big challenges for sustainable farming. Typically, feed cost for Tra catfish farming is approximately 80% of total cost of investment. Although protein and lipid sources are the key ingredients that supply essential nutrients for Catfish, these ingredients contribute to over 60% cost of feed. Hence, reducing feed cost will definitely help to improve profitability and sustainability.

Phospholipids act as emulsifiers and facilitate the digestion and absorption of fat and other lipid-soluble nutrients. The inclusion of phospholipids in the diet can improve culture performance of many fish species (Coutteau *et al.,* 1997). The beneficial effects of dietary phospholipids in fish include improved growth in both larvae and early juveniles, increased survival rates and decreased incidence of malformation in larvae, and perhaps increased stress resistance (Coutteau *et al.,* 1997; Cahu *et al.,* 2003). Dietary phospholipids play a significant role in lipid transport in crustaceans by transporting absorbed lipids from the gut epithelium into the hemolymph, and by facilitating lipid transport between tissues and organs (Coutteau *et al.,* 1997). Although initial studies indicated that the requirement of dietary phospholipids may be restricted to early life stages of fish, limited data is available to demonstrate the effect of phospholipids in adult fish (Dapra *et al.,* 2011; Taylor *et al.,* 2015).

LYSOFORTE™ is a natural bio-emulsifier enriched with lysophospholipids used widely in animal feeds to improve the digestion and absorption of fat. Earlier studies using LYSOFORTE™ demonstrated the efficacy of the

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product in improving performance of post larvae of the pacific white shrimp, *Litopaneaus Vannamei*, Sea bass, juvenile carp and *Penaeus monodon*. This paper summarises the results of a trial which evaluated the efficacy of LYSOFORTE™ for its growth promoting and fish oil sparing effect in Tra Catfish (*Pangasianodon Hypophthalmus*) diets.

Materials and Methods

The trial was conducted at the Laboratory of Fish Nutrition of Research Institute for Aquaculture No.2 (RIA2), Center for Fish Nutrition and Post-Harvest Technology (CEFIPOHATEC) in Vietnam

Experimental Design

Healthy and similar size Tra Catfishes originated from Mekong Delta were selected for the trial. Tra Catfish juveniles (initial mean body weight, 20 g ± 0.1 g) were stocked at the National Breeding Center for Southern Freshwater Aquaculture (An Thai Trung Commune, Cai Be District, Tien Giang Province) and then transferred to the Lab of fish nutrition, Research Institute for Aquaculture No.2 (RIA2), Center for Fish Nutrition and Post-harvest Technology (CEFIPOHATEC) in Vietnam. Tra Catfish juveniles were stocked in fiberglass tanks for acclimatization before the feeding trial. During the acclimatized period of 2.5 weeks, fish were fed commercial diet and then gradually adapted with the experimental feeds. Fish were starved for 24 hours before they were transferred into the experimental tanks. Water was stored for stabilization and sensory tested for quality before using for the experiments. Fiberglass tanks of 0.5 m^3 water volume were used for the trials.

Husbandry

Fish were fed twice daily (8:00 and 16:00) to satiation for 90 days. After 30 minutes feeding, uneaten feed, leftover feed in the bottle and feed consumed were recorded daily. During the feeding trial, faeces were siphoned and water supplied from 3 to 4 times every week.

Environmental parameters

- Temperature (^{0}C) and pH: measured using pH meter HANA (2 times per day at 8:30 and 14:30);
- Dissolved oxygen (DO) (mg/L): measured using DO meter– HANA (once a week at 10:00 am);
- Determination of NH_3-N (mg/L) and NO_2-N (mg/L) concentration: using SERA test kit (once a week)

Diets used for the trial are given in Table 1. Experimental diets were manufactured at the feed mill of RIA2 using extrusion system. Composition of the diets used for the trial is given in Table 2 and the proximate composition of diets is given in Table 3.

Parameters Tested

The following parameters were monitored.

- Growth performance and feed utilization
- Weight gain (WG): WG (g) = Wf/Nf – Wi/Ni
- Specific growth rate (SGR):
- SGR (% per day) = 100*[Ln (Wf/Nf) – Ln (Wi/Ni)]/T
- Survival rate definition (SUR): SUR (%) = Nf/Ni *100
- Feed conversion ratio (FCR): FCR = I/(Wf – Wi + Wd)
- Protein efficiency ratio (PER): PER = WG/MP
- Protein retention (PR): PR (%) = (Wf*CPt – Wi*CP0 + Wd*CP0)/I*CPf
- Lipid retention (LR): LR (%) = (Wf*CLt – Wi*CL0 + Wd*CL0)/I*CLf
- Feed intake (FI): FI (% per day) = 100*I/[(Wi + Wf)/2*T]

Where,

- I (g) is total feed consumption on a dry weight basis
- Wi (g) is total initial body weight
- Wf (g) is total final body weight
- Wd (g) is total body weight of the dead fish
- Ni is number of fish at the start
- Nf is number of fish at the end of the trial
- T (day) is duration of the trial
- MP (g/kg) is total protein content of the test diets
- CPt (g/kg) is protein content in whole fish body at the end of the trial
- CP0 (g/kg) is protein content in whole fish body at the start of the trial
- CPf (g/kg) is protein content of the test diets

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- CLt (g/kg) is lipid content in whole fish body at the end of the trial
- CL0 (g/kg) is lipid content in whole fish body at the start of the trial
- CLf (g/kg) is lipid content of the test diets

***Statistics*:** Data was analysed using Microsoft Excel and a statistic software named SPSS 16.0.

Table 1. Treatments used for the trial

Treatment	Group	Description
T1	Positive Control (PC)	Basal diet
T2	Negative Control (NC)	Basal diet – Less 1% Fish oil
T3	NC + LYSOFORTE™	Negative control diet + 0.05% LYSOFORTE™
T4	NC + LYSOFORTE™	Negative control diet + 0.1% LYSOFORTE™

Table 2. Composition of experimental diets (kg)

Feed ingredient	*Positive control (T1)*	*Negative control + LYSOFORTE™ (T2)*	*Negative control + 0.05% LYSOFORTE™ (T3)*	*Negative control +0.1% LYSOFORTE™ (T4)*
Cassava-Piece	120.000	120.000	120.000	120.000
Rice bran	359.580	373.590	372.090	371.590
Wheat bran	100.000	100.000	100.000	100.000
SBMHipro (USA)	249.000	249.000	249.000	249.000
Rape seed meal	30.000	30.000	30.000	30.000
MBM 50%	75.000	71.000	72.000	72.000
Fish Meal 60%	50.000	50.000	50.000	50.000
Fish Oil	10.000	0	0	0
Catfish Premix	2.500	2.500	2.500	2.500
Choline Chloride 60%	0.500	0.500	0.500	0.500
VIT.C Coated	0.500	0.500	0.500	0.500
Selen-glycinate	0.020	0.020	0.020	0.020
DL-Methionine	2.450	2.440	2.440	2.440
Alpha D3	0.250	0.250	0.250	0.250
LYSOFORTE™	0	0	0.500	1.000
Endox-C	0.200	0.200	0.200	0.200

Table 3. Proximate composition of experimental diets

Parameter	*Positive control (T1)*	*Negative control + LYSOFORTE™ (T2)*	*Negative control + 0.05% LYSOFORTE™ (T3)*	*Negative control + 0.1% LYSOFORTE™ (T4)*
Protein (%)	26.01	25.99	26.02	26.01
Fat (%)	6.95	6.11	6.10	6.10
Fiber (%)	5.10	5.20	5.19	5.19
Moisture (%)	10.10	10.22	10.21	10.20
Ash (%)	7.56	7.60	7.61	7.61

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Results

Results of supplementing LYSOFORTE™ to Tra Catfish diets are shown in Table 4 and 5. Water quality data is given in Figures 1 to 5.

Table 4. Effect of adding LYSOFORTE™ on body weight gain, growth rat and survival of Tra Cat fish

Treatments	*Initial Body weight (g)*	*Final body weight (g)*	*Weight gain (g)*	*Specific growth rate (% per day)*	*Survival (%)*
Positive Control (PC)	23.50	112.29	88.79	1.68	97.33
Negative Control (NC)	23.65	93.29	69.65	1.45	93.33
NC + LYSOFORTE™ 0.05%	23.71	109.43	85.73	1.63	100
NC + LYSOFORTE™ 0.1%	23.67	119.07	95.39	1.73	100
p-value	*0.9553*	*0.4382*	*0.4399*	*0.3988*	*0.5115*

Table 5. Effect of adding LYSOFORTE™ on Feed intake, feed conversion ratio, protein efficiency ratio, protein and lipid retention of Tra Catfish

Treatments	*Feed intake (g per day)*	*Feed conversion ratio*	*Protein efficiency ratio*	*Protein retention (%)*	*Lipid retention (%)*
Positive Control (PC)	1.15	1.20	2.84	43.76	156.95
Negative Control (NC)	0.96	1.31	2.76	42.11	163.30
NC + LYSOFORTE™ 0.05%	1.18	1.30	2.98	47.00	190.56
NC + LYSOFORTE™ 0.1%	1.23	1.20	2.87	45.74	200.30
p-value	*0.4574*	*0.1835*	*0.3740*	*0.0702*	*0.0000*

Figure 1. Changes in water pH recorded for the four treatments during the study

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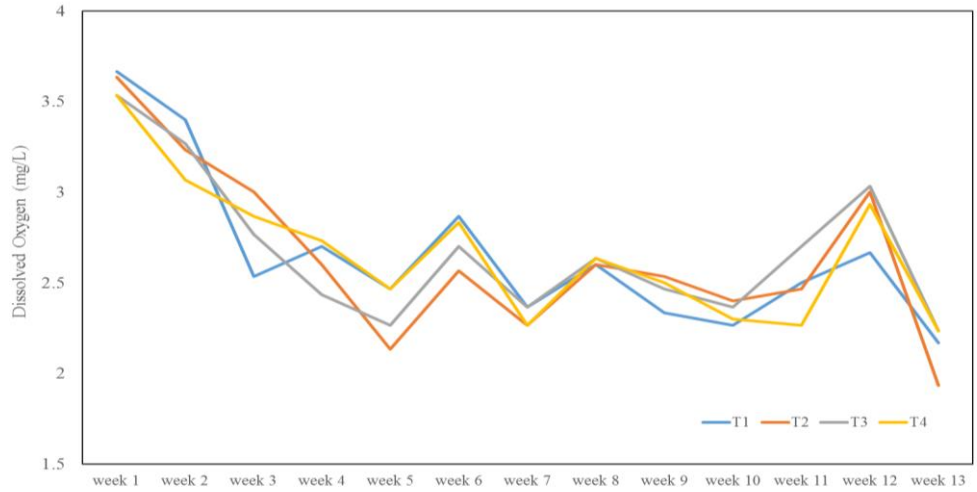

Figure 2. Dissolved oxygen content of treatments during the study

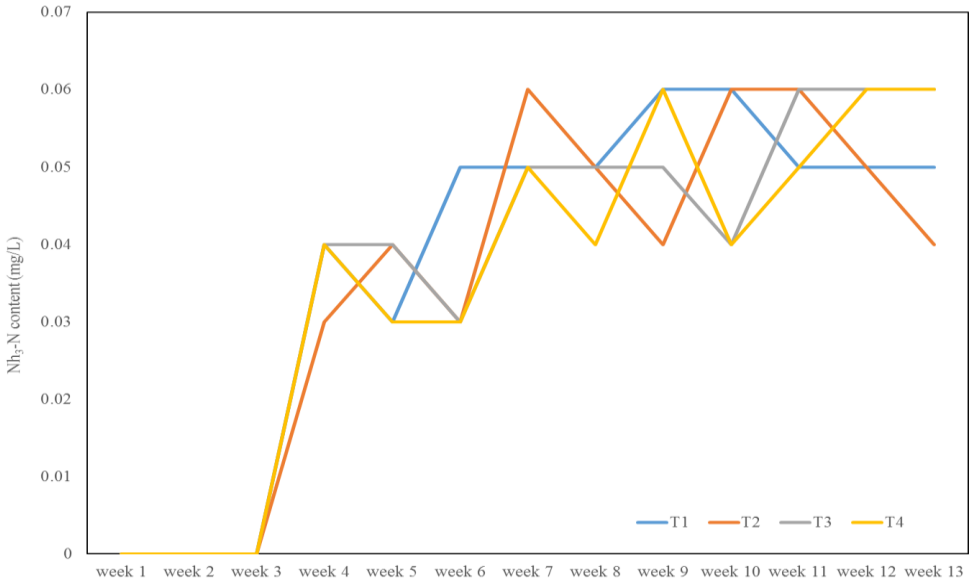

Figure 3. Changes in NH3-N content during the study

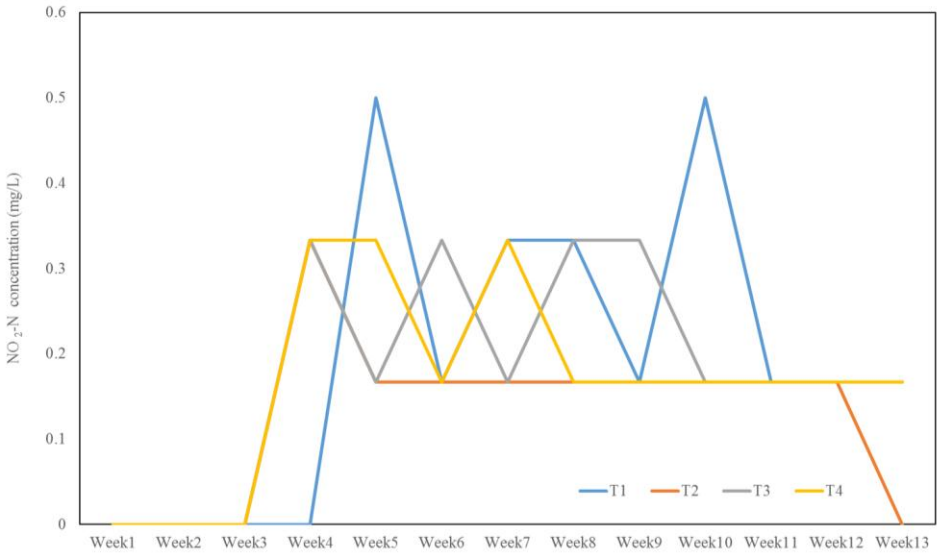

Figure 4. Changes in NO_2-N content monitored during the study

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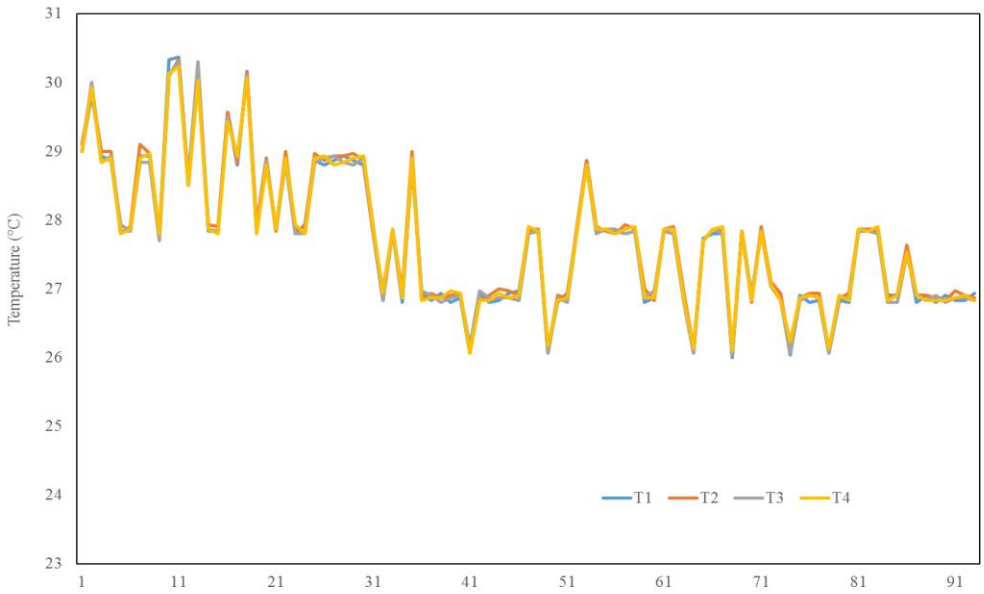

Figure 5. Variation in water temperature of the treatments monitored during the study

Discussion

Effect of reducing fish oil content on growth performance of Tra Cat fish

Decrease of fish oil in the negative control diet resulted in lower final body weight by 19g and body weight gain by 19.14 g compared to the positive control respectively (P>0.05). Specific growth rate decreased by 0.23%/day and the survival showed a decline by 4%. FCR increased by 11 points. Protein efficiency ratio reduced by 0.08 with a corresponding decrease in protein retention (%) by 1.65% (P>0.05). Lipid retention (%) was comparable to that of the positive control.

Reduction in the fish oil content of diet produced a significant impact on growth performance of Tra fish probably due to important role played by dietary lipid as a source of energy and essential fatty acids necessary for fish growth and development. A supplementation of lipid rather than carbohydrate as a non-protein energy source is generally more effective for increasing dietary energy level because lipid is an energy-dense nutrient that is readily metabolized by fish, especially by carnivorous fish (NRC, 1993).

Effect of LYSOFORTE™ addition on growth performance of diets with low fish oil conten

LYSOFORTE™ addition to negative control diets at 0.5% dosage helped to improve body weight by 16.14 g and body weight gain by 16.08 g respectively (P>0.05). Specific growth rate also improved by 0.18% day (P>0.05) with a corresponding improvement in survival by 6.7% compared to the negative control treatment. There was an improvement in FCR by 1 point. Protein efficiency ratio increased by 0.22 with a corresponding improvement in protein and lipid retention of 4.89% and 27.26% respectively (P>0.05).

At a higher dosage of 0.1%, LYSOFORTE™ supplementation improved the final body weight by 25.78 g and body weight gain by 25.74 g respectively (P>0.05). Improvement in specific growth rate with 0.1% LYSOFORTE™ was found to be 0.28%/day (P>0.05), with a 6.7% improvement in survival. FCR improvement was found to be higher (11 points). Protein efficiency ratio improved by 0.11 with a corresponding improvement in protein retention by 3.63% (P>0.05). Interestingly, lipid retention was found to improve by 37% compared to negative control (P>0.05).

Environmental changes during the study

Water quality measurements indicated that water temperature fluctuated from 26.0°C – 30.5°C during the experiment (Figure 5) This fluctuation is due to the change in temperature of the surrounding environment. However, this range of temperature is suitable for growth of Tra catfish. As shown in the Figure 1, there is no significant difference in pH values of all the treatments, and the pH range of water stays in the

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suitable condition for growth of Tra catfish. DO concentration of water was found to be between 2.0 to 3.8 mg/L during the experiment due to change of water 3 to 4 times per week (Figure 2). These DO values are in the best condition for well-being and growth of Tra catfish. The NH_3-N content of water at the treatments slowly increased from 0 to 0.06 mg/L during the experiment, which is still within the acceptable range for the growth of Tra catfish (Figure 3). NO_2-N content of water fluctuated from 0 – 0.5 mg/L and was within the acceptable range suitable for fish culture (Figure 4).

In general, the results of environmental measurements show that the environmental factors including daily temperature, daily pH, weekly DO, weekly NH_3-N and weekly NO_2-N were in the suitable range for health and growth of fish and in compliance with the standard of water for catfish culture

The positive effects of LYSOFORTE™ supplementation on growth performance and nutrient retention of Tra Catfish observed in this study may be due to the beneficial effects of phospholipids in improving fish larval development, including growth and survival, digestive functions and skeletal development. Dietary phospholipids have been reported to cause integrative effects on fish growth and survival. They are known to exert a signfiicant effect on lipid absorption and transport (Fontagné *et al.*, 2000; Izquierdo *et al.*, 2000; Morais *et al.*, 2006) and accumulation of lipid droplets in the enterocytes of fish larvae (Diaz *et al.*, 1997; Fontagné *et al.*, 1998; MacQueen-Leifson *et al.*, 2003; Gisbert *et al.*, 2005). In a recent study, the level and class composition of dietary phospholipids was found to impact several biochemical and morphological parameters including growth during the immature fry phase of atlantic salmon (De Santis *et al.*, 2015). The same study showed that, at the gene expression level, phospholipid biosynthetic pathways were not affected by dietary phospholipid level or composition in salmon fry at 1990°dpf, which could reflect that phospholipid biosynthesis pathways were not fully developed at this early life stage.

In conclusion, the observed improvements in growth performance and nutrient retention further supports the potential of LYSOFORTE™, a bio-emulsifier with a combination of lysophopsholipids and phospholipids in improving the digestion and absorption of nutrients.

Summary and conclusion

This study was designed to evaluate the beneficial effect of LYSOFORTE™, when used to replace the dietary fish oil in Tra Catfish diets. Results demonstrated that growth performance and nutrient retention of LYSOFORTE™ supplemented negative control diets were found to be better than negative control diets. LYSOFORTE™ supplementation @ 0.1% level performed on par with positive control diets. This result indicated that LYSOFORTE™ can help Catfish producers to formulate diets with less fish oil level and improve the profitability. From the results of this experiment, it can be concluded that LYSOFORTE™ can either be used to partially replace the expensive fish oil to improve profitability or be added “on top” in low fish oil diets to enhance growth performance. Future studies using Lysophospholpids should aim to further elucidate the biochemical and molecular changes triggered by different types of Lysophospholipids and their combination in fish and the mechanisms associated with lipid transport and phospholipid biosynthesis during different stages of fish growth.

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