QUARTERLY REPORT

Project Number:	44218
Project Title:	Evaluating the supplementation of soy lecithin for the production performance and physiological responses of channel catfish (Ictalurus punctatus) Year 2
Organization:	Mississippi State University
Project Lead Name:	Fernando Y. Yamamoto
Report Date:	07/14/2025

Project Summary:

Overview of project in non-proprietary lay language suitable to be shared publicly.

This project evaluated the dietary supplementation of soy lecithin (SL) and catfish oil (CFO) as lipid additives in plant-based diets for channel catfish (*Ictalurus punctatus*) and hybrid catfish (*I. punctatus* × *I. furcatus*), aiming to improve production performance, physiological resilience, and fillet quality. Two indoor feeding trials and one pond-based study were conducted under controlled and practical conditions.

In the first trial, channel catfish were fed diets with graded levels of SL (0–2%), replacing soybean oil. Fish fed 1.5% SL exhibited superior feed efficiency, and those fed 0.5% SL showed improved protein conversion efficiency. Although SL inclusion did not influence survival, blood parameters, intestinal morphology, or altered stress markers after fish were subjected to acute air exposure, SL-supplemented groups demonstrated a dose-dependent increase in survival following bacterial challenge with *Edwardsiella ictaluri*.

In the second trial, fish were fed diets containing soybean oil (SBO), 1% SL, or 1% CFO. Feed efficiency was enhanced in fish receiving SL, while fish fed CFO exhibited elevated hemoglobin levels and increased deposition of saturated and monounsaturated fatty acids in muscle tissue. Additionally, SL inclusion increased expression of the pro-inflammatory cytokine TNF-α, but did not alter IL-1β, IL-6, or IL-10 expression.

In the pond study, hybrid catfish were fed diets top-coated with CFO, SL+SBO, or plain feed. While growth performance, survival, and carcass yields were unaffected, fish fed CFO exhibited greater intraperitoneal fat and a reduced proportion of undersized fish. SL supplementation significantly increased the concentration of n-6 fatty acids, total PUFA, and LC-PUFA in fillets.

Overall, results indicate that SL can positively modulate nutrient utilization and fillet fatty acid composition, with SL showing additional benefits on immune function and disease resistance in catfish culture.

Detailed Project Status:

Expand upon the above section. What key activities were undertaken and what were the key accomplishments during this reporting period? List each key deliverable from the proposal and describe progress made (or not made) toward achieving it, including metrics where appropriate.

During the reporting period, fillet samples were further processed for fatty acid profile, and the remaining data for the pond study were computed and analyzed. Hybrid catfish offered feed top-coated with an additional 1% of catfish oil presented differences in the size frequency distribution, with more individuals binned in the 0.75-1.00 lb category. The supplementation of soy lecithin in the diets improved the fatty acid profile of catfish fillets by increasing the concentration of eicosapentaenoic acid (20:5 n-3), linoleic acid (18:2 n-6), total polyunsaturated fatty acids, and total long-chain polyunsaturated fatty acids.

The graduate student funded through this project successfully defended her thesis on June 9th and is expected to graduate in August 2025. Her research article (attached) is currently being reviewed by the co-authors, and it is expected to be submitted for consideration as a research article to the Journal of Animal Physiology and Animal Nutrition by the end of August. The data generated from the pond study was compiled, and the manuscript is currently being drafted (also attached). For this study, the material and methods, and results section are complete, and prospective journals for publication are the North American Journal of Aquaculture, Journal of Applied Aquaculture, or the Journal of the World Aquaculture Society.

Re-evaluating the dietary supplementation of soy lecithin for channel catfish (*Ictalurus punctatus*)

Running title: Dietary soy lecithin for channel catfish

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Statements & Declarations

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Data availability statement: The data can be provided by the corresponding author upon reasonable request.

Ethics approval: The authors confirm that the ethical policies of the journal were followed, as noted on the journal's author guidelines page, and all procedures have been approved by the ethical review committee (Mississippi State Institutional Animal Care and Use Committee).

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ABSTRACT

Soy lecithin (SL), a phospholipid-rich by-product of soybean oil (SBO), is used in aquafeed. This study evaluated SL as a feed additive to enhance growth, stress resilience, and disease resistance in channel catfish. In Trial 1, feeds were formulated using plant-based ingredients, with SL included at 0, 0.5, 1.0, 1.5, and 2.0%, replacing SBO. Catfish fed diets with 1.5% SL had better feed efficiency, while those supplemented with 0.5% SL had greater protein conversion efficiency. Following an acute air exposure stressor, glucose and lactate levels increased from 0 to 2 hours, then decreased by 6 hours. In contrast, cortisol and osmolality continued to increase over time, with cortisol showing an interaction effect. In Trial 1, survival after the bacterial challenge increased with increasing levels of SL. In Trial 2, three diets were tested: control (SBO), 1% SL, and catfish oil (CFO). Fish fed the SL diet had greater feed efficiency than fish fed the SBO diet. However, there were no differences between treatments in terms of survival, condition indices, intestinal microbiota, and blood parameters, except for hemoglobin, which was greater in the CFO group compared to the SBO group. The relative gene expression of cytokines in the intestine presented greater TNF-α production for the fish fed diets supplemented with SL, while no differences were observed in IL-1β, IL-6, or IL-10. In Trial 2, fish fed SL exhibited greater survival when compared to the SBO group when challenged. In conclusion, the data obtained suggest SL supplementation can improve the growth performance of channel catfish juveniles and potentially modulate their stress response and disease resistance.

Keywords: Channel catfish, Lipid sources, Plant-based diet, Soy lecithin oil, Fish nutrition, Stress in fish

1. Introduction

According to FAO (2024), global aquaculture production reached 130.9 million tonnes in 2022, with 94.4 million tonnes of aquatic animals farmed at an estimated value of $295.7 billion USD. In the United States, the annual production of catfish is 150,000 metric tons, with the majority being produced in the states of Alabama, Arkansas, and Mississippi (Kumar et al., 2020). Alternative nutritional approaches have been investigated to improve catfish production and enhance market competition (Sink and Lochmann, 2014). One of the primary sources of energy for fish is dietary lipids, which can also supply essential fatty acids to optimize fish growth and health (Liu et al., 2024). Feed additives, such as soy lecithin (SL), have been investigated to enhance lipid digestion and absorption (Liu et al., 2019). Soy lecithin is a by-product obtained when refining soybean oil, specifically from the degumming process, and has been a common source of phospholipids in aquaculture diets (Le et al., 2019). Commercial lecithin is primarily derived from soybean oil, while it can also occasionally be manufactured from other plant products and animal sources such as cereals, sunflowers, eggs, and milk (Wee et al., 2023).

All lipids containing phosphorus are referred to as phospholipids (PL; Bargui et al., 2021). Phosphoglycerides are the most common phospholipid type and are the main component of fish tissues. They are synthesized through esterification of choline, ethanolamine, serine, and inositol to the phosphate group (Zumbuehl, 2019). The supplementation of this nutrient in fish feeds has improved growth, survival, and stress resistance (Haghparast et al., 2019). Phospholipids have several physiological functions, including preserving the integrity and functionality of cellular membranes, acting as emulsifiers in the digestive tract, enhancing the intestinal absorption of long-chain fatty acids, inducing lipoprotein synthesis in intestinal enterocytes, and being crucial for the transportation of dietary lipids (Jiang, 2024).

Phospholipids play a key role in the absorption of dietary fats and fat-soluble vitamins, are vital for intermediary metabolism, and are important for fatty acid metabolism (List, 2015; Sink and Lochmann, 2014; Van Nieuwenhuyzen, 2008). Considering these benefits, two separate feeding trials were conducted to evaluate SL supplementation in catfish diets. The first study aimed to identify optimum inclusion levels of SL in plant-based diets for channel catfish fingerlings and their effects on production performance and physiological responses during an air exposure challenge. A follow-up feeding trial was conducted to compare diets containing

soybean oil, 1% SL supplementation, and catfish oil, and their potential to modulate expression of immune-related genes. At the end of both feeding trials, disease challenges were performed using *Edwardsiella ictaluri*.

2. Materials and Methods

2.1. Experimental diets for feeding Trials 1 and 2

Two comparative feeding trials (Trial 1 and Trial 2) were conducted for 70 days to evaluate the production performance of channel catfish juveniles when fed plant-based diets were supplemented with SL. All diets were formulated to be isoenergetic and isonitrogenous and to meet the nutrient requirements for catfish (Li and Robinson, 2021).

In Trial 1, five experimental plant-based diets were prepared with graded levels of SL (0.5%, 1%, 1.5%, and 2%) replacing soybean oil, with the 0% SL serving as the control (Table 1). In Trial 2, a plant-based formulation with three different lipid sources: CFO, SBO, and SBO with 1% SL was used (Table 2). In Trial 2, the fatty acid profile of the tested lipids and experimental diets was evaluated (Supplementary Table 1). All ingredients used in the study were sourced from local feed mills, and commercial SL was kindly donated by Bunge Ltd (Chester, MO, USA). The ingredients were individually weighed and mixed using a V-mixer machine (P-K Blend Master, Patterson-Kelley LLC, PA, USA) for 30 minutes, with intermittent operation of the inner bar every 5 min for a duration of 5 min. The mixture was then transferred to an orbital mixer and blended for an additional 15 min, during which the lipid sources were gradually added to the feed mash. Deionized water was subsequently incorporated, making up 30% of the total feed weight. The moistened mash was cold pelleted through a 3-mm die plate (Hobart Machine, Hobart, OH, USA) and dried overnight in a forced-air bench oven at room temperature. All ingredients and experimental diets were analyzed for nutrient composition and ash content according to Association of Official Analytical Chemists (AOAC, 2005) procedures. The experimental procedures for both feeding trials followed the guidelines set by the Mississippi State University Institutional Animal Care and Use Committee (MSU IACUC-23-254).

2.2.Feeding Trial 1

Each dietary treatment (Control, 0.5, 1, 1.5, and 2%) was randomly assigned to five aquaria (110 L; n=5), stocked with 30 catfish juveniles (~4.4 g) each. Feed rations were adjusted according to the tank biomass and provided twice daily for 70 days. Water quality parameters were measured three times a week. The water temperature and dissolved oxygen (DO) were monitored using an optical DO meter (ProSolo ODO, YSI, Yellow Springs, OH, USA), and the salinity and pH were measured by handheld meters (Hach Company, Loveland, CO, USA). Total ammonia nitrogen (TAN) and total nitrite nitrogen (TNN) were measured using a spectrophotometer (Hach Company). The water quality variables throughout the first feeding trial were as follows (mean ± SD): temperature (27.0 ± 1.2°C), DO (7.65 ± 0.39 mg/L), salinity (1.01 ± 0.38 mg/L), pH (8.28 ± 0.14), TAN (0.27 ± 0.19 mg/L), and TNN (0.030 ± 0.030 mg/L).

2.3.Production performance

After 70 days of feeding, the experimental fish were withheld for a day prior to sampling, and each tank was individually counted and group-weighed to calculate the production performance parameters:

Percentage of weight gain (% of initial) = [(average weight at the 70th day (g)-average initial weight (g))/average initial weight (g)] × 100

Feed efficiency (FE) = weight gain (g) /dry feed intake (g)

Survival (%) = (number of surviving fish/initial number of fish) × 100

2.4.Blood samples, condition indices, and intestinal histology

Three fish were randomly selected and anesthetized with MS-222 (150 mg/L) for blood collection using heparinized tuberculin syringes. Blood samples were collected from the caudal vasculature and immediately transferred to 1.5-mL centrifuge tubes. The red blood cell concentration (RBC) was counted using Countess 3 Automated Cell Counter (Thermo Fisher Scientific, Washington County, MA, USA) following the manufacturer's guidelines. The samples were diluted 1:400 (798 µL NaCl buffer solution and 2 µL of the whole blood) and homogenized with a pipette. From the resulting mixture, 20 µL was aliquoted into a 0.6-mL tube and mixed in a 1:1 ratio with a 0.4% Trypan blue solution. From this mixed tube, 10 µL of stained blood was added to each side of the slide and analyzed using an Invitrogen Thermo Fisher Countess II. To determine the hemoglobin (Hb) concentration, 5 µL of the collected blood

was homogenized with 1 mL of Drabkin's reagent. After 15 min, 800 µL was pipetted to a cuvette and read at 540 nm absorbance (Drabkin, 1946). The whole blood was centrifuged (3,500 × *g* for 15 min) in micro hematocrit capillary tubes and used to quantify hematocrit (Ht) (Goldenfarb et al., 1971).

The same fish were euthanized with an overdose of MS-222 (250 mg/L), and they were carefully dissected to compute the weight of the liver, intraperitoneal fat, and total viscera to compile the condition indices:

Viscerosomatic index (VSI) (%) = (viscera weight (g)/ body weight (g)) × 100

Hepatosomatic index (HSI) (%) = (liver weight (g)/ body weight (g)) × 100

Intraperitoneal fat ratio (IPF) (%) = (fat weight (g)/ body weight (g)) × 100

The gastrointestinal tracts from the same fish were carefully dissected and preserved in 10% neutral-buffered formalin (NBF), then transferred to 70% ethanol for eight days and returned later to 10% NBF. Samples were placed in plastic cassettes, embedded with paraffin, sectioned using a microtome to a 5 µm thickness, and mounted on glass slides. Slides were stained using hematoxylin and eosin, and images were captured using light microscopy (Olympus DP28, Evident Scientific, Inc., MA, USA), with scale bars representing 500 µm.

2.5. Whole-body proximate composition

An initial biomass of catfish was sampled at the beginning of the feeding trial and stored frozen at -20°C prior to proximate composition analyses. After 70 days of feeding, three fish per tank were sampled and euthanized with MS-222 and stored frozen at -20°C. Each composite sample was homogenized using a meat grinder, and a subset of the ground fish was dried for 24 hours at 105°C. The samples were removed from the oven, placed in desiccators until ambient temperature was reached, and mechanically homogenized for further analyses. The whole-body samples were measured for crude protein, crude fat, and ash following AOAC (2005), and protein conversion efficiency (PCE) was calculated as follows:

Protein conversion efficiency (PCE, %) = [(Final weight (g) × final protein (%) − (initial weight (g) × initial protein (%)] ÷ protein intake (g) × 100.

2.6. Air exposure challenge and analysis of stress markers in the plasma

Following the diet experiment, fish continued to be fed their assigned experimental diets. Five days later, the remaining fish were subjected to a stress challenge. Briefly, all fish from each tank were captured in a single net, air-exposed for two minutes, and immediately returned to their respective aquarium. The time of air exposure was selected to simulate the conditions associated with various handling procedures conducted in the Thad Cochran National Warmwater Aquaculture Center (NWAC), Stoneville, MS, USA. Four fish per tank were randomly selected and bled at 0 (without air exposure), 0.5, 1, 2, and 6 hours after the air exposure. Fish were bled following the same procedures previously described. After performing Ht, RBC, and Hb, blood samples were kept undisturbed in the refrigerator until analyzed (4°C) and centrifuged at 2,800 × *g* for 15 min at 4°C to collect the plasma. The plasma samples were stored at -80°C until further analysis.

Plasma cortisol levels were analyzed using an enzyme-linked immunosorbent assay (ELISA) commercial kit (Cortisol EIA Kit; EA65 Enhanced Immunoassay Buffer, Oxford Biomedical Research, Oxford, USA). Plasma glucose (QuantiChrom TM Glucose Assay Kit, BioAssay Systems, CA, USA) and lactate (L-Lactate Assay kit, Biomedical Research Service Center, NY, USA) were measured using commercial kits following manufacturers' instructions. Plasma osmolality was measured using a vapor pressure osmometer (Vapro 5.520; Wescor, Inc., Logan, UT, USA).

2.7. Intestinal microbiota

Digesta were collected 9 days after the end of the feeding trial to assess the posterior intestinal microbiota. One day before sampling, fish were fed to apparent satiation in three-minute intervals between each tank to ensure that the intestinal transit time would be similar during the collection for all experimental units. Three catfish per tank were euthanized with an overdose of MS-222, and digesta samples were aseptically removed using sterilized tweezers and collected from the distal intestine and pooled per tank into 15 mL tubes. Subsequently, the pool of collected digesta was homogenized in PBS at a 1:1 (w/v) ratio, and 400 µL was aliquoted into a 2 mL cryotube with silica beads. Afterwards, samples were kept at -80°C until they were further processed.

The sequencing data were analyzed using QIIME 2 (v. 2023.7; Bolyen et al., 2019). Primer sequences were eliminated using Cutadapt (Martin, 2011), followed by quality filtering

with DADA2 (Callahan et al., 2016). During this process, the forward and reverse reads were truncated to 280 and 180 nucleotides, respectively, and then joined with a 30-nucleotide overlap. Taxonomic classification was performed using a Naïve Bayes classifier trained on the SILVA v138.1 SSU Ref NR 99 full-length sequences (Quast et al., 2013), excluding any non-bacterial sequences. For phylogenetic analysis, sequences were mapped onto the SILVA 128 reference tree using SEPP (Janssen et al., 2018), and sequences that could not be inserted were removed.

2.8. Bacterial challenge using Edwardsiella ictaluri

The remaining fish from each tank were transferred to individual glass aquaria operating as a flow-through system and equipped with individual air stones. The experimental fish were offered their respective dietary treatment for an additional week prior to the bacterial challenge. On the eighth day, catfish were exposed to a virulent strain of *Edwardsiella ictaluri* (S97-773; GenBank ASM305480v2) through immersion. The isolate was originally isolated from an enteric septicemia outbreak from an industrial catfish operation and was obtained from the MSU College of Veterinary Medicine repository.

Bacteria were incubated overnight in Brain Heart Infusion porcine broth (Research Products International, RPI, 410 Business Center Dr., Mt. Prospect, IL, USA) for 16 hours at 27°C. After incubation, the bacterial culture was centrifuged at 5,000 × *g* for 8 min, and the resulting pellet was resuspended in PBS to a final target of 1×10^6 CFU/mL. Before the challenge, the water flow in all tanks was suspended, and 70 mL of the bacterial suspension was added to each tank. After 1 h exposure, the water flow was restored, and the cumulative mortality was monitored for 22 days.

2.9. Feeding Trial 2

A total of 540 channel catfish juveniles (initial weight ~4.9 g) were equally distributed to eighteen aquaria (110 L, 30 fish per tank; n=6) operating as a recirculating aquaculture system as previously described in Trial 1. The fish were acclimated to the control diet for two weeks before the commencement of the feeding trial. After the acclimation period, fish were fed to apparent satiation twice daily at 8:00 AM and 3:30 PM for 70 days. A 12-hour light/dark photoperiod was maintained and controlled by timers. Water quality was monitored three times as previously

described (mean ± SD): DO (7.05 ± 0.60 mg/L), temperature (29.3 ± 1.01°C), pH (8.41 ± 0.40), TAN (0.31 ± 0.28 mg/L), and TNN (0.09 ± 0.02 mg/L).

2.10. Production performance and sample collection

At the end of the feeding trial, the fish were fasted for one day, group-weighed, and individually counted. Three fish from each tank were anesthetized with MS-222 at 100 mg/L, and blood samples were collected and analyzed as described in Trial 1. Subsequently, the fish were euthanized, condition indices were computed, and posterior intestinal tissues were sampled for histology using the aforementioned procedures.

2.11. Fatty acid profile and proximate composition of channel catfish

An initial sample of 60 catfish was collected at the start of the comparative feeding trial and stored at -20°C for later analysis. At the conclusion of the feeding trial, three fish from each tank were euthanized with an overdose of MS-222 and stored at -20°C. Then, the same fish used for condition indices and histology were filleted and pooled for fatty acid composition analysis. The samples were freeze-dried, followed by cold lipid extraction using chloroform-methanol (Folch et al., 1957). The resulting lipid droplets were shipped to the University of Missouri Agricultural Experiment Station Chemical Laboratories (ESCL) for fatty acid composition analysis via gas chromatography. Additionally, three more fish were collected, and each was homogenized with a meat grinder. The subsamples were then dried for 24 hours at 105°C. After drying, the samples were placed in desiccators to cool to room temperature before being mechanically homogenized for further analysis. Whole-body samples were analyzed for crude protein, crude fat, and ash content following AOAC (2005) methods.

2.12. Intestinal microbiota

Intestinal digesta samples were collected 9 days after the end of the feeding trial to assess the composition of the microbiota. One day prior to sampling, fish were fed to apparent satiation in three-minute intervals to standardize intestinal transit times across experimental units. Three catfish per tank were euthanized with an overdose of MS-222, and the digesta was aseptically collected using the same methodology as described in Trial 1. These samples were stored at -

20°C until further processing, and then the sequencing data were processed using the same methodology previously described.

2.13. Gene expression analyses

At the end of the feeding trial, three fish from each tank had their posterior intestines collected and processed using the phenol-chloroform method for RNA extraction, following the protocol by Yamamoto et al. (2024). The relative expression of selected genes was determined using the ΔΔCt method based on real-time polymerase chain reaction (PCR) analysis. Four genes (TNF-α, IL-1β, IL-6, IL-10) were analyzed using designed primers, with β-actin as the reference gene for normalization. The cycling conditions included an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A melt curve analysis was performed at the end using the StepOne Plus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Each reaction was conducted in a total volume of 20 µL, containing 400 ng of template cDNA, 2 µL of forward/reverse primer mix (10 ng/µL each), and 2×SYBR Green PCR Master Mix (cat#430915, Thermo Scientific, Waltham, MA, USA). Data analysis followed the method described by Rao et al. (2013).

Approximately 200 mg of intestinal tissue from each tank was pooled and homogenized with 1 mL of TRI Reagent (cat#T9424, Sigma-Aldrich, St. Louis, MO, USA) for RNA extraction. The TRI Reagent was added to the tissue at a 1:1 ratio, and molecular-grade chloroform was added at a 1:5 ratio. The mixture was centrifuged at 12,800 ×g for 15 minutes, and the upper aqueous phase was carefully collected. An equal volume of isopropanol was added to precipitate the RNA. The supernatant was then centrifuged for 10 minutes at the same speed, and the RNA pellet was washed twice with 80% ethanol before being eluted in nuclease-free water. The extracted RNA was immediately used for cDNA synthesis using the iScript™ cDNA synthesis kit (cat#1708891, Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions. A total of 1000 ng of RNA was used as the template for cDNA synthesis.

2.14. Bacterial challenge

The challenge used the remaining fish from the comparative feeding trial, and each tank was moved to a flow-through system in aquaria containing 22 L, where oxygen levels were kept near saturation through continuous diffusion of air via air stones. The catfish were fed with

respective dietary treatments for one more week before being subjected to the bacterial challenge. On the eighth day, the catfish were exposed to *Edwardsiella ictaluri* (S97-773; GenBank ASM305480v2) via immersion. The isolate from the MSU stock was cultured following the previous methodology to a final concentration of 6.2×10^6 CFU/mL. Fish mortality was checked twice daily over 28 days.

2.15. Statistical analyses

Data collected from feeding Trials 1 and 2 were subjected to a Shapiro-Wilk test to validate their normal distribution and to a one-way ANOVA. If significant differences were observed ($P<0.05$), then a Brown-Forsythe test was performed to ensure the homogeneity of variances, followed by a Tukey-HSD test for comparison of means. The dataset from feeding Trial 1 was also subjected to regression analyses (linear and second-order polynomial), but no relationship ($P>0.05$) could be established between the variables tested and the supplementation levels of dietary SL. Due to the variance heterogeneity, the gene expression data set from Trial 2 was subjected to a Kruskal-Wallis test followed by a Dunn's test.

The results from the air exposure challenge performed in Trial 1 were analyzed as a 5×5 factorial model, having the dietary treatments and sampling time points as the main factors. If significant differences were observed for the main factors or their interaction, then a Tukey-HSD test was performed to compare the means.

Microbiota diversity was evaluated using alpha diversity metrics, including Faith's phylogenetic diversity, observed features, Shannon entropy, and Pielou's evenness, and beta diversity metrics such as Bray-Curtis dissimilarity, Jaccard distance, and both unweighted and weighted UniFrac. Alpha diversity was compared across treatments using the Kruskal–Wallis test, and beta diversity was assessed using an analysis of similarities (ANOSIM). The cumulative survival of bacterial challenge in Trial 1 and Trial 2 was analyzed using Kaplan–Meier and Cox proportional hazards models (Coxph) from the survminer and survival packages, using R Studio (R version 4.2.1 2022-06-23).

3. Results

3.1. Feeding Trial 1

No significant differences (P>0.05) were observed in final weight, weight gain, survival, condition indices, and hematological parameters (Table 3). However, significant differences were observed in feed efficiency, where the catfish fed 1.50% SL had a greater feed efficiency than the control group. No differences were observed among the dietary treatments for whole-body proximate composition (*i.e.*, dry matter, protein, lipid, and ash; Table 4). In contrast, significant differences were observed for PCE, where fish fed diets supplemented with 0.5% were more efficient in converting dietary protein to fish biomass than the control group. No significant differences were observed in the number and length of folds, and mucosa thickness across the different levels of SL inclusion (Supplementary Table 2).

The composition of the intestinal microbiota of channel catfish fed the experimental diets with different levels of SL was mainly comprised of *Cetobacterium* sp. (~76%), followed by *Plesiomonas* sp. (~10%), and *Turicibacter* sp. (~7%) (Figure 1). The gradual inclusion of SL in the experimental diets did not significantly affect the bacterial communities in the posterior intestine of channel catfish for beta-diversity metrics and for the linear discriminant analysis; however, there was a mild effect on alpha diversity (Pielou's evenness index; Supplementary Table 3).

3.2. Air exposure challenge

There was a main effect of time, with most of the whole-blood parameters from the experimental fish changing with time post-stressor. The Ht and Hb showed statistical significance in recovery time. However, plasma stress markers cortisol, glucose, lactate, and osmolality increased from time 0 to 1h (Table 5). Nevertheless, the inclusion of SL in diets did not influence most of the analyzed variables. Significant interactions between the dietary treatment and time were found for RBC and cortisol, with these parameters responding differently over time depending on the treatment group.

3.3. Bacterial challenge

After the 22 days of bacterial challenge, fish fed with different grades of SL, a significant treatment effect was observed in a dose dependent manner (Figure 2). The control group had a 35.8% survival rate which was significantly lower than fish treated with SL supplemented diets (0.50%: 52.5% survival; 1.00%: 59.2%; 1.50%: 60.8%; and 2.00%: 70.8%).

3.4. Feeding Trial 2

At the conclusion of feeding trial 2, no significant differences were observed in final weight, weight gain, survival, VSI, HSI, IPF, Ht, and RBC (Table 6). However, feed efficiency was significantly greater in the SL group compared to SBO. The Hb levels also exhibited a statistically significant difference among treatments, with catfish oil resulting in significantly greater levels compared to SBO. No differences were observed among the treatments (SBO, SL, and CFO) for whole-body proximate composition and protein conversion efficiency (Table 7).

The fatty acid composition of fillets from fish fed the different experimental diets was analyzed, and significant differences in the concentrations of fatty acids were observed (Table 8). For saturated fatty acids (SFA), significant differences were observed in 14:0, 20:0, 22:0 and 23:0. The total SFA of fish fed with CFO was significantly greater when compared to SBO fillets. In the monounsaturated fatty acids (MUFA) category, the CFO fillet exhibited significantly greater levels of 18:1, 18:1, 20:1, and 22:1 when compared to SBO and SL fillets. Additionally, the total MUFA was significantly greater in the CFO fillet compared to the other two groups. Polyunsaturated fatty acids (PUFA) in the CFO fillet contained significantly lower levels of 18:3 n-3, 18:4 n-3, and 20:3 n-3 compared to both the SBO and SL fillets. The total n-3 content was not affected by the dietary treatments. Moreover, 18:2 n-6 and 18:3 n-6 levels were lower in the CFO fillet than in the other two groups. No significant differences among the fillets were observed in the long-chain polyunsaturated fatty acids (LC-PUFA).

3.5 Intestinal histology and microbiota

In the analysis of the posterior intestine, no significant differences were observed in the number of folds, mucosa thickness, or length of folds across the different treatment groups (SBO, SL, CFO). Although there were variations between the groups, these differences were not statistically significant (Supplementary Table 4).

No significant differences were observed in the intestinal microbiota of channel catfish fed diets with the different dietary treatments, as determined by alpha- and beta-diversity metrics and linear discriminant analysis (Supplementary Table 5). The composition of the intestinal microbiota of channel catfish was mainly comprised of *Cetobacterium sp*. (~41%), followed by *Plesiomonas sp*. (~10%), and *Turicibacter sp*. (~8%; Figure 3).

433 *3.6 Gene expression*

434 In the analysis of gene expression, significant differences were observed for TNF-α. Fish
435 fed SL exhibited more relative gene expression for this cytokine compared to the SBO and the
436 CFO groups (Figure 4). No significant differences were observed among the dietary treatments
437 for relative gene expression of IL-1β, IL-6, and IL-10.
438
439 *3.7 Bacterial challenge*

440 A significant treatment effect was observed on survival after the bacterial challenge
441 (Figure 5). Fish fed SL exhibited a greater survival rate (60%), suggesting a protective effect of
442 this feed additive when compared to the SBO group (44.5%). However, when compared to the
443 CFO group, no significant differences were observed (54.2%).
444
445 **4. Discussion**

446 The supplementation of SL was evaluated as a phospholipid source in diets for channel
447 catfish, and improvements in production performance were observed in both studies. These
448 results agree with previous reports where the supplementation of SL improved the feed
449 conversion of juvenile channel catfish (Sink and Lochmann et al. 2014), and feed efficiency of
450 goldfish (*Carassius auratus*; Lochmann and Brown, 1997), Nile tilapia (*Oreochromis niloticus*;
451 El-Naggar et al., 2021), silvery-black porgy (*Sparidentex hasta*; Pagheh et al., 2019, European
452 sea bass (*Dicentrarchus labrax*; Cahu et al., 2003), milkfish (*Chanos chanos*; Balito-Liboon et
453 al., 2018; Sivaramakrishnan et al., 2021), and sea bream (*Sparus aurata*; Saleh et al., 2022). In
454 contrast with the present findings, Liu (2019) did not find significant differences when feeding
455 juvenile channel catfish diets supplemented with SL. It is hypothesized that these discrepancies
456 can be attributed to differences in life stages and husbandry conditions for the different studies.

457 Dietary lipids supply essential fatty acids needed for fish growth and development
458 (Fawole et al., 2021). The fatty acid profile of dietary lipids directly influences the fish lipid
459 storage in their tissues, as observed on channel catfish fed with dietary treatments containing 4%
460 corn oil or 4% canola oil (Manning et al., 2007) or when supplemented with the microalgae
461 *Schizochytrium* sp. (Li et al., 2009). Similar results have been reported in other fish species fed
462 with plant-based oils, such as rainbow trout (*Oncorhynchus mykiss*; Olsen et al., 2003; Güler and
463 Yildiz, 2011), brown trout (*Salmo trutta*; Tocher et al., 2001), juvenile Caspian brown trout

(*Salmo trutta caspius*; Sotoudeh et al., 2010), and Atlantic salmon (*Salmo salar*; Ruyter et al., 2006).

In this study, catfish fed CFO supplemented diets exhibited a greater total SFA content compared to those fed with SBO. These changes reflected the fatty acid profile of diets, where CFO had slightly greater SFA content (22.4%) than SBO (18.5%) and SL (18.9%). This has been consistently observed in channel catfish tissues when they were offered diets supplemented with different lipid sources, namely SBO, menhaden oil, or flaxseed oil (Suja et al., 2012). Furthermore, fish oils generally have greater concentrations of SFA than vegetable oils, and this has been highlighted by Tocher (2015). The greater levels of 14:0, 20:0, 22:0, and 23:0 in the fillets of fish fed with CFO also reflected the greater presence of these fatty acids with their dietary treatment.

The fish fed CFO had significantly greater concentrations of 18:1, 20:1, and 22:1 compared to the SBO and SL groups. These results are consistent with studies indicating that fish oil is rich in long-chain MUFAs, especially those belonging to the n-9 series (Glencross et al., 2020). The main difference observed between the dietary treatments was the deposition of PUFAs, especially those of the n-6 series. The fillets of fish fed with SL had a greater concentration of n-6 than the other groups, resulting in a greater deposition of 18:2 n-6 and 18:3 n-6. This result was also observed in a study conducted with Nile tilapia, where the total PUFA, including the n-6 PUFAs, significantly increased as dietary soy lecithin increased (Batista et al., 2023). Similarly, this pattern has been described by Turchini et al. (2010), who stated that oils and emulsifiers derived from soybeans are rich in n-6 PUFAs, which will promote their incorporation into the muscle tissues of the fish. Nevertheless, neither the n-3 concentration in the fillets nor the n-3:n-6 ratio was affected by the dietary treatments, indicating that fish may be able to regulate the retention of n-3 to maintain an adequate physiological balance (Tocher et al., 2019).

Differences in whole-body composition can be related to the diverse feeding behaviors of the fish (Breck, 2014), and they can also be indicators of enhancement of the nutrient composition in their feed (NRC, 2011). In both feeding trials, no differences were observed for whole-body proximate composition. Contrastingly, increasing dietary levels of SL (0, 21, 43, and 64 g/kg) in Nile tilapia led to a decrease in whole-body lipid content (Batista et al., 2023). Whereas channel catfish protein conversion efficiency (PCE) was significant in Trial 1, and the

improvement of protein utilization may be associated with the enhanced nutrient digestibility provided by the emulsifying properties of phospholipids (Kim et al., 2018; Tocher et al., 2008).

The supplementation of dietary SL did not affect the condition indices or most of the hematological parameters, except for Hb in Trial 2, which was greater in fish fed CFO diets compared to the ones fed SBO diets. Different than what has been reported in this study, Jafari et al. (2018) and Torbaso et al. (2023) observed an increase in RBC, Hb, and Ht levels in stellate sturgeon (*Acipenser stellatus*) and striped catfish (*Pangasianodon hypophthalmus*), respectively, when fed diets supplemented with 6% SL. Additionally, Nile tilapia fed with diets supplemented with 2 g/kg of granular soy phospholipids also had an increased RBC concentration (Ali et al., 2025). These conflicting results suggest that the effects of dietary SL on hematological parameters may be species-specific or dose-dependent. Further research is warranted to clarify whether SL supplementation in aquafeeds can play a significant role in modulating the catfish hematology under varying dietary and environmental conditions.

Hematological parameters are frequently used to evaluate the general health status of fish (Witeska et al., 2022). When the experimental fish were subjected to an acute stressor, Ht and Hb were significantly affected at the different sampling points, showing a decrease in values as recovery time increased. This was also observed in channel catfish stressed by management and capture practices, where their RBC concentration, Ht, Hb, and glucose levels were significantly greater at 6 hours post-stress compared to non-stressed fish (Aguirre et al., 2016). Furthermore, the RBC had an interaction between dietary treatment and time.

The main function of RBCs is to deliver oxygen to the body by absorbing oxygen in the gills and releasing it into tissues (Esmaeili, 2021). Under stress, the pentose phosphate route and the glycolysis reaction will supply energy for the cells, playing a major part in RBC metabolism (Kosmachevskaya et al., 2021). These findings showed that dietary phospholipids present in SL may enhance energy availability for metabolism, stabilize cell membrane structure and permeability, and potentially improve the catfish response to a stressor and return to their basal levels (Nagasaka et al., 2004).

Stress-response variables, such as plasma cortisol, glucose, lactate, and osmolality, were measured to assess the physiological conditions of the fish after being exposed to a stressor (Peterson, 2004). In teleost fish, cortisol is the main corticosteroid, and stress causes a significant spike in cortisol plasma concentrations (Sadoul and Geffroy, 2019). The recovery time of fish

from a stressor can be species-specific and will depend on the stress source (Sadoul and Vijayan, 2016; Schreck and Tort, 2016). The secretion of cortisol can stimulate gluconeogenesis and glycogenolysis, which will release glucose into blood to mobilize stored energy (Seibel et al., 2021). The influence of a stressor on fish plasma/serum markers over time has been described in channel catfish, where a decreased growth performance was observed due to the reduced feed intake and increased disease susceptibility (Bilodeau et al., 2005; Pankhurst et al., 1997; Small and Bilodeau, 2005). In contrast, the present study's findings showed no significant effect of stress on growth performance, likely due to the optimal conditions of the rearing system and the absence of chronic stressors throughout the feeding trial.

The examination of tissue morphology enhances the understanding of the digestive system and intestinal health by displaying the physiological alterations and intestinal responses to different dietary treatments (Gonçalves et al., 2024). In this study, the dietary supplementation of SL in both trials did not alter the intestinal histomorphometry of channel catfish juveniles. In contrast with the present findings, striped catfish (Amer et al., 2023), rock bream larvae (*Oplegnathus fasciatus*; Tan et al., 2022), and Caspian brown trout (Haghparast et al., 2019) increased their intestinal morphology when offered diets supplemented with SL. These discrepancies may be attributed to the channel catfish's robust and resilient intestinal mucosa, which may be less responsive to dietary SL supplementation compared to more sensitive species or during early life stages (Sis et al., 1979).

The intestinal microbiota can impact the metabolism of the host by generating beneficial components during food fermentation or even aiding in detoxifying potential harmful elements from the ingested materials (Yoon et al., 2024). The dietary treatments did not affect the channel catfish intestinal microbiota, despite marginally significant differences observed for alpha diversity (Pielou's evenness) in feeding Trial 1. In this study, sequences associated with the genera *Cetobacterium*, *Turicibacter*, and *Plesiomonas* have been frequently identified in the intestinal microbiota of channel catfish (Yamamoto et al., 2024; Older et al., 2025). *Cetobacterium sp*. are common bacteria present in the intestine of freshwater fish such as bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus nigricans*), and channel catfish (Larsen et al., 2014), and they can biosynthesize vitamin B12 (Ramírez et al., 2018). The *Turicibacter* sp. has also been identified in the intestinal microbiota of channel catfish (Burgos et al., 2018; Yamamoto et al., 2024; Older et al., 2025), Asian sea bass (*Lates calcarifer*; Morshed

et al., 2023), and red tilapia (*Oreochromis niloticus* × *O. mossambicus*; Amthungphong et al., 2025). *Plesiomonas* is a genus that belongs to the Enterobacteriaceae family (Dodd, 2017), in which *P. shigelloides* is the only species. This bacterium has demonstrated antibacterial properties that could benefit the fish intestinal health (Haruo et al., 1996). Studies on red claw crayfish (*Cherax quadricarinatus*) fed diets with SL have shown changes in the abundance and composition of their intestinal microbiota (Chen et al., 2024), aligning with findings by Liang et al. (2022), who suggested that dietary SL may promote intestinal immune homeostasis in female Pacific white shrimp (*Litopenaeus vannamei*). Additionally, the inclusion of SL in their feed improved disease resistance for both species, highlighting its potential as a nutraceutical additive.

The gene expression of inflammatory and anti-inflammatory cytokines in the posterior intestine of catfish was assessed to evaluate if the dietary treatments could modulate the immunological responses. The observed results in this study are similar with those reported by Tan et al. (2016), who investigated different dietary oil blends for large yellow croaker (*Larimichthys crocea*). In that study, fish fed with SBO displayed an increase in TNF-α levels and a decrease in the anti-inflammatory cytokine IL-10. In addition, Weng et al. (2022), also using juvenile large yellow croaker, tested the supplementation of lysolecithin, which is a hydrolyzed lecithin source with enhanced emulsification properties. Interestingly, dietary lysolecithin increased the expression of IL-10 and decreased the expression of TNF-α and IL-1β, suggesting that it can reduce inflammatory responses and promote beneficial health effects. Further studies are needed to understand better the relationship between different sources of phospholipids and their modulation of intestinal immune responses.

The dietary supplementation of SL has shown improvement in survival of European sea bass (*Dicentrarchus labrax*) during their larval stage (Cahu et al., 2009). However, the mechanisms of how dietary SL influences the immune responses during a bacterial infection are still unclear, as greater survival was observed for catfish fed supplemented diets. Similar to the present study, dietary lecithin supplementation (1%, 1.5%, and 2%) in diets for milkfish improved their survival after a *Vibrio parahaemolyticus* challenge (Kumar et al., 2024). A prospective mechanism for this improved survival could be the modulation of the innate immune response, as observed by the increase of the pro-inflammatory cytokine TNF-α to combat *E. ictaluri* (Santander et al., 2014). Alternatively, diets rich in phospholipids may promote the

structural integrity of immune cells and play a role in signaling the innate immune responses (Bargui et al., 2021).

5. Conclusion

This study demonstrates that dietary supplementation with SL in plant-based diets for channel catfish may provide benefits such as enhancing feed efficiency, influencing the expression of the cytokine TNF-α, and improving disease resistance. The addition of 0.5% and 1.5% dietary SL improved feed efficiency and protein conversion, respectively. Although no significant effects were found on condition indices, blood parameters, or body composition at the end of the feeding trial 1, the addition of SL significantly increased survival rates when fish were challenged with *Edwardsiella ictaluri*. For both feeding trials, neither the composition of the intestinal microbiota nor the intestinal morphology was modulated by dietary SL. After the air exposure challenge, plasma cortisol, glucose, lactate, and osmolality displayed a time-dependent increase and then returned to baseline, but no significant improvements were obtained through dietary SL. Overall, SL supplementation demonstrated the potential to improve production performance and disease resistance in channel catfish.

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889 Table 1: Feed formulation of experimental diets fed to channel catfish (*Ictalurus punctatus*)
890 juveniles for 70 days. Values are expressed as g/100 g of feed on a dry-matter basis, unless
891 otherwise stated.

Ingredients	Control	0.50%	1%	1.50%	2%
Soybean meal[1]	52.00	52.00	52.00	52.00	52.00
Corn meal[1]	17.43	17.43	17.43	17.43	17.43
Corn germ[1]	12.80	12.80	12.80	12.80	12.80
Cottonseed meal[1]	10.00	10.00	10.00	10.00	10.00
Carboxymethyl cellulose[2]	2.00	2.00	2.00	2.00	2.00
Wheat midds[1]	2.00	2.00	2.00	2.00	2.00
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10
Mineral premix[1]	0.10	0.10	0.10	0.10	0.10
DL-Methionine[4]	0.05	0.05	0.05	0.05	0.05
L-Lysine[5]	0.05	0.05	0.05	0.05	0.05
Soybean oil[6]	3.47	2.97	2.47	1.97	1.47
Soy lecithin[7]	0.00	0.50	1.00	1.50	2.00
Proximate composition					
Dry matter (%)	92.4	91.9	91.9	91.8	91.6
Crude protein (%)	35.4	35.1	34.9	35.1	35.2
Crude lipid (%)	5.88	5.69	5.91	5.95	5.48
Phospholipids (%)	16.5	18.9	21.3	23.9	24.5
Ash (%)	5.25	5.33	5.26	5.34	5.33
Phosphorus (%)	0.647	0.627	0.651	0.658	0.664

892 [1]Ingredients donated by Delta Western Feed Ingredients and Fish Belt Feeds, MS, USA
893 [2]MP Biomedicals, Solon, OH, USA
894 [3]DSM Chemicals, Heerlen, Netherlands
895 [4]Thermo Scientific, Waltham, MA, USA
896 [5]Beantown Chemical, Hudson, NH, USA
897 [6]Food grade soybean oil, USA
898 [7]Bunge, St. Louis, MO, USA
899

900 Table 2: Experimental diets formulated for channel catfish (*Ictalurus punctatus*) juveniles
901 evaluating soy lecithin (SL) supplementation at 1%, and a diet containing catfish oil (CFO),
902 soybean oil (SBO) serving as a negative control. Data is expressed as % on a dry-matter basis,
903 unless otherwise stated.

Ingredients	SBO	SL	CFO
Corn germ[1]	3.8	3.8	3.8
Cottonseed meal[1]	4.35	4.35	4.35
Soybean meal[1]	65.7	65.7	65.7
Corn meal[1]	10	10	10
Wheat midds[1]	9.9	9.9	9.9
Dicalcium phosphate[1]	1.1	1.1	1.1
Catfish oil[1]	0	0	2.45
Soybean oil[4]	2.45	1.45	0
Soy lecithin[5]	0	1	0
Vitamin premix[3]	0.1	0.1	0.1
Mineral premix[1]	0.1	0.1	0.1
Carboxymethyl cellulose[2]	2.5	2.5	2.5
Proximate composition			
Dry matter	94.4	95.3	95.3
Crude protein	39.7	39.8	40.8
Lipid	4.73	6.02	5.33
Ash	7.12	7.70	7.71
Phospholipids (% of lipid)	19.1	24.1	22.6

904 [1]Ingredients donated by Delta Western Feed Ingredients and Fish Belt Feeds, MS, USA
905 [2]MP Biomedicals, Solon, OH, USA
906 [3]DSM Chemicals, Heerlen, Netherlands
907 [4]Food grade soybean oil, USA
908 [5]Bunge, St. Louis, MO, USA

Table 3: Production performance, condition indices and red blood cell panel of channel catfish (*Ictalurus punctatus)* juveniles, fed experimental diets supplemented with graded levels of soy lecithin for 70 days.

	IW (g)	FW (g)	WG (%)	FE	Survival (%)	VSI (%)	HSI (%)	IPF (%)	Ht (%)	Hb (g dL^{-1})	RBC ($\times 10^6$ µL)
Control	4.38	29.7	590.9	0.81^{B}	98.7	8.92	1.80	3.62	29.0	3.95	4.95
0.50%	4.43	32.0	621.9	0.86^{AB}	98.7	8.96	1.65	4.12	29.0	4.47	5.00
1.00%	4.43	31.7	616.2	0.84^{AB}	98.7	8.65	1.65	3.55	28.2	4.41	4.89
1.50%	4.45	33.2	646.1	0.89^{A}	99.3	8.65	1.60	3.74	28.4	3.49	4.73
2.00%	4.41	31.9	624.5	0.84^{AB}	98.0	8.57	1.39	3.61	30.4	3.42	5.26
PSE	2.57	0.76	15.8	0.02	0.7	0.25	0.09	0.29	0.7	0.53	0.35
P-value	-	0.60	0.22	0.04	0.83	0.89	0.09	0.67	0.53	0.49	0.87

Abbreviations: FE: Feed efficiency; FW: Final weight (g); Hb: Hemoglobin; Ht: Hematocrit; HSI: Hepatosomatic index; IPF: Intraperitoneal fat; IW: Initial weight; RBC: Red blood cell concentration; VSI: Viscerosomatic index; PSE: Pooled standard error; WG: Weight gain.

Table 4: Whole-body proximate composition and protein conversion efficiency of channel catfish (*Ictalurus punctatus*) juveniles fed experimental diets supplemented with graded levels of soy lecithin for 70 days. Values are expressed as g/100 g of sample on a wet basis, unless otherwise stated.

	Dry matter	Protein (%)	Lipid (%)	Ash (%)	PCE (%)
Control	29.5	20.5	11.7	2.17	32.2^{B}
0.50%	30.5	21.7	12.4	2.43	38.4^{A}
1.00%	30.2	20.8	12.3	2.33	34.9^{AB}
1.50%	29.8	20.9	11.5	2.21	36.5^{AB}
2.00%	29.6	20.7	12.3	2.21	33.7^{AB}
PSE	0.5	0.43	0.6	0.07	1.2
P-value	0.71	0.35	0.83	0.11	0.02

Abbreviations: PCE: Protein conversion efficiency; PSE: Pooled standard error. Different letters indicate statistically significant differences among groups ($P < 0.05$)

Table 5: Whole blood parameters and plasma stress markers from channel catfish (Ictalurus punctatus) juveniles fed the experimental diets for 74 days and subjected to an air exposure stress challenge.

	Ht (%)	Hb (g/dL)	RBC ($\times 10^6$ cells/μL)	Cortisol (ng/mL)	Glucose (mg/dL)	Lactate (mmol/L)	Osmolality (mmol/kg)
Time							
0 h	28.5^{AB}	3.93^{AB}	4.28	3.78	43.6^{B}	5.37^{B}	256.5^{B}
0.5 h	29.4^{A}	4.23^{A}	4.39	5.08	57.1^{A}	11.74^{A}	268.9^{A}
1 h	26.9^{BC}	3.23^{C}	3.90	5.20	58.2^{A}	13.50^{A}	269.6^{A}
2 h	26.4^{C}	3.29^{C}	4.37	3.96	54.2^{AB}	6.90^{AB}	276.2^{AB}
6 h	26.1^{C}	3.58^{BC}	4.21	7.81	43.4^{B}	4.57^{B}	276.7^{AB}
Treatment							
Control	27.1	3.47	4.15	3.50	49.5	7.76	268.8
0.50%	27.6	3.76	4.29	4.63	54.2	7.98	270.5
1.00%	27.5	3.59	4.13	5.82	51.1	8.7	262.6
1.50%	27.3	3.65	4.10	5.57	50.3	8.47	273.8
2.00%	27.9	3.80	4.48	5.06	51.3	9.48	273.0
P-values							
Time	<0.01	<0.01	0.38	<0.01	0.03	<0.01	0.01
Treatment	0.79	0.30	0.16	0.12	0.83	0.44	0.44
Interaction	0.08	0.31	0.01*	0.03	0.37	0.57	0.06

Abbreviations: Hb: Hemoglobin; Ht: Hematocrit; RBC: Red blood cells

*Despite significant differences for the interaction, the Tukey-HSD test did not show differences among the time*treatments.

Table 6: Production performance, condition indices, and hematology of channel catfish (*Ictalurus punctatus*) fed the experimental diets for 70 days.

	IW (g)	FW (g)	WG (%)	FE	Survival (%)	VSI (%)	HSI (%)	IPF (%)	Ht (%)	Hb (g/dL)	RBC ($\times 10^6$ cells/μL)
SBO	4.98	40.6	717.6	0.63^{B}	90.5	6.14	1.17	2.01	34.2	5.34^{B}	4.76
SL	5.00	43.4	767.0	0.69^{A}	93.9	6.15	1.18	1.90	34.3	5.68^{AB}	4.99
CFO	4.99	42.0	743.8	0.67^{AB}	93.3	6.08	1.23	1.86	34.8	7.16^{A}	4.87
PSE	0.05	2.9	28.1	0.01	1.8	0.25	0.03	0.18	1.5	0.26	0.56
P-value	-	0.33	0.48	0.01	0.41	0.97	0.44	0.83	0.96	0.01	0.96

Abbreviations: CFO: Catfish oil; FE: Feed efficiency; FW: Final weight; Hb: Hemoglobin; Ht: Hematocrit; IW: Initial weight; SBO: Soybean oil; SL: Soy lecithin; PSE: Pooled standard error; VSI: Viscerosomatic index; RBC: Red blood cells; WG: Weight gain. Different letters indicate statistically significant differences among groups ($P < 0.05$).

Table 7: Whole-body proximate composition and protein conversion efficiency of channel catfish (*Ictalurus punctatus)* fed the experimental diets for 70 days. Data for whole-body proximate composition is expressed in % on a wet-basis.

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	PCE (%)
Soybean oil	71.6	15.3	10.0	2.70	27.6
Soy lecithin	72.4	15.0	8.9	3.16	29.7
Catfish oil	71.3	15.6	10.1	2.66	29.7
PSE	0.67	0.29	0.5	0.25	0.7
P-value	0.50	0.40	0.17	0.32	0.10

Abbreviations: PCE: Protein conversion efficiency; PSE: Pooled standard error.

Table 8: Fatty acid composition (%) present in muscle of channel catfish (*Ictalurus punctatus)* fed with lipids source (Soybean oil, soy lecithin, catfish oil) for 70 days.

	SBO fillet	SL fillet	CFO fillet	PSE	ANOVA P-value
Saturated					
14:0	0.58[a]	0.63[ab]	0.72[b]	0.03	<0.01
16:0	18.3	18.5	19.2	0.27	0.06
17:0	0.12	0.12	0.13	0.1	0.46
18:0	5.05	4.72	5.00	0.13	0.20
20:0	0.16[a]	0.15[ab]	0.14[b]	0.01	0.01
SFA[1]	24.4[a]	24.3[a]	25.5[b]	0.59	0.03
Monounsaturated					
16:1	1.49[a]	1.70[b]	1.99[c]	0.04	<0.01
17:1	0.35	0.32	0.38	0.02	0.20
18:1	38.8[a]	38.9[a]	44.1[b]	0.7	<0.01
20:1	1.22[a]	1.19[a]	1.71[b]	0.03	<0.01
MUFA[2]	43.4[a]	43.7[a]	49.9[b]	1.0	0.01
Polyunsaturated					
18:3 n-3	1.91[a]	1.86[a]	1.04[b]	0.05	<0.01
18:4 n-3	0.10[a]	0.09[a]	0.05[b]	0.01	0.01
20:3n-3	0.12[a]	0.13[a]	0.09[b]	0	0.01
20:5 n-3	0.20	0.18	0.20	0.03	0.88
22:5 n-3	0.19	0.18	0.18	0.01	0.81
22:6 n-3	0.8	0.76	0.87	0.07	0.60
n-3	3.32	3.21	2.43	0.17	0.90
18:2 n-6	19.8[a]	19.9[a]	13.0[b]	0.37	<0.01
18:3 n-6	0.61[a]	0.63[a]	0.43[b]	0.02	<0.01
20:3 n-6	2.04	1.80	1.63	0.14	0.15
20:4 n-6	1.08	0.99	1.08	0.08	0.65
n-6	23.8[a]	23.6[a]	16.4[b]	0.62	0.01
PUFA[3]	28.2[a]	27.9[a]	19.7[b]	0.23	0.01
LC-PUFA	4.61	4.22	4.22	0.10	0.44
n-3: n-6	0.14	0.14	0.15	0.00	0.14
n-6:n-3	7.18	7.38	6.84	0.16	0.12

Abbreviations: Total unsaturated fatty acids. SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid. [1]SFA: Summary of all fatty acids with no double bones; [2]MUFA: Sum of all fatty acids with one double bone; [3]PUFA: PUFA: Summary of all fatty acids with 2 or more double bonds. Catfish oil (CFO), Soybean oil (SBO), and Soy lecithin oil (SL). Total polyunsaturated also included: 9c-14:1,18:2t, 9t-18:1, 20:4 n-3, 20:5 n-3, and 22:3 n-3.

Figures

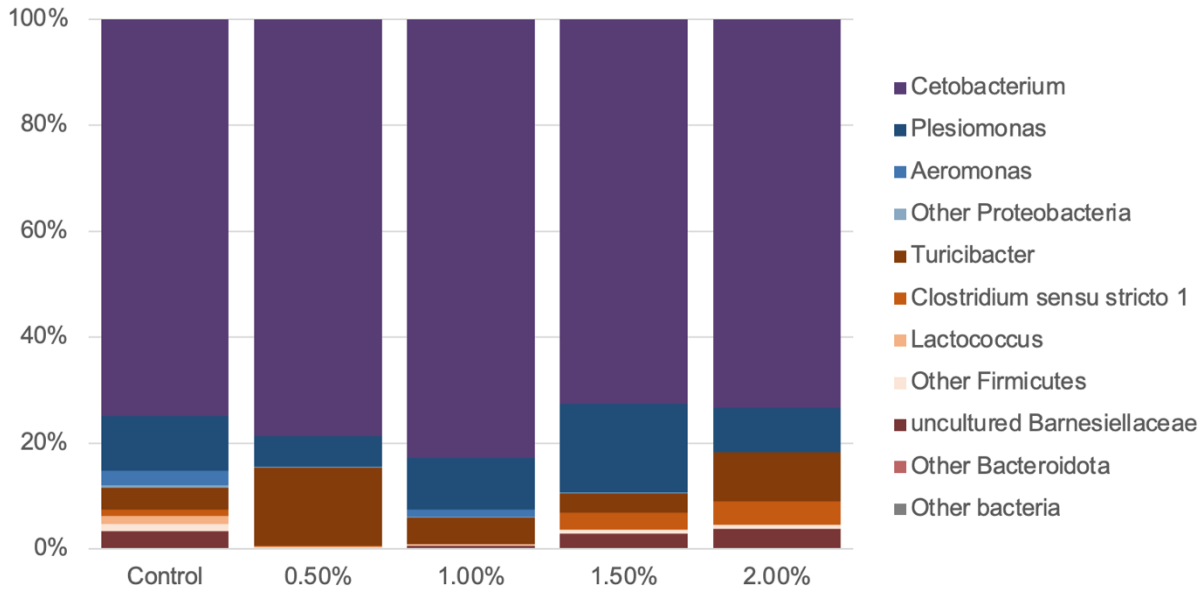

Figure 1: Average bacterial relative abundance in the posterior intestinal segment of channel catfish (*Ictalurus punctatus*) fed diets containing different levels of inclusion of soy lecithin (Control, 0.5%, 1.0%, 1.5%, 2.0%) for 79 days.

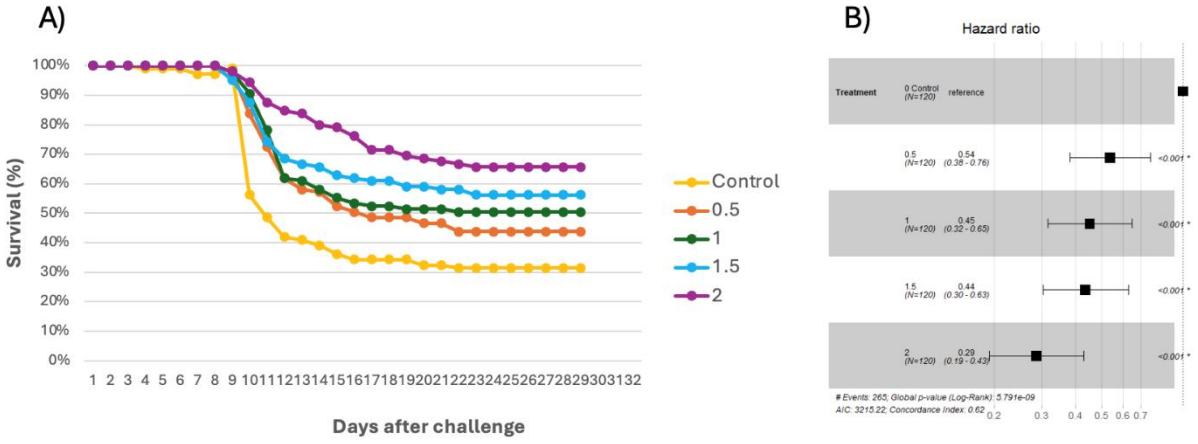

Figure 2: A) Survival curve of channel catfish (*Ictalurus punctatus*) juveniles exposed to an *Edwardsiella ictaluri* challenge through immersion**.** B) Hazard ratio plot using the 0% (control) treatment as a reference.

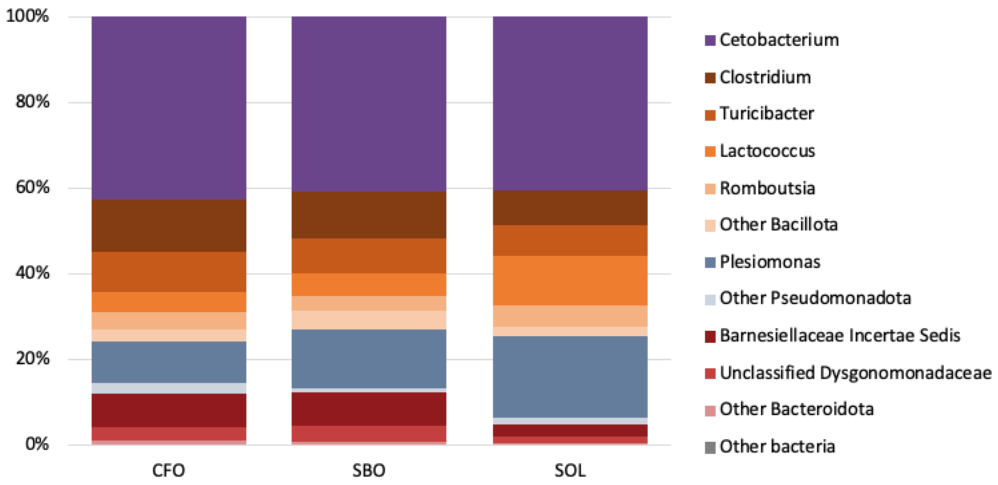

Figure 3: Average of microbiome diversity of the posterior intestinal segment of channel catfish (*Ictalurus punctatus*) fed experimental diets containing fish oil (CFO), soybean oil (SBO), and soybean lecithin (SL) for 79 days.

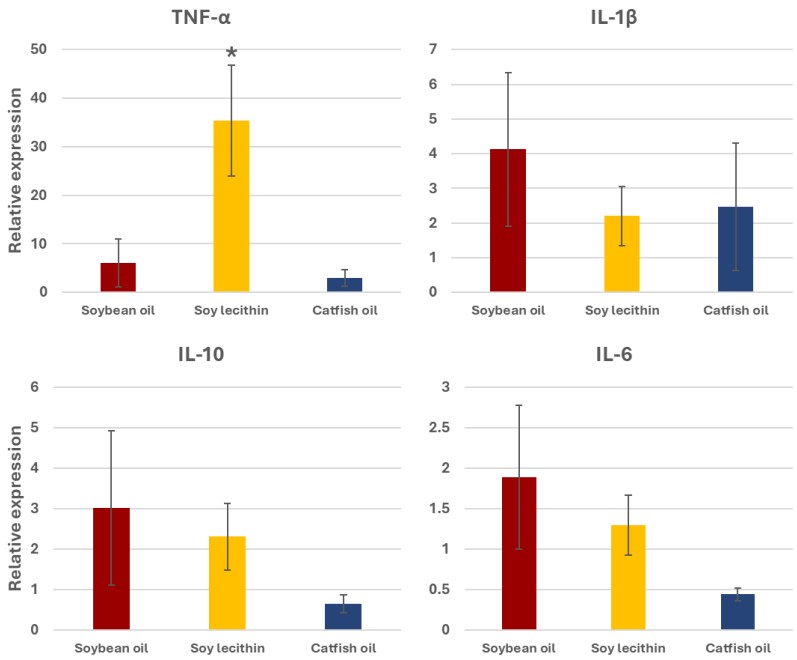

Figure 4: Relative gene expression of cytokines in the intestinal tissue of experimental channel catfish (*Ictalurus punctatus)* after the 70-day feeding trial. Data is represented by averages with standard error bars.

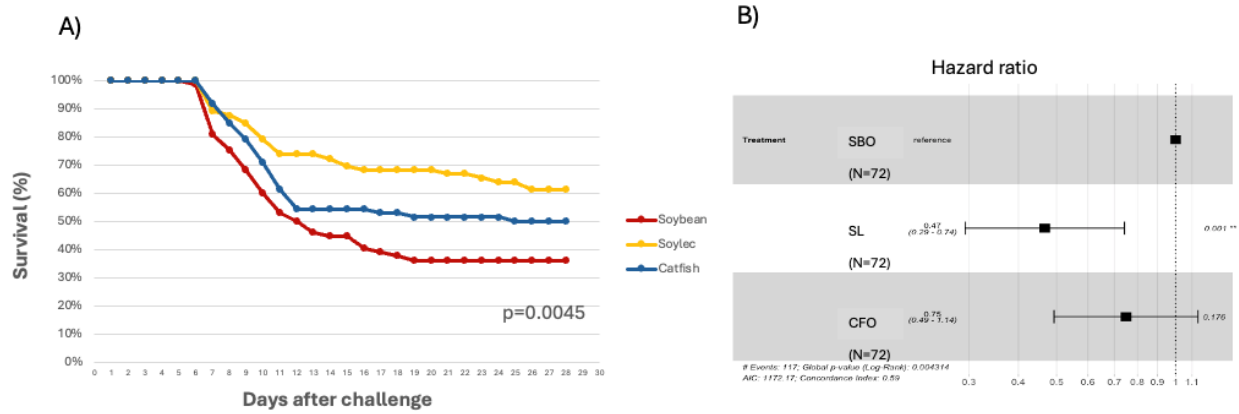

Figure 5: Survival curve of remaining experimental channel catfish (*Ictalurus punctatus*) juveniles after feeding the experimental diets for 82 days. Fish were exposed to *Edwardsiella ictaluri* (6.2×10^6 CFU/mL) through bath immersion; **6B)** Hazard ratio plot using the soybean treatment as a reference.

Supplementary Table 1: Fatty acid composition (%) of lipids from soybean oil (SBO), soy lecithin (SL), and catfish oil (CFO) used in experimental diets for channel catfish (*Ictalurus punctatus*).

	SBO Oil	SL Oil	CFO Oil	SBO Feed	SL Feed	CFO Feed
Saturated						
14:0	0.07	0.08	1.00	0.14	0.13	0.60
16:0	10.3	14.6	17.7	13.5	14.2	16.7
17:0	0.10	0.13	0.18	0.11	0.12	0.15
18:0	4.37	4.34	5.48	3.79	3.75	4.17
20:0	0.33	0.23	0.17	0.31	0.30	0.25
SFA[1]	15.3	20.0	24.9	18.5	18.9	22.4
Monounsaturated						
16:1	0.09	0.12	2.32	0.13	0.14	1.18
17:1	0.01	0.01	0.04	0.00	0.00	0.04
18:1	20.9	13.4	45.7	18.9	17.8	30.0
20:1	0.70	0.17	1.62	0.36	0.32	0.85
MUFA[2]	23.1	15.1	52.0	20.9	19.7	34.0
Polyunsaturated						
18:3 n-3	6.47	7.23	1.01	6.02	6.01	3.37
18:4 n-3	0.00	0.05	0.07	0.00	0.05	0.06
20:3 n-3	0.00	0.01	0.08	0.00	0.00	0.04
20:5 n-3	0.00	0.00	0.01	0.00	0.04	0.20
22:5 n-3	0.00	0.00	0.19	0.00	0.00	0.09
22:6 n-3	0.00	0.00	0.50	0.00	0.00	0.4
n-3	6.47	7.3	1.44	6.02	6.14	4.36
18:2 n-6	52.9	56.3	14.3	53.2	53.4	34.8
18:3 n-6	0.5	0.09	0.32	0.13	0.09	0.14
20:4 n-6	0.00	0.00	0.55	0.00	0.00	0.26
n-6	53.4	56.4	16.1	53.3	53.5	35.7
PUFA[3]						
PUFA	0.06	0.05	0.91	0.04	0.03	0.06
n-3:n-6	0.12	0.13	0.08	0.11	0.11	0.12
n-6:n-3	8.25	7.72	11.18	8.85	8.71	8.18

Abbreviations: SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid. [1]SFA: Summary of all fatty acids with no double bones; [2]MUFA: Sum of all fatty acids with one double bone; [3]PUFA: PUFA: Summary of all fatty acids with 2 or more double bonds. Total polyunsaturated also included: 9c-14:1,18:2t, 9t-18:1, 20:4 n-3, and 22:3 n-3. SBO: soybean oil, SL: soy lecithin, CFO: catfish oil

970 Supplementary Table 2: Intestinal fold numbers, length, and mucosa thickness from channel
971 catfish (*Ictalurus punctatus*) juveniles fed the experimental diets for 70 days.

	Number of folds		Mucosa thickness (μm)		Length of folds (μm)	
	Anterior intestine	Posterior intestine	Anterior intestine	Posterior intestine	Anterior intestine	Posterior intestine
Control	38.2	27.2	84.5	155.7	566.6	593.5
0.50%	37.7	29.6	86.1	128.6	628.6	635.2
1.00%	36.9	33.2	92.8	135.7	610.3	610.8
1.50%	35.9	30.4	84.5	172.4	614.3	611.4
2.00%	34.9	27.7	95.8	159.0	639.5	573.3
PSE	1.7	1.9	11.1	23.9	29.1	28.8
P-value	0.74	0.72	0.93*	0.69	0.68*	0.64

972 *Data was not normally distributed; thus, it is reported Prob>ChiSquare values from the non-parametric Kruskal-Wallis test.
973 Different letters indicate statistically significant differences among groups ($P < 0.05$)
974
975 Supplementary Table 3: Alpha and beta diversity of intestinal microbiota from channel catfish
976 (*Ictalurus punctatus*) digesta fed the experimental diets supplemented with graded doses of soy
977 lecithin for 78 days. Alpha diversity was compared across treatments using the Kruskal-Wallis
978 test, and beta diversity was performed using the analysis of similarities test (ANOSIM).

Alpha diversity	Kruskal-Wallis P-value
Faith phylogenetic diversity	0.86
Observed features	0.90
Pielou's Evenness	0.04
Shannon entropy	0.08

Beta diversity	ANOSIM R	ANOSIM P-value
Bray Curtis	0.02	0.30
Jaccard	0.01	0.40
Unweighted UniFrac	0.001	0.45
Weighted UniFrac	0.02	0.31

979

Supplementary Table 4: Intestinal fold numbers, length and mucosa thickness from the posterior intestine of channel catfish (*Ictalurus punctatus)* juveniles fed the experimental diets for 70 days.

	Number of folds	Mucosa thickness (μm)	Length of folds (μm)
	Posterior intestine		
SBO	22.7	126.5	3395.0
SL	25.4	117.2	1381.9
CFO	26.3	112.8	1336.9
PSE	2.0	10.1	1147.3
P-value	0.77	0.42*	0.30*

Abbreviations: PSE: Pooled standard error. SBO: Soybean oil, SL: Soy lecithin oil, CFO: Catfish oil
*Data was not normally distributed; thus, it is reported Prob>ChiSquare values from the non-parametric Kruskal-Wallis test.

Supplementary Table 5: Microbiome diversity of the posterior intestinal segment of channel catfish (*Ictalurus punctatus)* fed diets containing catfish oil, soybean oil, and soybean lecithin.

Alpha diversity	Kruskal-Wallis	
	P-value	
Faith phylogenetic diversity	0.38	
Observed features	0.18	
Pielou's Evenness	0.51	
Shannon entropy	0.63	
Beta diversity	**ANOSIM R**	**ANOSIM P-value**
Bray Curtis	0.13	0.06
Jaccard	0.07	0.15
Unweighted UniFrac	0.05	0.19
Weighted UniFrac	0.10	0.12

Evaluating the dietary supplementation of soy lecithin and catfish oil in feeds for hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) raised in earthen ponds

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ABSTRACT

This study evaluated feeding hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) with a commercial feed top coated with 1% catfish oil (CFO), 1% mixture of soybean oil and soy lecithin (SL; 0.5%), or the plain commercial feed as a control. Thirty thousand advanced hybrid catfish juveniles (~54 g) were equally distributed in 30 earthen ponds (0.04 ha), and the three dietary treatments were randomly assigned to each experimental unit (n=10). Fish were fed to satiation once a day for 119 days, and at the end of the study, fish were harvested, group weighed, and individually counted. A subset of 100 fish per pond were individually weighed to assess if the dietary treatments could affect the size distribution of the population. Thirty fish per pond were selected for processing, where carcass and fillet yield, and intraperitoneal fat were sampled. Fish fillets were subjected to proximate composition and fatty acid profile. No differences were observed for final weight, weight gain, feed conversion ratio, survival, and fillet, carcass and nugget yield. The proximate composition of the hybrid catfish fillets was unaffected by the dietary treatments. However, fish fed CFO presented higher fat accumulation in their intraperitoneal cavity, as well as a higher number of individuals between 0.75-1.00 lb when compared to the other treatments. The supplementation of SL modulated the fatty acid profile of hybrid catfish fillets, by increasing the concentration of eicosapentaenoic acid (20:5 n-3), linoleic acid (18:2 n-6), total polyunsaturated fatty acids and total long-chain polyunsaturated fatty acids. Dietary lipid supplementation presented to be a beneficial approach to catfish culture as a positive modulation in the fatty acid profile has been promoted by SL, and a reduction of undersized fish was observed for the CFO group.

Keywords: crude protein, hybrid catfish, pond production, fillet yield, ammonia nitrogen

Material and Methods

Experimental conditions, animals and feeding

Thirty experimental earthen ponds, 0.04 ha, at the National Warmwater Aquaculture Center (Stoneville, MS) were stocked each with 1,000 hybrid catfish juveniles (*Ictalurus punctatus* × *I. furcatus*) (initial weight of 54.4 ± 1.0 g).

A commercial diet with 28% crude protein and 4% lipid was used to prepare the dietary treatments. Batches of 15 kg of feed were loaded in concrete mixers (50 kg capacity), and either soy lecithin mixed with soybean oil or 1% catfish oil was sprayed to topcoat the feed. The feed was allowed to mix for an additional 15 min and stored at room temperature in containers sheltered from light. Fish were fed to apparent satiation, once a day, and the feeding trial lasted 123 days. Because of the weather conditions, fish were fed for 119 days under normal conditions, while feeding was skipped for 4 days due to heavy rain. Dissolved oxygen and temperature were sampled from 5 randomly selected experimental ponds every weekday, and total ammonia-nitrogen, nitrite-nitrogen, chlorides, and pH were sampled from all ponds once a week as described by Mischke et al. (2023). Each pond had additional aeration at 18:00 until 8:00 using 0.37 kW electrical aerators (Air-O-Lator Corp., Kansas City, MO, USA) to ensure adequate levels of dissolved oxygen were provided during the nighttime.

Feeding ceased for 2 days before harvest, and each pond was seined thrice using tractors, and fish were loaded on a truck equipped with hauling tanks. Each tank compartment had supplemental liquid oxygenation to ensure O_2 levels were above saturation, where fish were transported to concrete vats flowing with pond water and stocked in cages within the vats. Groups of 25 fish were counted and weighed in plastic baskets to compute the production performance metrics. A subset of 100 fish per experimental pond was anesthetized with MS-222 (200 mg/L) (Topic Popovic et al. 2012) to obtain individual weight (kg). A group of 30 fish per pond was selected, with individual weights close to the population average, and subjected to processing for fillet and nuggets, following the procedures previously outlined by Li et al. (2018). The fillets were mechanically ground and freeze-dried for 24 h. The resulting sample was homogenized and subjected to cold lipid extraction using chloroform and

methanol (Folch et al. 1957). The resulting solvent suspension was evaporated under N_2, and samples were shipped frozen to the ESCL University of Missouri for fatty acid profile using gas chromatography. The fillet samples were subjected to proximate analyses to compute total protein, crude lipid, and ash using AOAC procedures (2005).

The fish that were not captured during the seining day were euthanized and counted, and the estimated biomass from each pond was computed using their respective average individual weight. The calculations for production performance and carcass yield metrics were performed as follows:

Weight Gain (%)=[(Final weight (g)-Initial weight (g)) / Initial weight (g)]×100

Feed Conversion Ratio (FCR)=Feed intake (g) / Weight gain (g)

Intraperitoneal fat ratio (IPF) (%)=[(intraperitoneal fat (g))/(body weight (g))]×100

Carcass yield (%) = [(weight of the fish (g) - beheaded and gutted fish (g))/weight of the fish (g)]×100

Fillet or nugget yield (%) = (weight of the fillets or nugget (g) / weight of the fish (g))

All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Statistical analysis

The production performance and carcass yield data were validated for normality using the Shapiro-Wilk test, and for homogeneity of variances using the Brown-Forsythe test (1974). If the data were normally distributed and had no heterogeneity in variances, they were subjected to a one-way ANOVA, and if significant differences were detected ($P<0.05$), then they were further subjected to the Tukey-HSD for comparison of means. If the data were not normally distributed or had heterogeneity of variances, they were subjected to the nonparametric Kruskal-Wallis test, and if Prob>ChiSq significance (<0.05) was observed, then a Dunn all-pairs joint rank test was performed. Data from fish individually weighed were grouped by treatment, and differences in size frequency were evaluated using the Pearson

Chi-Square test. All statistical analyses were performed using JMP Software (v. 18.0.0, SAS Institute, CA, USA).

Results

Throughout the feeding trial, the water temperature averaged 26.7 ± 4.1°C, and dissolved oxygen was 6.64 ± 2.12 mg/L. Total ammonia nitrogen = 0.25 ± 0.39; total nitrite nitrogen = 0.074 ± 0.148; pH = 7.81 ± 0.23.

No significant differences ($P>0.05$) were observed for final weight, weight gain (%), FCR, and survival (Table 1). Nevertheless, it is important to highlight that a reduced P-value was observed for WG and FCR ($P=0.07$ and 0.06, respectively). The IPF was significantly affected by the dietary treatments ($P<0.05$), where fish fed CFO diets presented higher fat accumulation in the intraperitoneal cavity when compared to the control group.

Carcass, fillet and nugget yield were unaffected by the dietary treatments (Table 2), as well as the fillet proximate composition (dry matter, protein, lipid and ash; Table 3). Significant differences were observed for the fillet's fatty acid profile depending on the dietary treatment (Table 4). Fish fed diets topcoated with CFO exhibited higher levels of myristic acid (14:0) and palmitoleic acid (16:1) compared to the SL group. On the other hand, heptadecenoic acid (17:1) level in the fillets fed the control diet was significantly higher when compared to the fish fed SL diets. Higher levels of eicosapentaenoic acid (20:5 n-3), linoleic acid (18:2 n-6), total n-6, total PUFA, and total LC-PUFA were observed for fish treated with SL when compared to the control group.

At the end of the study, the dietary treatments significantly affected the frequency distribution of class sizes of hybrid catfish, where catfish oil presented a lower number of individuals under <0.5 lb, and a higher frequency on the 0.75-1.00 lb.

Disclosure statement

The authors declare there are no competing interests.

Data availability statement

Data for this study can be shared upon reasonable request.

Acknowledgments

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Table 1: Production performance and carcass yield of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) fed the dietary treatments for 119 days.

	Initial weight (g/fish)	Final weight (g/fish)	WG (%)	FCR	IPF (%)	Survival (%)
Control	54.5	316.6	422.2	1.87	3.72^{B}	90.0
CFO	54.3	339.9	476.8	1.79	4.06^{A}	92.4
SL	54.4	326.4	450.9	1.77	3.89^{AB}	91.8
PSE	0.3	10.4	15.9	0.03	0.07	1.0
P-value	-	0.29	0.07	0.06	0.006	0.23

Abbreviations: CFO: Catfish oil; FCR: Feed conversion ratio; IPF: Intraperitoneal fat; PSE: Pooled standard error; SL: Soy lecithin; WG: Weight gain.

Table 2: Processing yield of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) fed for 119 days the dietary treatments.

	Carcass yield (%)	Fillet yield (%)	Nugget yield (%)
Control	68.1	32.7	9.43
CFO	67.9	32.7	9.40
SL	67.6	32.8	9.47
PSE	0.2	0.3	0.10
P-value	0.12	0.91	0.88

Abbreviations: CFO: Catfish oil; PSE: Pooled standard error; SL: Soy lecithin.

Table 3: Proximate composition of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) fillets fed the experimental treatments for 119 days. Values are expressed as g/100 g of sample on a wet basis.

	Dry matter	Protein	Lipid	Ash
Control	22.6	15.3	4.73	0.24
CFO	23.3	14.7	5.29	0.25
SL	22.9	14.6	4.93	0.24
PSE	0.4	0.4	0.26	0.006
P-value	0.51	0.42	0.32	0.32

Abbreviations: CFO: Catfish oil; PSE: Pooled standard error; SL: Soy lecithin.

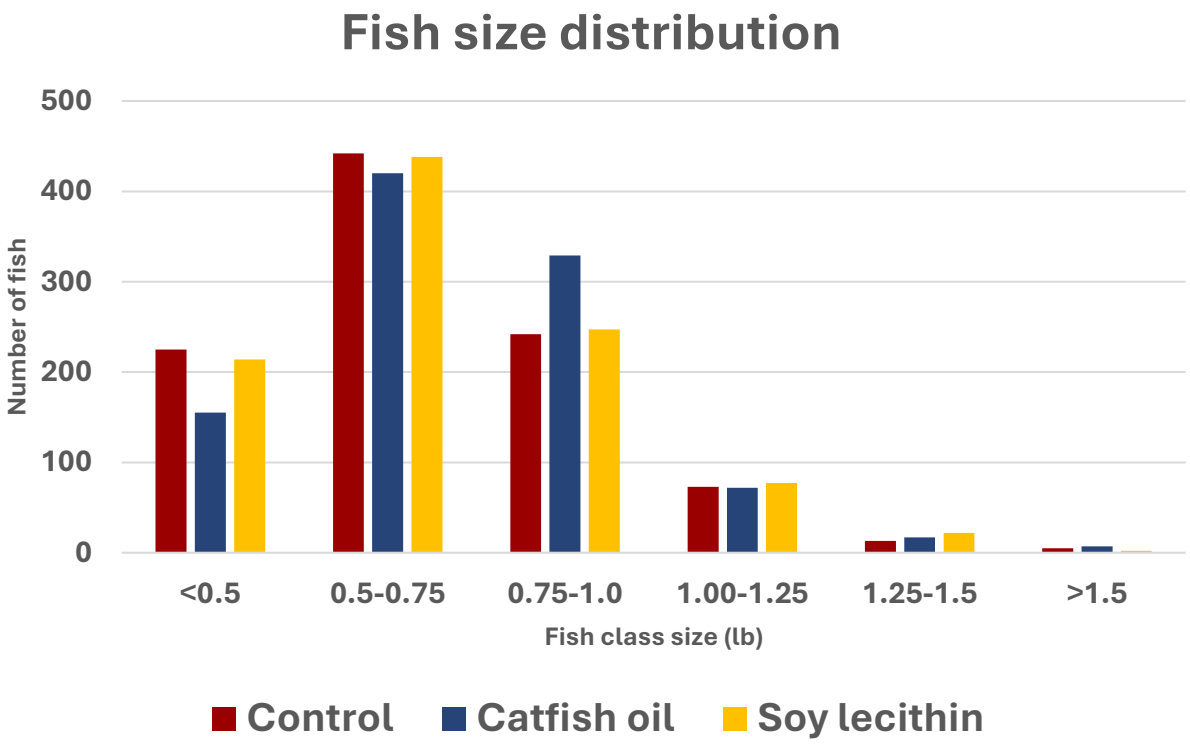
Table 4: Fatty acid profile of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) fed the experimental treatments for 119 days. Data is expressed as g/100 g of lipid.

	Control	CFO	SL	PSE	P-value/ Prob>ChiSq
Saturated					
14:0	0.60^{AB}	0.64^{A}	0.58^{B}	0.01	0.003*
16:0	18.0	18.0	17.9	0.1	0.68
18:0	6.49	6.55	6.49	0.08	0.86
SFA	25.7	25.8	25.6	0.2	0.63
Monounsaturated					
16:1	1.50^{AB}	1.53^{A}	1.43^{B}	0.02	0.01*
17:1	0.26^{A}	0.17^{AB}	0.13^{B}	0.03	0.01*
18:1	45.4	45.7	44.6	0.3	0.13
20:1	1.54	1.55	1.48	0.03	0.17
MUFA	50.4	50.8	49.5	0.4	0.08
Polyunsaturated					
18:3 n-3	0.66	0.62	0.78	0.04	0.01**
19:4 n-3	0.31	0.24	0.18	0.04	0.13
20:5 n-3	0.02^{B}	0.09^{AB}	0.11^{A}	0.02	0.02*
22:5 n-3	0.13	0.12	0.14	0.01	0.40
22:6 n-3	0.43	0.42	0.44	0.02	0.76
n-3	1.61	1.56	1.71	0.07	0.27
18:2 n-6	14.2^{B}	14.0^{AB}	15.2^{A}	0.2	0.02*
18:3 n-6	0.39	0.38	0.4	0.01	0.35
20:3 n-6	1.26	1.19	1.28	0.03	0.11
20:4 n-6	0.38	0.42	0.87	0.11	0.03**
n-6	16.5^{B}	16.3^{B}	18.0^{A}	0.25	0.001*
PUFA	19.0^{B}	18.7^{B}	20.6^{A}	0.3	0.01*
LC-PUFA	3.44^{B}	3.45^{B}	4.01^{A}	0.01	0.01
n-3/n-6	0.09	0.09	0.09	0.003	0.85
n-6/n-3	10.4	10.5	10.6	0.4	0.94

Abbreviations: CFO: Catfish oil; LC-PUFA: Long-chain polyunsaturated fatty acids; MUFA: Monounsaturated fatty acids; PSE: Pooled standard error; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids; SL: Soy lecithin.

*Data was not normally distributed and/or variances were heterogeneous; thus, a Nonparametric Kruskal-Wallis test was performed, followed by a Dunn all pairs for joint ranks.

**Data was analyzed using the aforementioned nonparametric test, but no differences were detected among the ranks.



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141 Figure 1: Frequency of hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) weight (lb) when fed the
142 experimental treatments for 119 days.

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