Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.155

RESEARCH ARTICLE

Effect of Graded Levels of Dietary L- Methionine on Growth Performance, Body Composition, Amino Acid Requirements, Serum Biochemical Parameters and Gut Microbiota in Coho Salmon (*Oncorhynchus Kisutch*) Alevins Cultured in Freshwater

Hairui Yu[1*], Abdur Rahman [1,2,3,*], Leyong Yu[4] , Govindhrajan Sattanathan[1,2], Lingyao Li[1 ,5], Muhammad Younus[6*], Muhammad Waqas[7], Asghar Abbas[8], Hafiz Muhammad Ishaq[8], Xiaojing Wu[1,9]

[1]Key Laboratory of Biochemistry and Molecular Biology in Universities of Shandong (Weifang University), Weifang Key Laboratory of Coho Salmon Culturing Facility Engineering, Institute of Modern Facility Fisheries, College of Biology and Oceanography, Weifang University, Weifang 261061, China; [2]Weifang Key Laboratory of Salmon and Trout Health Culture, Conqueren Leading Fresh Science and Technology Inc., Ltd., Weifang 261205, China; [3]Department of Animal Sciences, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan; [4]College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China; [5]Shandong Collaborative Innovation Center of Coho Salmon Health Culture Engineering Technology, Shandong Conqueren Marine Technology Co., Ltd., Weifang 261108, China; [6]Department of Pathology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan; [7]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Atakum, 55139, Samsun, Türkiye; [8]Department of Pathobiology and Biomedical Sciences, Muhammad Nawaz Sharif University of Agriculture Multan; [9]College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
*Corresponding author: yhr6003@hotmail.com, abdurrehman@uvas.edu.pk, younusrana@uvas.edu.pk

ARTICLE HISTORY (25-236)

Received: March 22, 2025
Revised: April 20, 2025
Accepted: April 23, 2025
Published online: April 27, 2025

Key words:
Body composition
Coho salmon alevins
Growth performance
Gut microbiota
L-methionine
Serum biochemistry

ABSTRACT

This study aimed to evaluate the methionine requirement of coho salmon (*Oncorhynchus kisutch)* alevins. A total of 1,800 alevins (initial body weight: 0.338±0.002 g) were used in the experiment, distributed across six dietary treatments with three replicates per treatment and 100 alevins per replicate. Six dietary concentrations levels of L-methionine were as follows: 1.09%, 1.64%, 2.15%, 2.63%, 3.14%, and 3.67% of the feed, or 1.98%, 3.01 %, 3.91%, 4.75%, 5.74% and 6.66% of the feed protein. ANOVA test was applied to statistically analyze the data. The results of the study revealed that body weight and specific growth rate were higher at 2.15% methionine level. While body protein increased with the increase of methionine level in the feed, reaching the highest ($P<0.05$) level at 2.63 % methionine, the arginine content was significantly lower at 1.09% and 1.64% levels; the lysine and methionine content was significantly higher at 2.15% and 2.63% methionine levels. Body fat, ash content, and essential amino acid requirements were nonsignificant. Significantly higher ALT, aspartate AST, *Lactobacillus*, and Bacillus, and lower ($P<0.05$) total cholesterol and triglyceride, *Coliform*, and *Vibrio* were recorded in 2.15% and 2.63% L-Methionine group. It was concluded that dietary L-methionine levels of 2.15% and 2.63% effectively enhanced performance, body composition, serum biochemical parameters, and gut microbiota in coho salmon alevins. Furthermore, the quadratic polynomial model analysis indicated that a methionine requirement between 2.55% and 2.69% is optimal for achieving the best results in specific growth rate, feed conversion ratio, protein efficiency ratio, and body protein deposition.

To Cite This Article: Yu H, Rahman A, Yu L, Sattanathan G, Li L, Younus M, Waqas M, Abbas A, Ishaq HM and Wu X, 2025. Effect of Graded Levels of Dietary L- Methionine on Growth Performance, Body Composition, Amino Acid Requirements, Serum Biochemical Parameters and Gut Microbiota in Coho Salmon (*Oncorhynchus Kisutch*) Alevins Cultured in Freshwater. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.155

INTRODUCTION

A nutritionally balanced feed is fundamental for optimizing fish growth and health. Among essential nutrients, protein and amino acids are crucial in regulating physiological functions, immune responses, and overall growth performance in fish (Li *et al*., 2009; Teles *et al*., 2020). In modern aquaculture, the high feed cost remains a

significant challenge for fish nutritionists, accounting for approximately 70% of total operational expenses (Hossain *et al*., 2020). Although fish meat is the primary protein source in aqua feeds, its limited global availability has led to increased costs and restricted supply. These economic constraints and sustainability concerns have necessitated the inclusion of alternative protein sources, such as processed plant proteins and by-products from agriculture, fisheries, and terrestrial animal processing. However, these alternatives often result in an imbalance of essential amino acids (EAAs), adversely affecting fish growth and metabolism (Nunes *et al*., 2014; Rolland *et al*., 2015). Soybean meal (SBM), a plant-based protein source, is considered one of the most promising alternatives due to its high protein content and relatively balanced amino acid profile; in 2020 SBM was the most widely grown crop, with 343 million metric tonnes of production (Singh *et al*., 2024). However, the complete replacement of fish meal with SBM has often been associated with reduced growth performance in fish (Pervin *et al*., 2020; Liu *et al*., 2021). This reduction in performance is attributed to SBM's lower methionine content and the presence of anti-nutritional factors (Ollie *et al*., 1994). Methionine and lysine are recognized as the most limiting amino acids in fish diets (Robinson and Wilson, 1985; Lovell, 1989). Several studies have demonstrated that methionine supplementation enhances growth and feed efficiency in various fish species, including blue catfish (*Ictalurus furcatus*) (Webster *et al*., 1995), rainbow trout (*Oncorhynchus mykiss*) (Kaushik *et al*., 1995; Cheng *et al*., 2003), Nile tilapia (*Oreochromis niloticus*) (El-Saidy and Gaber, 2002), and Indian major carp (*Cirrhinus mrigala*) (Khan *et al*., 2003). However, similar benefits have not been consistently observed in catfish (Andrews and Page, 1974), grass carp (Dabrowski and Kozak, 1979), or red drum (*Sciaenops ocellatus*) (Reigh and Ellis, 1992). A deficiency of dietary methionine has been linked to reduced weight gain, lower protein deposition, poor feed conversion efficiency, and increased fat accumulation (Abidi and Khan, 2011; Khan and Abidi, 2011). To formulate nutritionally balanced aqua feeds with an optimal amino acid profile, the inclusion of crystalline amino acids (CAA) has been proposed as most effective strategy than simply increasing dietary protein levels from conventional sources (Chu *et al*., 2014; Nunes *et al*., 2014). Recent studies have highlighted the significant physiological roles of amino acids in protein and hormone synthesis, immune function, and anti-oxidative defence. For instance, arginine (Hoseini *et al*., 2020), taurine (El-Sayed, 2014), methionine hydroxy analog (MHA) (Guo *et al*., 2020a), and tryptophan (Hoseini *et al*., 2019) have all been shown to play critical roles in fish nutrition and health. Lu *et al.* (2014) reported a decline in triglyceride in fish and Zhao *et al.* (2022) reported an increase in beneficial microbiota after supplementing methionine. Methionine, an indispensable amino acid, is unique among EAAs due to its sulfur-containing structure. It serves as the primary methyl donor in biological systems via the S-adenosylmethionine pathway, which is essential for the synthesis of key metabolites such as phosphatidylcholine and creatine. Research on aquatic animals shows that methionine deficiency triggers oxidative stress as well as liver damage and growth performance deterioration (Bin *et al.*, 2017; Lee *et al.*, 2019). The replacement of fish-meal with 25% soybean protein concentrate led to decreased weight gain together with reduced protein retention efficiency and elevated feed conversion ratios in whiteleg shrimp (*Litopenaeusvannamei)* as found by Chen *et al.* (2018). The research into methionine requirements within fish-meal aqua feeds demands further exploration due to its crucial nature. Aqua feeds incorporate methionine primarily in three forms: crystalline DL-methionine, crystalline L-methionine and DL-2-hydroxy-4-methylthiobutanoic acid (methionine hydroxy analog). The supplement Methionine hydroxy analog (MHA) exists commercially as hydroxy analog free acid (MHA-FA) together with methionine hydroxy analog salts including methionine hydroxy analog calcium (MHA-Ca) (Huyghebaert, 1993; Nunes *et al.*, 2014; Powell *et al.*, 2017; Niu *et al.*, 2018). However, crystalline amino acids are highly susceptible to leaching in aquatic environments, particularly for slow-feeding crustaceans. Moreover, the bioavailability of amino acids from intact proteins remains greater than the bioavailability of separate free amino acids because free amino acids undergo quick absorption followed by rapid metabolism which lowers their utilization rate (Gu *et al.*, 2013; Zheng *et al.*, 2023).

The present study aims to evaluate the effects of dietary L-methionine supplementation on coho salmon (*Oncorhynchus kisutch*) alevins. Coho salmon is one of the seven recognized Pacific salmon species within the *Oncorhynchus* genus, with a broad natural distribution and significant commercial and recreational value (Ruggerone and Irvine, 2018). Global production of coho salmon is estimated at approximately 120,000 metric tons annually, primarily sourced from aquaculture and wild fisheries, with Chile and Norway contributing to 80% of the farmed production (Anonymous, 2016). In recent years, China has expanded its coho salmon aquaculture sector. Researchers have extensively studied coho salmon's nutritional needs to develop better commercial fish diets, according to Yu *et al.* (2024). The precise mandatory amounts of EAA for coho salmon remain unknown despite research efforts. With the growing demand for cost-effective and nutritionally balanced feeds in coho salmon aquaculture, understanding methionine requirements is vital for optimizing early growth and health. However, data on dietary L-methionine needs during the alevin stage remain scarce. This study aimed to assess the effects of graded dietary L-methionine levels on survival, growth, body composition, amino acid profile, serum biochemistry, and gut microbiota of coho salmon alevins and to determine their optimal methionine requirement. The findings are expected to support efficient and sustainable feed formulation for commercial fish production.

MATERIALS AND METHODS

Ethical statement: The current study was carried out according to the recommendations (animal handling, care, and housing) in the "Guide for the Ethical Use of Experimental Animals" of the Weifang University, Weifang, Shandong, China (No. 20200601). It was approved by the Institutional Animal Care and Use Committee of the Institute of Modern Facility Fisheries, Weifang University, China (IACUC NO. 20220516003).

Experimental feed: The feed formula and the essential amino acid composition of the conventional ingredients and raw materials are shown in Tables 1 and 2, respectively. The experimental feed for coho salmon alevins was prepared with reference to the amino acid composition of coho salmon alevins (Yu *et al.*, 2023). The basal feed used super-steamed Peruvian fish meal, shrimp meal, soy protein concentrate, wheat gluten protein, and brewer's yeast as protein sources; α - α-starch as sugar source; fish oil, soybean oil, and soybean lecithin as fat sources, and added with complex vitamins and complex inorganic salts.

Table 1: Formulation and proximate composition of the experimental diets (% dry matter)

Dietary groups	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Methionine levels % of diet	1.09	1.64	2.15	2.63	3.14	3.67
Methionine levels % of diet protein	1.98	3.01	3.91	4.75	5.74	6.66
Ingredient composition (%)						
Fish meal (Peruvian)	25.00	25.00	25.00	25.00	25.00	25.00
Shrimp meal	10.00	10.00	10.00	10.00	10.00	10.00
Soy protein concentrate	25.00	25.00	25.00	25.00	25.00	25.00
Wheat gluten meal	6.00	6.00	6.00	6.00	6.00	6.00
Beer yeast	5.00	5.00	5.00	5.00	5.00	5.00
Methionine	0.00	0.50	1.00	1.50	2.00	2.50
Glycine	0.90	0.72	0.54	0.36	0.18	0.00
Glutamic acid	0.70	0.56	0.42	0.28	0.14	0.00
Cellulose	0.90	0.72	0.54	0.36	0.18	0.00
Amino acid mixture[1]	5.25	5.25	5.25	5.25	5.25	5.25
α - starch	7.82	7.82	7.82	7.82	7.82	7.82
Fish oil	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00
calcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix [2]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix [3]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Ascorbyl polyphosphate	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.03	0.03	0.03	0.03	0.03	0.03
Proximate composition						
Crude protein (%)	55.02	54. 53	54.97	55.39	54.75	55.15
Crude lipid (%)	15.75	15.37	15.61	15.44	15.82	15.47
Ash (%)	9.77	9.91	9.63	9.89	9.82	9.96
Methionine (% of diet)	1.09	1.64	2.15	2.63	3.14	3.67
Methionine (% of dietary protein)	1.98	3.01	3.91	4.75	5.74	6.66
energy (KJ g^{-1} DM)	22.15	21.93	22.34	22.26	22.52	22.20

Note: [1] Amino acid complex (g per 100 g dry diet): arginine, 0.02 ; histidine , 0.45; isoleucine, 0.43; leucine, 0.86; lysine, 0.91; phenylalanine, 0.31 ; threonine , 0.55 ; tryptophan , 0.14 ; valine , 0.88 ; 2 Vitamin premix (IU or g / kg vitamin ^{pre} mix) : VA palmitate, 10,000IU ; VD3 , 4,000IU ; DL - α - tocopherol, 75.0g ; menadione , 22.0g ; thiamine - HCl, 40.0g ; riboflavin, 30.0g ; D- calcium pantothenate, 150.0g ; pyridoxine- HCl, 20.0g ; Inositol, 300.0g ; D - biotin, 1.0g ; Folic acid, 15.0g ; Niacin, 200.0g ; VB_{12} , 0.3g ; [3] Mineral premix (g/kg mineral pre mix): $AlK(SO_4)_2 \cdot 12H_2O$, 124.0; $CaCl_2$, 17,880.0; $CoCl_2 \cdot 6H_2O$, 49.0; $FeSO_4 \cdot 7H_2O$, 707.0; KCl, 1192.0; KI, 5.0; $MgSO_4 \cdot 7H_2O$, 4317.0; $MnSO_4 \cdot 4H_2O$, 31.0 ; NaCl, 4934.0; $Na_2SeO_3 \cdot H_2O$, 3.0 ; $ZnSO_4 \cdot 7H_2O$, 177.0 ; 9930.0 .

The methionine in the basal feed (Diet 1) comes from Peruvian fish meal, shrimp meal, soy protein concentrate, wheat gluten protein, and brewer's yeast in the raw materials, with the lowest content, accounting for 1.09% of the dry matter of the feed or 1.98 % of the protein of the feed. The other five experimental diets (Diet 2 to Diet 6) were prepared by supplementing the basal feed with graded levels of L-crystalline methionine at 0.5% increments, resulting in final methionine concentrations of 1.64, 2.15, 2.63, 3.14, and 3.67% of the feed's dry matter, corresponding to 3.01, 3.91, 4.75, 5.74, and 6.66% of the feed protein, respectively. A total of 1,800 coho salmon alevins (initial body weight: 0.338±0.002g) were used in the experiment. The fish were randomly assigned to six dietary treatments, each with three replicates (18 replicates in total), with 100 alevins per replicate. Each replicate group was housed in a 240-L tank and reared under identical experimental conditions. The experiment lasted for 84 days. The experimental diet of coho salmon alevins was kept isonitrogenous and isoenergetic, when the methionine gradient was increased by reducing the corresponding contents of glycine and glutamate. The diet's pH was adjusted through the addition of 6N NaOH solution between 7.0-7.5. The diets were prepared into pellets of 150-425μm in size, packaged in aluminum foil bags, and stored at -20°C until use.

Experimental fish and daily management: The experiment was conducted at the Silver Salmon Seedling Breeding Center of Shandong Silver Salmon Engineering Technology Collaborative Innovation Center (Linyi). The experimental fish were artificially bred silver salmon juveniles in April of the same year. At the beginning of the experiment, 18,000 alevins with normal appearance, strong physique, and good vitality and weighing (0.338±0.002g) were selected and randomly divided into 18 groups, with 100 alevins in each group, and placed in n=18, 240L blue rectangular plastic barrels (80cm × 60cm × 60cm). All barrels were placed in the same indoor nursery pool (700cm × 500cm × 150cm), and there was a drainage pipe at the bottom of the pool. The underground spring water used was precipitated in an outdoor water storage tank, filtered in a secondary sand filter, and filtered through a filter bag before entering the nursery pool. During the experiment, the water temperature and pH were controlled at 16.0±1.0°C and 7.3±0.2, respectively, and the dissolved oxygen was maintained at 7.5mgO_2/L. Natural light was used during the experiment.

From the beginning of the experiment, the alevins were fed with experimental feeds (Diet 1 - Diet 6). Artificial feeding was given 4 times daily (7:00, 10:30, 14:00, 17:30).

Sampling and sample analysis: At the end of the experiment, the remaining fish in each tank were counted to measure their survival rate. Before feeding in the morning, the fish in each tank were weighed. Thirty fish were taken from each tank and freeze-dried at -21°C for at least 48 h for subsequent analysis. Feed and fish samples were then dried at 105°C to constant weight to determine dry matter content. Nitrogen (N), fat, and ash were analyzed according to AOAC (2005). Protein content was calculated as N × 6.25. Gross energy was measured using a Parr 1281 automatic oxygen bomb (Parr, Moline, IL, USA).

The amino acid content in the samples was determined using an amino acid automatic analyzer (Model A300, GmbH, Frankfurt, Germany). Hydrolysis of samples through 6N HCl solution at 110°C for 22h followed by filtration step was conducted except for the tryptophan sample. 2mL was taken and evaporated to dryness under reduced pressure after constant volume, and then 2mL of 0.0N HCl solution was added, and the samples were continuously shaken to dissolve all the amino acids before being subjected to chromatographic

Table 2: Essential amino acid (EAA) composition of ingredients in the experimental diets (% dry matter)

Essential Amino Acids	25% fish meal	10% Shrimp Powder	25% Soy Protein Concentrate	6 % wheat gluten Meal	5% brewer's yeast	55.78% Coho salmon egg protein	Supplied EAA
Lysine	1.50	0.44	0.93	0.10	0.18	4.06	0.91
Histidine	0.44	0.11	0.42	0.11	0.06	1.59	0.45
Arginine	1.18	0.33	1.35	0.20	0.12	3.18	0.02
Phenylalanine	0.89	0.27	0.88	0.21	0.22	2.78	0.31
Leucine	1.27	0.45	1.40	0.20	0.25	4.43	0.86
Isoleucine	0.83	0.30	0.79	0.36	0.15	3.07	0.63
Threonine	0.89	0.28	0.72	0.14	0.12	2.70	0.55
Valine	0.88	0.32	0.85	0.22	0.18	3.82	1.38
Tryptophan	0.16	0.07	0.17	0.04	0.01	0.59	0.14
Methionine	0.58	0.16	0.23	0.08	0.04	2.28	Variable

analysis. After the samples were hydrolyzed with 5mol/L NaOH solution at 110℃ for 20h, the tryptophan content was determined.

Growth performance and survival rate: Body weight and fish numbers were recorded for all fish from each replicate both at the beginning and end of the experiment. At the conclusion of the trial, 30 fish from each replicate were randomly selected, and their final body length and weight were measured. These fish were then immediately dissected to collect whole-body muscle, liver, and viscera samples. The recorded values were subsequently used to calculate the weight gain (WG), survival rate (SR), weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), body protein deposition rate (BPD),Fulton's condition factor (K), viscerosomatic index (VSI), and hepatosomatic index (HSI), using the equations provided below:

SR (%) = (final fish number/initial fish number) × 100
SGR (%/d) = (In (final body weight) – In (initial body weight) / days) × 100
FCR = total feed intake (g) / (final body weight) – (initial body weight)
Protein intake (g) = total feed intake (g) × protein percent in feed
PER = weight gain (g) / protein intake (g)
Body protein deposition rate (BPD, %) = 100 × (BWf (g) × CP_f (%) − BWi (g) × CP_i (%))/(FI (g) × FP (%))
K (%) = (W/L^3) × 100; [(W=weight (g); L=length (cm)]
HSI (%) = (liver weight / final weight) × 100
VSI (%) = (visceral weight / final weight) × 100

Where BW_f and BW_i, and CP_f and Cp_i were the final and initial numbers, final and initial body weight, and final and initial carcass protein content of fish, respectively; FI and FP were the feed intake and feed protein content, respectively

Feed samples and muscle chemical composition analysis: The feed samples and stored bodymuscle and liver samples from the dissected fishes were analyzed by the methods prescribed in AOAC (2005) for nutrient composition analysis; crude protein was determined by Kjeldhal apparatus (Automatic Apparatus Kjeltec 8400, FOSS, Denmark) by calculating nitrogen (N × 6.25); crude fat was determined by Soxhlet (ST 243, FOSS, Denmark); moisture was determined at 105℃ to constant weight; and samples were burnt at 550℃ for 12h for crude ash analysis.

Essential amino acid requirements estimation: The A/E ratio (essential amino acids to total amino acids) was calculated using the formula proposed by Kaushik (1998). This approach allowed for the comparison of muscle tissue amino acid composition in the studied species with that of 16 other fish species documented in the literature through cluster analysis: A/E ratio = (content of each essential amino acid / total essential amino acids) × 1000

Analysis of serum biochemical profile: Blood samples were collected from three fish per replicate via the caudal vein using a sterile, disposable 22-gauge plastic syringe. The samples were transferred into non-heparinized Eppendorf tubes and centrifuged at 5,000 × g for 5 minutes to obtain serum for biochemical analysis. The collected serum was then stored at −20 °C for further analysis. Serum biochemical parameters were analyzed using commercial reagent kits obtained from Nanjing Jiancheng Bioengineering Institute in China. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measurement activities in serum samples were evaluated with the Reitman and Frankel (1957) procedure. Total cholesterol (TC) levels and triglycerides (TG) were measured using the methods described by Hardisari and Koiriyah (2016).

Analysis of gut microbiota: The gut microbial count of coho salmon alevins was performed following the method described by Hussein *et al*. (2023). The assembled intestines of three fish (weighing ≃ 5g per treatment) underwent cleaning with phosphate-buffered saline solution 0.90% at pH 7.5 before homogenization into a 250mL sterile Erlenmeyer flask containing 95mL of peptone saline solution with peptone concentration at 0.10% and NaCl at 0.85%, then the mixture was shaken for five minutes. The experiment was performed under sterile conditions where 0.1mL quantities of suitable diluted solutions were spread on poured culture plates with different media types alongside negative reference plates without intestinal material. At 30°C, total bacterial count (Log CFU/g) was examined, while measuring growth periods between 24–72 hours using 0.1mL of 10-7 dilutions on plate count agar (PCA). The researcher employed the pouring plate procedure and selective culture media [thiosulfate-citrate-bile salt-sucrose (TCBS), MaConkey, and MRS] to count total bacterial count, *Lactobacillus*, *Bacillus* and *Vibrio* sp., and *Coliform* bacteria.

Statistical analysis: The experimental data were expressed as mean±standard deviation (mean±S.D., *n = 3).* All percentage data were converted to arcsine before analysis. All the data were analyzed using SPSS 25.0 software and the one-way analysis (ANOVA) technique. If the difference was significant (P<0.05), Tukey's multiple comparison (Tukey's HSD test) was performed to compare the means. Quadratic regression analysis was also applied using

Microsoft Excel 97-2003 Worksheet and Chart Design to the data of the SGR, FCR, PER, and BPD to find the predicted suitable dietary requirement of the methionine.

Microsoft Excel 97-2003 Worksheet and Chart Design: to the data of the SGR, FCR, PER, and BPD to find the predicted suitable dietary requirement of the methionine.

RESULTS

Growth performance: At the end of the experiment, there was no significant difference in the survival rate of alevins fish in each methionine treatment group (P>0.05). At the end of the experiment, among the methionine treatment groups, the 1.09% group had the slowest growth of alevins, with a body weight of only 4.73g; the growth of alevins increased significantly with the increase of dietary methionine (P<0.05), reaching the maximum body weight (5.94g) at 2.15%. The growth showed a downward trend when the methionine content exceeded 2.15%. The SGR of alevins had a similar trend to that of body weight. Feed conversion ratio (FCR) and protein efficiency were significantly better in the diet of groups of 2.15% and 2.63%. The body protein deposition (BPD) rate was also higher in the diet groups of 2.15% and 2.63%. condition factor (K), HSI, and VSI were non-significant among the treatment groups (Table 3). The relationship between SGR, FCR, PER, BPD, and dietary methionine level was analyzed by quadratic polynomial model, showing that the optimal methionine requirement for coho salmon alevins was 2.55%, 2.59%, 2.56%, and 2.69% of the feed respectively (Fig. 1-4).

Fish mucle composition: The level of methionine in the feed had no significant effect on the fat and ash content of the fish muscle but significantly affected the protein content of the fish (Table 4). Among them, the protein content of the alevins fish in the 1.09% group and the 1.64% group was not different (P>0.05) but was significantly lower than that in the other groups (P<0.05). The content increased significantly with the increase of the methionine level in the feed (P<0.05), and the protein content of the fish reached the highest in the 2.63% group and then showed a downward trend (Table 4).

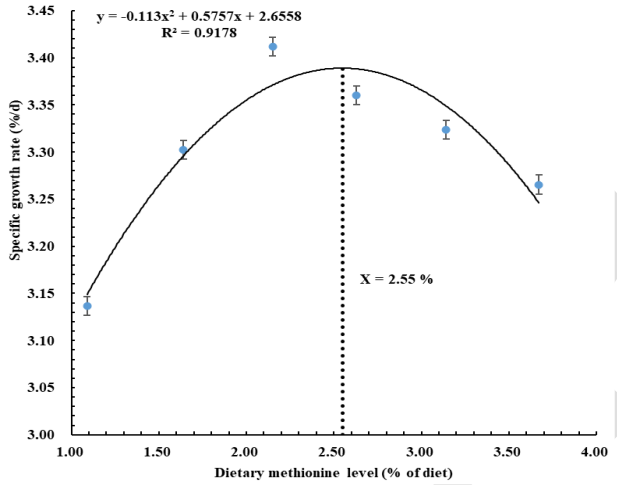

Fig.1: Polynomial quadratic regression analysis of SGR with graded levels of L-Methionine in coho salmon (*Oncorhynchus kisutch*) alevins. The predicted L-methionine requirement for SGR was 2.55% of the diet. Abbreviations SGR, specific growth rate.

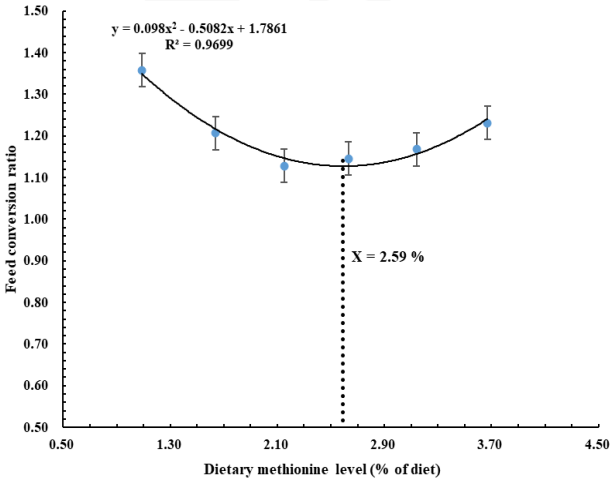

Fig. 2: Polynomial quadratic regression analysis of FCR with graded levels of L-Methionine in coho salmon (*Oncorhynchus kisutch*) alevins. The predicted L-methionine requirement for FCR was 2.59% of the diet. Abbreviations FCR, feed conversion ratio.

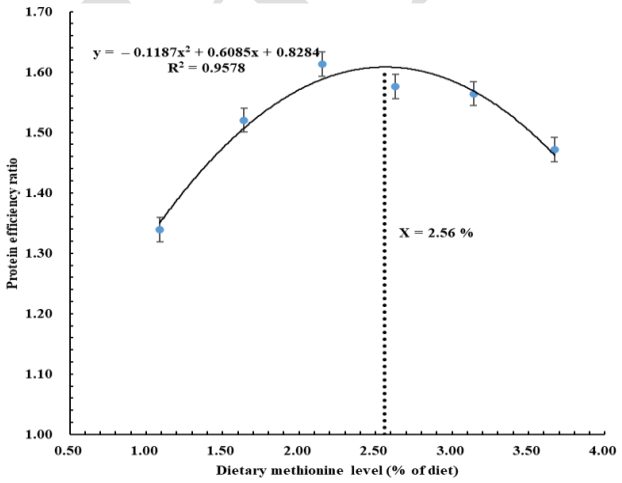

Fig. 3: Polynomial quadratic regression analysis of PER with graded levels of L-Methionine in coho salmon (*Oncorhynchus kisutch*) alevins. The predicted L-methionine requirement for PER was 2.56% of the diet. Abbreviations PER, protein efficiency ratio.

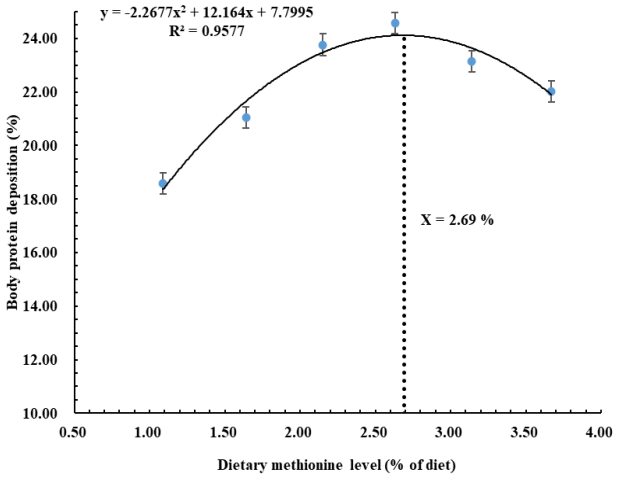

Fig. 4: Polynomial quadratic regression analysis of BPD with graded levels of L-Methionine in coho salmon (*Oncorhynchus kisutch*) alevins. The predicted L-methionine requirement for BPD was 2.69% of the diet. Abbreviations BPD, body protein deposition.

Table 3: Growth performance parameters of coho salmon alevins fed with the experimental diets of graded levels of L-methionine for 84 days

Items	Diets (L – methionine level, %)					
	Diet 1 (1.09)	Diet 2 (1.6 4)	Diet 3 (2.15)	Diet 4 (2.63)	Diet 5 (3.14)	Diet 6 (3.67)
Survival rate (%)	97.00±1.00	97.33±0.58	97.67±1.53	97.67±0.58	98.00±1.00	97.33±1.53
Initial BW [2] (g)	0.339±0.01	0.336±0.01	0.338±0.01	0.34±0.01	0.338±0.00	0.337±0.01
Final BW (g)	4.73±0.06^{c}	5.39±0.10^{b}	5.94±0.08^{a}	5.72±0.07^{ab}	5.51±0.04^{b}	5.26±0.11^{b}
Specific growth rate SGR (%/d)	3.14±0.01^{c}	3.30±0.02^{b}	3.41±0.01 ^{a}	3.36±0.02^{ab}	3.32±0.01^{b}	3.27±0.02^{b}
Feed Coefficient (FCR)	1.36±0.02^{a}	1.21±0.02^{b}	1.13±0.02^{c}	1.15±0.01^{c}	1.17±0.01^{bc}	1.23±0.03^{b}
Protein efficiency (PER)	1.34±0.03^{c}	1.52±0.03^{b}	1.61±0.04^{a}	1.58±0.02^{a}	1.56±0.01^{ab}	1.47±0.05^{b}
Body protein deposition rate (BPD, %)	18.59±0.39^{c}	21.04±0.45^{b}	23.76±0.54^{a}	24.58±0.34^{a}	23.14±0.20^{ab}	22.02±0.71^{ab}
Condition factor (K, %)	1.25±0.16	1.17±0.22	1.34±0.23	1.27±0.10	1.16±0.19	1.35±0.26
Liver to body ratio (HIS, %)	1.67±0.05	1.72±0.05	1.59±0.07	1.82±0.11	1.77±0.13	1.62±0.08
Viscera to body ratio (VSI, %)	7.29±0.08	7.25±0.0 6	7.18±0.03	7.05±0.07	7.11±0.05	7.10±0.06

Note: [1] Means±SD (*n* = 3) with the same superscript letter in the same row are not significantly different (P>*0.05*); the [same] as Tab. 6; 2 BW: body weight, PER: protein efficiency ratio, SGR: specific growth rate; FCR: feed conversion ratio; CF: condition factor; HSI: hepatosomatic index; VSI: viscerosomatic index; [2]BW=body weight

Table 4: Proximate composition (% dry matter) of body muscle of coho salmon alevins fed with the experimental diets of graded levels of L-methionine for 84 days

Dietary groups (Methionine level, %)	Moisture	Crude Protein	Crude fat	Ash
The alevins at the start of the feeding trial				
	76.89±0.43	13.72±0.28	5.33±0.20	4.00±0.19
The alevins at the end of the feeding trial				
Diet 1 (1.09)	77.42±0.26	13.87±0.11^{c}	4.95±0.32	3.77±0.27
Diet 2 (1.6 4)	77.54±0.17	13.83±0.18^{c}	5.14±0.24	3.96±0.32
Diet 3 (2.1 5)	76.85±0.13	14.67±0.21^{b}	5.11±0.20	3.74±0.21
Diet 4 (2.6 3)	76.64±0.15	15.48±0.26^{a}	5.28±0.37	3.85±0.23
Diet 5 (3.1 4)	75.77±0.22	14.73±0.27^{b}	5.07±0.26	4.08±0.34
Diet 6 (3.6 7)	76.29±0.14	14.88±0.19^{b}	5.32±0.34	3.68±0.25

Note: Means±SD (*n* = 3) with the same superscript letter in the same column are not significantly different determined by Tukey's test (P>0.05)

Table 5: Essential amino acid (EAA) profile (% dry matter) in the whole body of coho salmon alevins fed diets with graded levels of L-methionine for 84 days

Essential Amino Acids	Dietary groups (Methionine level, % of diet)					
Essential amino acids	Diet 1 (1.09)	Diet 2 (1.6 4)	Diet 3 (2.1 5)	Diet 4 (2.6 3)	Diet 5 (3.1 4)	Diet 6 (3.6 7)
Arginine	3.11±0.04^{b}	3.17±0.04^{b}	3.35±0.03^{a}	3.27±0.02^{a}	3.29±0.05^{a}	3.37±0.04^{a}
Histidine	1.03±0.02	0.99±0.02	1.07±0.02	1.05±0.02	1.10±0.0 3	0.94±0.02
Isoleucine	2.53±0.03	2.67±0.03	2.68±0.03	2.62±0.02	2.59±0.02	2.52±0.02
Leucine	4.27±0.04	4.12±0.05	4.17±0.04	4.14±0.03	4.21±0.05	4.19±0.05
Lysine	4.09±0.04^{b}	4.14±0.03^{b}	4.33±0.06^{a}	4.36±0.0 2^{a}	4.21±0.04^{ab}	4.24±0.04^{ab}
Methionine	1.68±0.02^{b}	1.74±0.05^{ab}	1.81±0.03^{a}	1.85±0.03^{a}	1.82±0.02^{a}	1.77±0.02^{ab}
Phenylalanine	2.38±0.03	2.45±0.02	2.36±0.02	2.40±0.02	2.34±0.03	2.26±0.03
Threonine	2.21±0.03	2.10±0.04	2.19±0.03	2.09±0.03	2.25±0.03	2.33±0.04
Valine	4.41±0.03	4.54±0.03	4.65±0.04	4.59±0.05	4.52±0.07	4.47±0.05
Tryptophan	0.57±0.01	0.59±0.02	0.60±0.02	0.62±0.02	0.61±0.02	0.59±0.01
Total EAA	26.29±0.23^{b}	26.51±0.13^{ab}	27.23±0.13^{a}	27.00±0.12^{a}	26.95±0.24^{a}	26.68±0.10^{ab}

Note: Means±SD (*n* = 3) with the same superscript letter in the same row are not significantly different determined by Tukey's test (P>0.05)

The arginine content was significantly lower at 1.09% and 1.64% levels; the lysine and methionine content were significantly higher at 2.15% and 2.63% methionine levels. In contrast, other amino acids showed no difference (P>0.05) with all levels of L-methionine (Table 5).

Dietary amino acid requirements: Based on the methionine requirement obtained in this experiment and the amino acid composition of alevins, the requirements of the six essential amino acids for coho salmon alevins were estimated using the A/E ratio. There was no significant difference (P>0.05) in essential amino acid requirements in all groups (P>0.05), as shown in Table 6.

Blood serum biochemical profile: Serum biochemical parameters have been presented in Table 7. Results showed that significantly (P<0.05) lower TG, TC, AST, and ALT levels were recorded in 2.15% and 2.63% L-methionine levels compared to the rest of the groups.

Gut microbiota count: In the present study, significantly lower total bacterial count (TBC), *Coliform*, and *Vibrio* counts were observed in 2.15 and 2.63% L-methionine levels compared to the rest of the groups. In comparison, significantly (P<0.05) higher *Lactobacillus* and *Bacillus* were found in 2.15 and 2.63% L-methionine levels compared to other treatment groups. Results have been presented in Table 8.

Table 6: Essential amino acid requirements for coho salmon alevins based on L-methionine requirements and amino acid composition of the alevins

Essential Amino Acids	Estimated requirement (% feed protein)	A/E [Ratio2]
Methionine	4.6 4	77.98
Arginine	6.47	108.76
Histidine	2.83	47.54
Isoleucine	6.25	104.99
Leucine	9.83	165.18
Lysine	8.26	138.85
Phenylalanine	5.66	95.08
Threonine	5.49	92.34
Tryptophan	1.20	20.18
Valine	7.77	130.64

Note: [1] The amino acid requirement other than methionine is calculated using the following formula: Requirement = methionine requirement × (A/E/ 77.98); [2] A / E ratio = [Content of each essential amino acid/Total amount of essential amino acids (including tyrosine)] × 1000

Table 7: Effect of L-methionine diets on serum biochemical parameters of coho salmon alevins

Parameters	Diets (L – methionine level, %)						
	Diet 1 (1.09)	Diet 2 (1.6 4)	Diet 3 (2.15)	Diet 4 (2.63)	Diet 5 (3.14)	Diet 6 (3.67)	P-value
TG (nmol/mL)	1.55±0.12^{a}	1.46±0.17^{b}	1.32±0.12^{c}	1.38±0.10^{c}	1.48±0.10^{b}	1.52±0.19^{ab}	<0.016
TC (nmol/mL)	6.18±0.15^{a}	6.15±0.19^{a}	5.53±0.12^{b}	5.78±0.16^{ab}	5.96±0.17^{b}	6.21±0.18^{a}	<0.029
AST (U/mL)	8.65±0.42^{e}	9.97±0.24^{c}	12.33±0.29^{a}	11.53±0.23^{b}	9.75±0.21^{bc}	8.91±0.14^{d}	<0.011
ALT (U/mL)	3.35±0.14^{f}	3.50±0.15^{e}	5.30±0.14^{a}	4.75±0.10^{b}	3.92±0.15^{d}	4.01±0.12^{c}	<0.021

$^{a-f}$ within the same row mean with different alphabets differ significantly (P<0.05); Mean±SD. TG: total triglycerides; TC: total cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase

Table 8: Effect of L-methionine diets on intestinal microbiota of coho salmon alevins (log CFU/g)

Microbiota count	Diets (L – methionine level, %)					
	Diet 1 (1.09)	Diet 2 (1.6 4)	Diet 3 (2.15)	Diet 4 (2.63)	Diet 5 (3.14)	Diet 6 (3.67)
TBC	59.31±3.3^{a}	18.33±3.0^{d}	13.00±0.9^{e}	18.67±2.6^{d}	25.35±2.2^{c}	37.36±2.7^{b}
Lactobacillus count	8.32±0.2^{e}	8.52±0.2^{c}	9.25±0.4^{a}	9.05±0.4^{ab}	8.71±0.2^{b}	8.43±0.2^{d}
Bacillus count	7.35±0.3^{e}	7.82±0.2^{d}	8.70±0.0^{a}	8.53±0.1^{b}	8.06±0.2^{c}	7.80±0.3^{d}
Coliform count	13.12±1.1^{a}	12.50±0.9^{a}	6.50±2.02^{d}	8.77±1.4^{c}	11.20±1.5^{ab}	11.75±2.1^{b}
Vibrio count	42.11±3.4^{a}	33.29±3.4^{c}	23.30±3.5^{e}	19.68±1.5f	26.57±4.1^{d}	38.30±1.3^{b}

$^{a-e}$ within the same row mean with different alphabets differ significantly (P<0.05); Mean±SD TBC: total bacterial count; CFU: colony forming unit

DISCUSSION

Several studies have evaluated balancing amino acid proportions by using synthetic amino acid supplements with plant-protein diets for aquaculture (Ren *et al.,* 2019; Li *et al.,* 2021; Zheng *et al.,* 2023). Scientific research shows that both L-methionine and MHA-Ca serve as methionine supplements and enhance animal growth performance (Goff and Gatlin, 2004; Luo *et al.,* 2005; Powell *et al.,* 2017), and this research supports these findings. However, literature is scarce about determining L-methionine requirements in coho salmon. After the 84-day feeding trial, dietary L-methionine supplementation positively affected the growth performance of coho salmon by improving the BWG, SGR, FCR, and PER. The research results of Niu *et al.* (2018) showed that 0.3% DL-methionine (8.2g/kg) positively affected the weight growth rate (WGR), SGR, and survival rate of whiteleg shrimp. Xie *et al.* (2018) found that 0.3% DL-methionine supplementation at 10.1 g/kg in the diet provided better SGR and WGR performance to whiteleg shrimp than feeding shrimp the lower-methionine diet with 7.3g/kg methionine contents. Feeding red seabream (*Pagrus major*) with diets containing 25% soy protein isolate and supplemented with DL-Met or Met-Met resulted in improved FCR, (Mamauag *et al.,* 2012). Goff and Gatlin (2004) conducted a study comparing the efficacy of L-methionine and methionine hydroxy analogue calcium (MHA-Ca) in red drum (Sciaenops ocellatus), and reported that both methionine sources provided comparable benefits in terms of growth performance. The growth of channel catfish required higher L-methionine amounts than MHA-Ca-based supplementation (Robinson *et al.'* 1978). Patro *et al.* (2011) reported that the estimated dietary methionine requirement for Florida pompano was 1.60% at a dietary crude protein level of 3.48%, based on second-order polynomial regression analysis (R^2 = 0.71), while the broken-line regression model (R^2 = 0.72) indicated a requirement of 1.17% at 2.54% crude protein. According to Corby *et al.* (2024), Florida Pompano achieved minimum weight gains while maintaining the highest FCR when fed diets containing the lowest methionine content between 0.57 and 0.66% of diet weight (as-is basis). The feeding patterns of Florida pompano and their feeding mechanism typically create higher FCR rates than cultured species in most research studies. The deficiency of dietary methionine in diets causes decreased growth parameters in fish. The strong relationship between methionine and metabolic functions exists because this essential amino acid serves as the primary methyl donor (Nguyen *et al.,* 2019). Decreased metabolic activity led to a lower growth rate in fish because dietary methionine needs were not met correctly, which means additive growth promoters demonstrated improved outcomes. Mai *et al.* (2006) published research on yellow croakers (*Pseudosciaena crocea*) that tested methionine concentration levels in their provided diets. Different experimental dietary methionine levels directly influenced yellow croaker growth variables, including specific growth rate and feed conversion efficiency, until reaching their estimated requirement threshold, according to Mai *et al.* (2006). Statistical modeling has been employed for several decades to detect the nutritional requirements of multiple important species (Corby *et al.,* 2024). The required minimum amount of dietary methionine for Florida pompano assessed at 0.68% (as-is basis) was determined through model selection of the five growth parameters, including percent weight gain (PWG), mean weight (MW), thermal growth coefficient (TGC), methionine retention (MR) and apparent net protein retention (ANPR) (Corby *et al.,* 2024). Previous datasets from the Auburn University aquatic animal nutrition facility validate these research outcomes (results unpublished). The estimated essential methionine content needed for Florida pompano diets proves significantly lower compared to silver and golden pompano (1.16-1.18% and 1.06-1.27%, respectively) (Ebeneezar *et al.,* 2020; Niu *et al.,* 2013). Wang *et al.* (2022) discovered that the literature reports necessary methionine levels between 0.49% and 2.50% of the diet or between 1.49% and 4.70% of dietary protein for most species.

The measured methionine requirements from this research align with accepted values for economic finfish species, according to Wang *et al.* (2023). This study provided different levels of L-methionine to measure distinct responses in coho salmon growth dynamics, while previous research supplied equal amounts of methionine during experimentation.

The use of L-methionine during this study did not significantly change the HSI and VSI values of coho salmon. The study of Yang *et al.* (2010) suggested that supplementation with EAA (methionine and lysine) led to decreased VSI and HSI and intra peritoneal fat ratio and whole-body lipid levels and improved muscle protein which indicated better health condition and economic value in grass carp. The hepatopancreas, analogous to the liver and pancreas in mammals, plays a key role in nutrient metabolism and health for crustaceans (Verri *et al.*, 2001). A study conducted by Zheng *et al.* (2023) established that shrimp eating a diet with low methionine content displayed elevated HSI because of their methionine-limiting diet. Swelling in the liver leads to elevated hepatic somatic index levels, as reported by Li *et al.* (2016). Laboratory experiments conducted by Gu *et al.* (2013) showed that removing methionine from plant protein diets caused liver swelling by creating fat buildup or disturbing sulfur metabolism processes. No statistically significant HSI changes were observed when using L-methionine supplementation. However, both HSI and VSI values showed numerical reductions in the 2.15% and 2.63% methionine-supplemented groups when compared to the control treatment. Research shows that dietary supplementation of methionine leads to improved amino acid ratios, while scientists agree that proper nutrient proportions determine liver size (Hansen *et al.*, 2007). Research shows that adding the appropriate amount of methionine to animal feed effectively minimizes hepatic fat buildup (Chiji *et al.*, 1990). This current study revealed that the K measurements of liver fatness did not demonstrate significant differences between L-methionine supplement levels. According to Oz *et al.* (2007), methionine deficiency in the diet triggers several physiological changes, which include liver harm and reduced intestinal epithelial cell development. In the present study, results for HSI, VSI, and CF demonstrated that different methionine levels from 1.09% to 3.67% showed no adverse effects since they remained in the normal range (Wang *et al.*, 2019).

The analysis of fish whole body muscle composition showed statistically different protein contents between diets containing methionine doses between 1.09% to 3.67% (as-is basis). Eukaryotic cells depend on methionine for protein biosynthesis as an essential amino acid because it starts protein synthesis (Brosnan *et al.*, 2007; Savino *et al.*, 2022). The fish group with 2.63% methionine content showed the most outstanding protein retention outcome among all dietary groups. According to Teodósio *et al.* (2022), protein retention was higher in the DL-methionine group than it was in the basal diet and methionine hydroxyl analogue groups. The study conducted by Corby *et al.* (2024) revealed that protein retention showed a peak level of 0.94% methionine concentration. The growth rate of aquatic animals shows a direct correlation with protein accumulation within their bodies. The research by Chen *et al.* (2023) demonstrated that abalone soft body crude protein levels rose progressively when methionine feeding rates increased from 0.46% to 1.19%. Similar findings have been reported in various fish species, including rainbow trout (*Oncorhynchus mykiss)* (Kim *et al.*, 1992), yellowtail (*Seriola quinqueradiata)* (Ruchimat *et al.*, 1997), grouper (*Epinephelus coioides)* (Luo *et al.*, 2005) and olive flounder (*Paralichthys oli vaceus)* (Alam *et al.*, 2000). Consistent with the findings of Chen *et al.* (2023), the present study observed a downward trend in crude protein content within the soft body of coho salmon as dietary L-methionine content increased from 2.63% to 3.67%, with a significant reduction occurring in the group receiving 3.67% L-methionine. A possible explanation is that an excess of methionine in the diet disrupts the balance of amino acid profiles, reducing the efficiency of amino acid utilization and shifting their metabolism toward catabolic rather than anabolic processes (Zhou *et al.*, 2011).

The amino acid profiles in muscle, blood, or other tissues are generally used to assess the utilization of dietary amino acids (Kaushik and Seiliez, 2010). This study found a gradual increase in muscle methionine levels in coho salmon as dietary methionine content rose from 1.09% to 3.67%, followed by a decline at the highest level of 3.67%. A similar trend was reported in yellow catfish (Wang *et al.*, 2016). Other muscle amino acids, including lysine, arginine, and total essential amino acids, were significantly influenced by dietary methionine levels (2.15% or 2.63%), with notable reductions in groups receiving 3.14% or 3.67%. These findings support the hypothesis that excessive dietary methionine affects amino acid utilization efficiency, leading to a decrease in crude protein content in the body (Zhou *et al.*, 2011). However, this result contrasts with studies on yellow catfish (Elmada *et al.*, 2016) and grouper (Luo *et al.*, 2005), where dietary methionine did not significantly alter muscle amino acid profiles. Differences in findings may be attributed to variations in experimental species, feed composition, dietary protein sources, and environmental conditions.

Overall, the retention levels of essential amino acids (EAAs) followed a similar pattern, increasing up to the required methionine level before stabilizing. This trend is partly attributed to methionine's role in reducing the oxidation rates of other EAAs (Nunes *et al.*, 2014) and the imbalance of EAAs. Unlike other amino acids that were not supplemented, higher dietary methionine levels led to a decline in retention, as excess methionine was no longer efficiently utilized by the body. An oversupply of methionine disrupts the transamination pathway involved in its metabolism (Stipanuk, 2020). The diet containing 2.63% methionine resulted in the highest methionine retention in fish. While this is slightly above the estimated requirement in this study, diets supplemented with 2.15% methionine demonstrated efficient retention.

Aspartate aminotransferase (AST) and ALT function as crucial agents that facilitate transamination, producing NEAAs useful for protein synthesis and fish development when fish obtain adequate energy from non-protein sources (Walton and Cowey, 1982). The newly produced non-essential amino acids will be broken down into energy by the body when there is insufficient non-protein energy, resulting in reduced growth potential. The results of the present study demonstrated that optimal dietary methionine (2.15% and 2.63%) increased the activities of AST and ALT until concentrations surpassed the optimum point. Researchers have suggested that excessive methionine allocation does not support protein synthesis since the body directs these molecules toward energy production during times when necessary amino acids for protein synthesis are inadequate (Li *et al.*, 2009). The

optimal methionine supplement in test diets stimulated aminotransferase activities, increased growth performance, and gain in carcass protein content. The research work by Xiao *et al*. (2011) and Wu *et al*. (2017) revealed identical outcomes while studying Jian carp (*Cyprinus carpio var. Jian*) and grass carp (*Ctenopharyngodon idella*), respectively. Methionine supplementation at an optimal level increased the activities of liver and muscle aminotransferases in juvenile sea cucumbers, according to Li *et al*. (2021). Lu *et al*. (2014) reported that serum activities of AST and ALT were lower in the control group of Juvenile Black Sea Bream (*Acanthopagrus schlegelii*) than those of low and high methionine levels.

The level of dietary nutrition is closely related to the metabolic function, physiology, and overall health of fish, as reflected by their serum biochemical indicators. serum triglycerides provide a key source of energy and are stored in fish. Additionally, total cholesterol is essential for forming cell membranes and serves as a precursor for bile acids, vitamin D, and hormones. Thus, the serum levels of TC and TG can offer valuable insights into lipid absorption (Soltanzadeh *et al*., 2016). In the present study, optimum methionine levels of 2.15% and 2.63% showed significantly reduced triglycerides and total cholesterol. Similar results were reported by Lu *et al*. (2014), who reported a significantly lower TG concentration in fish fed high methionine (3.4%) diet than those of fish fed no or low methionine diets. It is possibly shown that the supplementation of amino acids reduced fat metabolism as an energy consumption, which is beneficial to weight gain (Regost *et al*., 1999). Lu *et al*. (2014) reported that fish fed with no methionine and low methionine diets had higher triglyceride concentrations than those fed the high methionine and fish meal diets. In contrast to the present study's findings, Garg *et al*. (2023) also reported that serum triglycerides and cholesterol concentrations did not change significantly in farmed tilapia juveniles at different dietary methionine levels. Similar findings were also reported in other species due to variations in dietary methionine levels (Yan *et al*., 2007; Ebeneezar *et al*., 2020).

Fish digestion primarily depends on the function of their intestinal system. The latest research shows that some nutrient supplements can potentially enhance digestive system functionality and positive microbiota balance. The microbial population in fish intestinal tissue shows direct relationships with fish dietary elements. Bacteria in fish digestive systems release digestive enzymes to break down nutrient substances and produce necessary nutrients for the fish (Okutani, 1996). Therefore, this study determined the effect of methionine on gut microbiota. Methionine benefits intestinal bacterial communities. The intestinal microbiota is vital for nutrition harvest, immunity, and resistance to pathogens (Wang *et al*., 2018). To our knowledge, no information is available on the effect of methionine on fish intestinal micro-organisms. The results of the present study demonstrated that optimal dietary methionine (2.15% and 2.63%) increased the *Lactobacillus* and *Bacillus* bacterial count and decreased the *Coliform* and *Vibrio* count. Other researchers also found similar findings. Tang *et al*. (2009) evaluated the effect of methionine on the gut microbiota of juvenile Jian carp. They found that adding 1% to 1.6% methionine led to a significant increase in *Lactobacillus* and *Bacillus* colony-forming units per gram of intestinal contents but caused a decrease in *E. coli* and *Aeromonas sp.* counts. Zhao *et al*. (2022) reported that the inclusion of MHA in the diet of largemouth bass improved the intestinal microbiota richness and diversity and increased the abundances of potential probiotics of *Bifidobacterium* and *Bacillus* but decreased the abundance of Bacteroides, potential pathogenic bacteria at the genus level (Zhao *et al*., 2022). The highest Firmicutes: Bacteroidetes (F:B) ratio in Nile tilapia emerged after supplementing with optimal dietary Met-Met levels (Guo *et al*., 2020b). This increase in F: B led to enhanced resistance to pathogens (Mariat *et al*., 2009) along with improved energy extraction from the intestines (Wang *et al*., 2022). The mechanism of the effects on microbial populations is unclear, but the authors suggest that it may be related to using methionine as a nutrient source.

Conclusions: The findings of this study clearly demonstrate that dietary L-methionine plays a crucial role in supporting optimal growth performance, nutrient utilization, and health status in coho salmon (*Oncorhynchus kisutch*) alevins. Among the tested levels, 2.15% dietary methionine (equivalent to 3.91% of feed protein) was identified as the optimal inclusion level, resulting in the highest body weight and specific growth rate. Moreover, enhanced body protein content and improved amino acid profiles, particularly methionine and lysine, were observed at 2.15% and 2.63% methionine levels. Additionally, these levels promoted better liver enzyme activity (AST and ALT), beneficial gut microbiota (Lactobacillus and Bacillus), and lipid metabolism, as evidenced by reduced serum cholesterol, triglycerides, and pathogenic bacteria (Coliform and *Vibrio*). Overall, dietary supplementation with 2.15–2.63% L-methionine not only improves growth and body composition but also enhances the physiological and microbial health of coho salmon alevins, making it a recommended range for optimal performance during early developmental stages. Furthermore, quadratic polynomial model analysis indicated that a methionine requirement between 2.55% and 2.69% is optimal for achieving the best results in specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and body protein deposition (BPD). Future research should focus on validating these findings in large-scale commercial settings across different life stages and environmental conditions to support practical application in aquaculture feed formulation for coho salmon

Funding: This study was funded by Shandong Provincial Key Research and Development Programs (Major Scientific and Technological Innovation Projects, MSTIP), Grant/Award Numbers: 2018CXGC0102 and 2019JZZY020710; Scientific and Technologic Development Program of Weifang, Grant/Award Number: 2019ZJ1046; Shandong Provincial Natural Science Foundation, Grant/Award Number: ZR2020MC174; Technical Development (Entrustment) Project between University and Enterprise (Grant / Award Number: 20240701CLFWFU01).

Conflicts of interest: The authors hereby declare that there are no conflicts of interest.

Data availability statement: All the data in the article are available from the corresponding author upon reasonable request.

Authors contribution: The project administration, the experiment design, and the manuscript revision, HY; software, manuscript editing, LY and GS; manuscript original draft writing, software analysis and review editing, AR, MY, MW, AA, HMI; feeding trial, sampling, assays and data analyses, LL and XW All authors have read and approved the final version of the manuscript.

REFERENCES

Alam MS, Teshima SI, Ishikawa M, *et al*., 2000. Methionine requirement of juvenile Japanese flounder Paralichthys olivaceus. J World Aquacult Soc 31:618-626.

Andrews JW and Page JW, 1974. Growth factors in the fish meal component of catfish diets. J Nutr 104:1091–1096.

Anonymous, 2016. Salmon Farming Industry Handbook 2023.

AOAC (Association of Official Analytical Chemists), 2005. Official Methods of Analysis of Official Analytical Chemists International, 16th ed. Association of Official Analytical Chemists: Arlington, VA, USA.

Bin P, Huang R, Zhou X, 2017. Oxidation resistance of the sulfur amino acids: Methionine and cysteine. Biomed Res Int 2017:9584932.

Brosnan JT, Brosnan ME, Bertolo RF, *et al*., 2007. Methionine: a metabolically unique amino acid. Livest Sci 112):2-7.

Chen H, Fan W, Zhang H, *et al*., 2023. Effects of dietary methionine on growth and body composition, indicators of digestion, protein metabolism and immunity, and resistance to heat stress of abalone *Haliotis discus hannai*. Aquaculture 563:738978.

Chen J, Li X, Huan D, *et al*., 2018. Comparative study on the utilization of crystalline methionine and methionine hydroxy analogue calcium by Pacific white shrimp (*Litopenaeus vannamei Boone*). Aquacult Res 49:3088–3096.

Cheng ZJ, Hardy RW, Usry JL, 2003. Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. Aquaculture 215:255–265.

Chiji H, Harayama K, Kiriyama S, 1990. Effects of feeding rats low protein diets containing casein or soy protein isolate supplemented with methionine or oligo-L-methionine. J Nutr 120:166–171.

Chu ZJ, Gong Y, Lin YC, *et al*., 2014. Optimal dietary methionine requirement of juvenile Chinese sucker, *Myxocyprinus asiaticus*. Aquacult Nutr 20):253-264.

Corby TL, Ngo T, Riche M, *et al*., 2024. Dietary Methionine Requirements for Juvenile Florida Pompano *(Trachinotus carolinus*). J Mar Sci Eng 12:1206.

Dabrowski K and Kozak B, 1979. The use of fish meal and soybean meal as a protein source in the diet of grass carp fry. Aquaculture 18:107–114.

Ebeneezar S, Vijayagopal P, Srivastava PP, *et al*., 2020. Optimum dietary methionine requirement of juvenile silver pompano, *Trachinotus blochii* (Lacepede, 1801). Anim Feed Sci Tech 268:114592.

Elmada CZ, Huang W, Jin M, *et al*., 2016. The effect of dietary methionine on growth, antioxidant capacity, innate immune response and disease resistance of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Aquacult Nutr 22:1163-1173.

El-Saidy DMSD and Gaber MMA, 2002. Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia Oreochromis niloticus (L.) fingerlings. *J World Aquacult Soc* 33:297–305.

El-Sayed AFM, 2014. Is dietary taurine supplementation beneficial for farmed fish and shrimp? A comprehensive review. Rev Aquacult 6:241-255.

Garg CK, Sardar P, Sahu NP, *et al*., 2023. Effect of graded levels of dietary methionine on growth performance, carcass composition and physio-metabolic responses of genetically improved farmed tilapia (GIFT) juveniles reared in inland saline water of 10 ppt. Anim Feed *Sci Tech* 298:115602.

Goff JB and Gatlin DM, 2004. Evaluation of different sulfur amino acid compounds in the diet of red drum, Sciaenops ocellatus, and sparing value of cystine for methionine. Aquaculture 241:465–477.

Gu M, Zhang WB, Bai N, *et al*., 2013. Effects of dietary crystalline methionine or oligo-methionine on growth performance and feed utilization of white shrimp (*Litopenaeus vannamei*) fed plant protein-enriched diets. Aquacult Nutr 19:39–46.

Guo J, Bao Y, Davis R, *et al*., 2020a. Application of meta-analysis towards understanding the effect of adding a methionine hydroxy analogue in the diet on growth performance and feed utilization of fish and shrimp. Rev Aquacult 12:2316-2332.

Guo TY, He JY, Liao SY, *et al*., 2020b. Dietary dl-methionyl-dl-methionine supplementation increased growth performance, antioxidant ability, the content of essential amino acids and improved the diversity of intestinal microbiota in Nile tilapia (Oreochromis niloticus). Br J Nutr 123:72-83

Hansen A-C, Rosenlund G, Karlsen Ø, *et al*., 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I-effects on growth and protein retention. Aquaculture 272:599–611.

Hardisari R, Koiriyah B, 2016. Gambaran kadar Trigliserida (*Metode Gpo-Pap*) pada sampel serum dan plasma EDTA. J Teknol Lab 5:27–31.

Hoseini SM, Ahmad Khan M, Yousefi M, *et al*., 2020. Roles of arginine in fish nutrition and health: insights for future researches. Rev Aquacult 12):2091–2108.

Hoseini SM, Pérez-Jiménez A, Costas B, *et al*., 2019. Physiological roles of tryptophan in teleosts: current knowledge and perspectives for future studies. Rev Aquacult 11:3–24.

Hossain MM, Rahman MH, Ali ML, *et al*., 2020. Development of a low-cost polyculture system utilizing *Hygroryza aristata* floating grass in the coastal wetlands of Bangladesh. Aquaculture 527:735430.

Hussein EE, Habiba MM, Ashry AM, *et al*., 2023. Effects of dietary supplementation with organic acids mixture on growth, feed efficiency, hematobiochemical parameters, immunity, and intestinal microbiota of Gilthead seabream (*Sparus aurata*) juveniles. Aquacult Rep 33:101846.

Huyghebaert G, 1993. Comparison of DL-methionine and methionine hydroxy analogue-free acid in broilers by using multi-exponential regression models. Br Poult Sci 34:351 359.

Kaushik SJ, 1998. Whole body amino acid composition of European bass (Dicentrarchus labrax), gilthead bream (*Sparus auratus*), and turbot (Psetta maxima) with an estimate of their IAA requirement profile. Aquat Living Resour 11:555–558.

Kaushik SJ, Cravedi JP, Lalles JP, Sumpter J, Fauconneau B, Laroche M, 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic of antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 133:257–274.

Kaushik SJ, Seiliez I, 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. Aquacult Res 41(3):322–332.

Khan MA, Jafri AK, Chadha NK, *et al*., 2003. Growth and body composition of rohu (*Labeo rohita*) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. Aquacult Nutr 9:391–396.

Kim KI, Kayes TB, Amundson CH, 1992. Requirements for sulfur amino acids and utilization of D-methionine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 101:95–103.

Lee S, Kwak JH, Kim SH, *et al*., 2019. Comparative study of liver injury induced by high-fat methionine- and choline-deficient diet in ICR mice originating from three different sources. Lab Anim Res 35:15.

Li BS, Han XJ, Wang JY, *et al*., 2021. Optimal dietary methionine requirement for juvenile sea cucumber Apostichopus japonicus selenka. Aquacult Res 52:1348–1358.

Li M, Lai H, Li Q, *et al.,* 2016. Effects of dietary taurine on growth, immunity and hyperammonemia in juvenile yellow catfish Pelteobagrus fulvidraco fed all-plant protein diets. Aquaculture *450*:349-355.

Li P, Mai K, Trushenski J, *et al*., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37:43–53.

Liu X, Chi S, Li S, *et al*., 2021. Substitution of fish meal with enzyme-treated soybean in diets for juvenile largemouth bass (*Micropterus salmoides*). Aquacult Nutr 27(5):1569–1577.

Lovell RT, 1989. Nutrition and feeding of fish. New York, NY: Van Nostrand Reinhold.

Lu J, Hua Y, Fu WZ, *et al*., 2014. Effects of supplementation coated lysine and methionine in mixture protein diets on growth performance, digestibility and serum biochemical indices of juvenile black sea bream(*Acanthopagrus schlegelii)*. Turk J Fish Aquat Sci 14(3):633–642.

Luo Z, Liu YJ, Mai KS, *et al*., 2005. Dietary L-methionine requirement of juvenile grouper Epinephelus coioides at a constant dietary cystine level. Aquaculture 249(1–4):409–418.

Mai K, Wan J, Ai Q, *et al*., 2006. Dietary methionine requirement of large yellow croaker, Pseudosciaena crocea R. Aquaculture 253:564–572.

Mamauag REP, Gao J, Thanh Nguyen B, *et al*., 2012. Supplementations of dl-methionine and methionine dipeptide in diets are effective for the development and growth of larvae and juvenile Red Sea Bream, Pagrus major. J World Aquacult Soc 43:362–374.

Mariat D, Firmesse O, Levenez F, *et al*., 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol* 9:1–6.

Nguyen MV, Espe M, Conceição LE, *et al*., 2019. The role of dietary methionine concentrations on growth, metabolism and N-retention in cobia (*Rachycentron canadum*) at elevated water temperatures. Aquaculture Nutr 25:495–507.

Niu J, Du Q, Lin HZ, *et al*., 2013. Quantitative dietary methionine requirement of juvenile golden pompano Trachinotus ovatus at a constant dietary cystine level. Aquaculture Nutr 19:677–686.

Niu J, Lemme A, He JY, *et al*., 2018. Assessing the bioavailability of the novel Met-Met product (AQUAVI® Met-Met) compared to DLmethionine (DL-Met) in white shrimp (*Litopenaeus vannamei*). Aquaculture 484:322–332.

Nunes AJ, Sá MV, Browdy CL, *et al*., 2014. Practical supplementation of shrimp and fish feeds with crystalline amino acids. Aquaculture 431:20–27.

Okutani K, 1996. The chintinolytic enzyme present in the digestive tracts of Japanese seabass, Lateo abraxjaponicus. Bull Misa Jimar Biol Inst 10:1–47.

Ollie JJ, Krogdahl A, Hjelmeland K, 1994. Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar*): Effects on nutrient digestibilities and trypsin in pyloric caeca homogenate and intestinal content. Comp Biochem Physiol 109:923–928.

Oz HS, Chen TS, Neuman M, 2007. Methionine deficiency and hepatic injury in a dietary steatohepatitis model. Dig Dis Sci 53:767–776.

Patro B, Reigh RC, Williams MB, 2011. Dietary methionine requirement of Florida pompano. Aquaculture America.

Pervin MA, Jahan H, Akter R, *et al*., 2020. Appraisal of different levels of soybean meal in diets on growth, digestive enzyme activity, antioxidation, and gut histology of tilapia (*Oreochromis niloticus*). Fish Physiol Biochem 46:1397–1407.

Powell CD, Chowdhury MAK, Bureau DP, 2017. Assessing the bioavailability of L-methionine and a methionine hydroxy analogue (MHA-Ca) compared to DL-methionine in rainbow trout (Oncorhynchus mykiss). Aquacult Res 48:332–346.

Regost C, Arzel J, Kaushik S, 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (Psetta maxima). Aquaculture 180:99–117.

Reigh RC, Ellis SC, 1992. Effects of dietary soybean and fish protein ratios on growth and body composition of red drum (*Sciaenops ocelutus*) fed isonitrogenous diets. Aquaculture 104:279–292.

Reitman S, Frankel SA, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63.

Ren M, He J, Liang H, *et al.,* 2019. Use of supplemental amino acids on the success of reducing dietary protein levels for Jian carp (*Cyprinus carpio* var. Jian). Aquacult Nutr *25*:567-576.

Robinson EH, Allen OW Jr, Poe WE, Wilson RP, 1978. Utilization of dietary sulfur compounds by fingerling channel catfish: L-methionine, DL-methionine, methionine hydroxy analogue, taurine, and inorganic sulfate. J Nutr 108:1932–1936.

Robinson EH, Wilson RP, 1985. Nutrition and feeding. In: Tucker CS, ed. Channel catfish culture. New York: Elsevier, pp. 323–404.

Rolland M, Dalsgaard J, Holm J, *et al*., 2015. Dietary methionine level affects growth performance and hepatic gene expression of GH–IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets. Comp Biochem Physiol B Biochem Mol Biol 181:33–41.

Ruchimat T, Masumoto T, Hosokawa H, *et al*., 1997. Quantitative methionine requirement of yellowtail (*Seriola quinqueradiata*). Aquaculture 150:113–122.

Ruggerone GT, Irvine JR, 2018. Numbers and biomass of natural- and hatchery-origin pink salmon, chum salmon, and sockeye salmon in the North Pacific Ocean, 1925–2015. Mar Coast Fish 10:152–168.

Savino RJ, Kempisty B, Mozdziak P, 2022. The potential of a protein model synthesized absent of methionine. Molecules 27:3679.

Singh SK, Pawar L, Thomas AJ, *et al*., 2024. The current state of research and potential applications of insects for resource recovery and aquaculture feed. Environ Sci Pollut Res 31:62264–62282.

Soltanzadeh S, Esmaeili Fereidouni A, Ouraji H, *et al*., 2016. Growth performance, body composition, hematological, and serum biochemical responses of beluga (Huso huso) juveniles to different dietary inclusion levels of faba bean (*Vicia faba*) meal. Aquacult Int 24:395–413.

Stipanuk MH, 2020. Metabolism of sulfur-containing amino acids: how the body copes with excess methionine, cysteine, and sulfide. J Nutr 150:2494S–2505S.

Tang L, Wang GX, Jiang J, *et al*., 2009. Effect of methionine on intestinal enzymes activities, microflora and humoral immune of juvenile Jian carp (*Cyprinus carpio var. Jian*). Aquaculture Nutr 15:477–483.

Teles AO, Couto A, Enes P, *et al*., 2020. Dietary protein requirements of fish–a meta-analysis. Rev Aquacult 12:1445-1477.

Teodósio R, Engrola S, Cabano M, *et al*., 2022. Metabolic and nutritional responses of Nile tilapia juveniles to dietary methionine sources. Br J Nutr 127:202-213.

Verri T, Mandal A, Zilli L, *et al*., 2001. D-Glucose transport in decapod crustacean hepatopancreas. Comp Biochem Physiol Mol Integr Physiol 130:585-606.

Walton MJ, Cowey CB, 1982. Aspects of intermediary metabolism in salmonid fish. Comp Biochem Physiol B Comp Biochem 73:59-79.

Wang AR, Ran C, Ringø E, *et al*., 2018. Progress in fish gastrointestinal microbiota research. Rev Aquacult 10:626-640.

Wang L, Gao C, Wang B, *et al*., 2023. Methionine in fish health and nutrition: potential mechanisms, affecting factors, and future perspectives. Aquaculture 568:739310.

Wang L, Sagada G, Wang C, *et al*., 2022. Berberine in fish nutrition: impact on hepatoenteric health, antioxidative and immune status. Front Mar Sci 9:967748.

Wang L, Ye L, Hua Y, *et al*., 2019. Effects of dietary dl-methionyl-dl-methionine (met-met) on growth performance, body composition and haematological parameters of white shrimp (*Litopenaeus vannamei*) fed with plant protein-based diets. Aquacult Res 50:1718-1730.

Wang W, Jiang D, Yi G, *et al*., 2022. An Evaluation of Replacing Soybean Meal with Proteolytic Soybean Meal in Low-Fish-Meal Diet on Growth Performance, Expression of Immune-Related Genes, and Resistance against Vibrio alginolyticus in White Shrimp (*Litopenaeus vannamei*). Aquacult Nutr 2022:8384917.

Wang YY, Che JF, Tang BB, *et al*., 2016. Dietary methionine requirement of juvenile Pseudobagrus ussuriensis. Aquacult Nutr 22:1293-1300.

Webster CD, Goodgame-Tiu LS, Tidwell JH, 1995. Total replacement of fish meal by soybean meal, with various percentages of supplemental L-methionine, in diets for blue catfish. Ictalurus furcatus (Lesueur). Aquacult Res 26:299-306.

Wu L, Liang H, Hamunjo CMK, *et al*., 2021. Culture salinity alters dietary protein requirement, whole body composition and nutrients metabolism related genes expression in juvenile genetically improved farmed tilapia (GIFT)(Oreochromis niloticus). Aquaculture 531:735961.

Xiao WW, Feng L, Liu Y, *et al.,* 2011. Effects of dietary methionine hydroxy analogue supplement on growth, protein deposition and intestinal enzymes activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquacult Nutr 17:408-417.

Xie JJ, Lemme A, He JY, *et al*., 2018. Fishmeal levels can be successfully reduced in white shrimp (*Litopenaeus vannamei*) if supplemented with DL-Methionine (DL-Met) or DL-Methionyl-DL-Methionine (MetMet). Aquac Nutr 24:1144-1152.

Yan Q, Xie S, Zhu X, *et al*., 2007. Dietary methionine requirement for juvenile rockfish, Sebastes schlegeli. Aquacult Nutr 13:163-169.

Yang HJ, Liu YJ, Tian LX, *et al*., 2010. Effects of supplemental lysine and methionine on growth performance and body composition for grass carp (*Ctenopharyngodon idella*). Am J Agric Biol Sci 5:222-227.

Yu H, Zhang X, Yuan Z, *et al*., 2024. Dietary Pyridoxine Requirements of Coho Salmon (Oncorhynchus kisutch) Post-Smolts. Aquacult Nutr 2024:3862563.

Yu L, Yu H, Yuan Z, *et al*., 2023. Dietary L-Lysine Requirement of Coho Salmon (*Oncorhynchus kisutch*) Alevins. Animals 13:3670.

Zhao Y, Yang C, Zhu XX, *et al*., 2022. Dietary methionine hydroxy analogue supplementation benefits on growth, intestinal antioxidant status and microbiota in juvenile largemouth bass Micropterus salmoides. Aquaculture 556:738279.

Zheng L, Liu Y, Zhang Y, *et al*., 2023. Comparative study on the effects of crystalline L-methionine and methionine hydroxy analogue calcium supplementations in the diet of juvenile Pacific white shrimp (*Litopenaeus vannamei*). Front Physiol 14:1067354.

Zhou F, Xiao JX, Hua Y, *et al*., 2011. Dietary l-methionine requirement of juvenile black sea bream (*Sparus macrocephalus*) at a constant dietary cystine level. Aquacult Nutr 17:469-481