



بست

علمي او څېړنيزه مجله



گڼه: لومړۍ

ټوک: څلورم

کال: ۱۴۰۴

بسم الله الرحمن الرحيم



بُست علمي او خپرنيزه مجله

بُست پوهنتون
څلورم ټوک – لومړۍ گڼه
کال – ۱۴۰۴

بُست علمی او خپرنیزه مجله بُست پوهنتون

د امتیاز خاوند: بُست پوهنتون

مسئول مدیر: ډاکټر ذبیح الله انوري

سرديیران: پوهندوی دوکتور علي احمد احمدي او خان محمد وفا

کتنپلاوي

د مجلې بورډ

- ✓ پوهنوال دوکتور احمد جاوید پویش
- ✓ پوهنوال نقيب الله مجددي
- ✓ پوهندوی دوکتور نجيب الله مجددي
- ✓ پوهندوی دوکتور علی احمد
- ✓ پوهندوی دوکتور غلام رسول فضلي
- ✓ پوهندوی نیاز محمد زاهدي
- ✓ پوهندوی گل محمد اعظمي
- ✓ پوهندوی عبدالولي هجران
- ✓ پوهنیار عبدالولي همت
- ✓ خان محمد وفا
- ✓ ډاکټر ذبیح الله انوري

- ✓ پوهندوی دوکتور عبدالوهاب حکمت
- ✓ پوهندوی عبدالعزيز صابر
- ✓ پوهنمل حنيف الله باوري
- ✓ پوهاند دوکتور خال محمد احمدزی
- ✓ پوهندوی رضوان الله مملوال
- ✓ ارسلان وطندار
- ✓ پوهنیار بشیر احمد بابازوی

ډیزاین: د بُست پوهنتون د خپرنیزو او فرهنګي چارو مدیریت

د خپرولو کال: ۱۴۰۴

درک: بُست پوهنتون، لښکرګاه، هلمند، افغانستان

د بُست پوهنتون د رئیس پیغام

په نني ژوند کې د یوې علمي مؤسسي یو له مسئولیتونو څخه دا دی ، چې نه یواځې خپل محصلان د پوهې په ګاڼه سمبال کړي ، بلکې د پوهنتون د لوړو زده کړو لرونکو پوهانو او استادانو د علمي زیرمتون څخه داسې څه وخت په وخت راوباسي ، چې د ټولني د ژوند د اړتیاوو د پوره کولو لپاره او یا لږ تر لږه د ټولنې د لوستي قشر د خبرولو او که وکولای شي له هغوی څخه د عمل په ډګر کې د ګټې اخیستنې په موخه ، په کار واچول شي .

و دې موخې ته د رسیدلو لپاره پوهنتون باید یو داسې علمي خپرندویه ارګان ولري ، چې په هغه کې د پوهنتون ټول با صلاحیته منسوبین که هغه استاد وي ، که کارکوونکی او که زده کړه یال ، خپلې علمي او څیړنيزي مقالې او لیکنې د کاغذ پر مخ باندې کښیښودلای شي .

زما په شخصي آند پدې مجله کې لکه له نوم څخه چې یې ښکاري ، باید داسې مسائل را برسیره شي ، چې نه یواځې په پوهنتون پورې راګیر پاتې شي ، بلکې په عام ډول سره د افغانې ټولنې او په ځانګړي ډول سره د هلمند ولایت د اوسیدونکو و نني او سبا ژوند ته په کتلو سره ، بریالیتونونه ، ستونزې ، وړاندیزونه او د حل لارې-چارې ، وړاندې کړل شي . هغه وخت به د بُست پوهنتون علمي مجله یواځې د بست پوهنتون نه ، بلکې د ټول هلمند ولایت ، آن د سیمې او ټول افغانستان په کچه د پوهې او څیړنې په برخه کې د وخت د غوښتنو سره سم ، د پاملرنې وړ او و خوان نسل ته د یوې سمې لارې د ښودلو په موخه ، یوه محبوه او پر زیاتو خلکو باندې ګرانه مجله وي او په ټول هیواد کې به خپل مینه وال ولري .

دا مجله به د بُست پوهنتون د مشرتابه ، استادانو ، محصلانو ، فارغانو او ټولو مینه د علمي او څیړنيزو مقالو د خپرولو لپاره که هغوی د پوهې په هر ډګر کې چې وي ، یو خپرنیز ارګان وي ، چې و خپریدلو ته به یې ټول مینه وال په تمه ناست وي . څومره به پرځای او ښه خبر وي ، چې د ټولنې لوستی قشر په تیره بیا د بست پوهنتون محترم استادان ، فارغ شوي او بر حاله محصلان د علمي او څیړنيزو مقالو و لیکلو ته و هڅول شي .

زه د بُست پوهنتون د ټولو منسوبینو په استازیتوب ویاړ لرم ، چې د بُست پوهنتون د علمي مجلې د خپریدلو له امله د محترم مؤسس ، محترم علمي مرستیال او د څیړنې له محترم آمر او همدا رنگه د مجلې له ټولو کارکوونکو او پرسونل څخه د زیار او زحمت په ګاللو سره چې مجله یې و خپریدلو ته چمتو کړې ده ، مننه او قدرداني وکړم ، ټولو ته د زړه له کومې مبارکي وایم او هیله لرم چې د بُست پوهنتون د علمي مجلې کارکوونکي به خپل رسالت د پوهنتون او ټول هلمندې ولس او په اخری تحلیل کې د ټول افغان ملت پر وړاندې په پوره او ټینګ عزم سره سرته ورسوي .

په درنښت

ډیپلوم انجنیر محمود سنگین

د بُست پوهنتون رئیس

سريزه

بُست پوهنتون وياړ لري چې د خپل علمي پرمختگ په لاره کې يې يو بل ډير مهم او اړين گام پورته کړ او هغه د بُست د علمي او څيړنيزي مجلې د څلورم ټوک، لومړۍ گڼې خپرېدل دي. تر هر څه دمخه د پوهنتون ټولو استادانو، محصلانو او د علم او پوهې د لوی کور مينه والو ته د بُست د علمي او څيړنيزي مجلې د خپرېدلو مبارکي وړاندې کوم او ددې سره جوخت د ټولو ملگرو څخه چې ددې مجلې د جواز په تر لاسه کولو، ترتيبولو او خپرولو کې يې نه سترې کېدونکې ونډه اخيستې ده د زړه له کومې مننه کوم.

د علمي کور کهول او اړوند کسانو ته ښکاره ده او پوره باور لري چې د نننۍ نړۍ هر اړخيزه پرمختگ د پوهانو د علمي څيړنو د زيار له برکته ممکن سوی او د لوړو زده کړو مؤسسي، اکادميک انستيتوتونه او څيړنيز علمي مرکزونه پکښې مرکزي او پريکنده رول لوبولی دی.

همدې اصل او ارزښت ته په کتو سره بُست پوهنتون غواړي د پرمختللو اکاډميکو نورمونو په رعايت د تدريس، علمي څيړنو او نوښتونو له لاري مسلکي کادرونه وروزي او د معياري تحصيلي اسانتياوو او زمينو په برابرولو سره د ټولنې ځوانانو ته معياري او د لوړ کيفيت لوړې زده کړې وړاندې او د علمي څيړنو پر بنسټ د کره پوهنيزو اثارو د توليد زمينه برابره کړي، ترڅو د لوړو زده کړو او مسلکي پوهې په ډگر کې د گټورو مهارتونو په تر لاسه کولو او د خپلو رښتينو اهدافو په لاسته راوړلو سره د ټولنې او هيواد په پرمختگ او رغونه کې رغنده ونډه واخلي او د رښتيني خدمت جوگه شي.

ژمن يو چې د هلمند ولايت، گاونډيو ولايتونو او په ټول هيواد کې ځوان نسل ته د اسلامي، ملي او کلتوري ارزښتونو په رڼا کې معياري د علمي او مسلکي لوړو زده کړو او پراخو علمي څيړنو زمينه برابره او ټولني او هيواد ته ژمن او روزل سوي کادرونه وړاندې کړو.

د اوس لپاره د بُست علمي او څيړنيزه مجله يوازي د سائنسي علومو په برخه کې علمي او څيړنيزي مقالې او ليکنې د چاپ او نشر د تگلارې سره سم مني او خپروي او هيله مند يو چې په راتلونکې کې به نورې برخې هم ور زياتي کړل سي.

ډاډ لرم چې د بُست پوهنتون استادان، محصلان او علمي کارمندان به انشاء الله، نن، سبا او په راتلونکې کې د خپلې علمي څيړنيزي مجلې د خپرولو له لاري خپل دغه دروند خو وياړلی دين (پور) ادا کړي. همدا ډول ټولو د علم او پوهې څښتنانو او مينه والو ته په مينه سره بلنه ورکوو چې ددې علمي او څيړنيزي مجلې او د بُست پوهنتون د پرمختگ په لاره کې خپلې علمي او څيړنيزي ليکنې، آندونه، وړاندیزونه او رغنده نيوکي او مرستي د تل په شان راولورو او د علم ددې ستر کور په ودانولو کې د خپلې ديني، او ملي برخې د ادائينې وياړ راوبخښی.

موږ هوډ کړيدي او هيله مند يو چې انشاء الله د وخت په تيريدو سره به د خپل هيواد و بچيانو او ځوان نسل ته د تدريس، ښه روزني او څيړنيز هاند لپاره اړيني او د پام وړ اسانتياوې برابرې کړو تر څو په لومړي پړاو کېنې خپلو هلمندوالو بيا د سهيل لويديځي حوزې او په پای کېنې و ټولو هيوادوالو ته د يو داسې چوپړ مصدر وگرځي چې زموږ د ځوريدلي اولس او ويجاړشوي هيواد اقتصادي، فرهنگي، سياسي او ټولنيزي ستونزې حل او افغانستان د نړي د پرمختللو هيوادونو په ليکه کې ودريري.

لړلیک

د مقالې عنوان	د صفحې شمېره
په پراخه شبکه کې د IPV6 پروتوکول د بدلون ستراتیژي	1
خان محمد وفا	
Psoriasis: A Comprehensive Overview of Its Etiology, Clinical Features, and Preventive Strategies	10
Dr. Zabihullah Anwary ¹ and Dr. Ali Ahmad ^{2*}	
د مجازي شخصي شبکې د امنیت تطبیق او ډیزاین	24
خان محمد وفا	
Green Marketing: A Review of Sustainable Consumer Preferences	41
Dr. Ali Ahmad ^{1*} and Mir Wais Nazari ²	
Livestock Development, Marketing, and Expansion in Afghanistan: Key Challenges and Strategic Approaches	51
Dr. Ali Ahmad ^{1*} Mir Wais Nazari ² and Mustafa Amin ³	
د بیسیم د منځني سطحي شبکې ډیزاین او د اجراتو تحلیل	66
بشیر احمد هادی	
په هلمند ولایت کې د کوچنیو او منځنیو تصدیو د کارمندانو په رضایت باندې د مجموعي کیفیت د مدیریت اغیزې	86
سردار ولي الهام ^۱ ، میرویس نظري ^۲ ، پوهنیار حمید الله الهام ^۳	
د هلمند ولایت د عامه ادارو پر کړنو باندې د مسلکي کارمندانو د ګمارنې اغیزې	99
ن. پوهنیار حکمت الله خادم ^۱ ، ن. پوهنیار مجتبی امین ^۲ ، ارسلان وطندار ^۳ ، محب الله امیني ^۴ ، محمد یوسفی ^۵	
د مدیریت پر اغېزمنتیا باندې د همکارۍ د کلتور اغېزې (هلمند - ښاروالي)	113
نوماند پوهنیار مجتبی امین ^{۱*} ، نوماند پوهنیار امان الله نیازی ^۲ ، عبدالهادي حقیار ^۳	
په عامه ادارو کې د مسلکي کارمندانو د هڅونې اغیزې	124
نوماند پوهنیار عبدالقدیر خادم ^{۱*} ، فیض محمد فیضي ^۲ ، مصطفی امین ^۳	
Effects of dietary phenylalanine on growth performance and digestion of common carp (<i>cyprinus carpio</i>)	136
Saifullah Sharifi ^{1*} , Naqeebullah Ejaz ² , Abdul wali Hemat ³	

**Effects of dietary phenylalanine on growth performance and digestion
of common carp (*Cyprinus carpio*)**

Saifullah Sharifi^{1*}, Naqeebullah Ejaz², Abdul wali Hemat³

^{1,2,3}Teaching Assistant, Animal Science Department, Agriculture Faculty, Helmand University

Corresponding Author Email: saifullahsharifi1369@gmail.com

Abstract

This study was conducted to determine the optimal dietary requirement of phenylalanine, also to investigate the effects of different levels of dietary phenylalanine on the growth performance and digestion of *Cyprinus carpio*. Six isonitrogenous ($37.92 \pm 0.28\%$ crude protein) and isolipidic ($7.14 \pm 0.15\%$ crude lipid) diets were formulated with incremental levels of phenylalanine 0.38% (Control), 0.94%, 1.32%, 1.75%, 2.23% and 2.7% in the diet. 1200 fishes were divided into 24 cylindrical polypropylenes with 50 individuals in each tank (individual initial weight of fishes was $0.20 \pm 0.07\text{g}$) into four replicates in a completely randomized design for 8 weeks. The results showed that the final weight of the groups fed with 1.76%, was significantly higher than other Phe groups ($P < 0.05$). WGR and SGR was significantly higher in groups fed with 1.76% ($P < 0.05$). While FCR was significantly lower in the groups fed 1.76% than 0.38% group ($P < 0.05$). PER was significantly higher in the group fed 1.76% than the group fed 0.38% Phe ($P < 0.05$). The optimum phenylalanine requirement was determined to be 1.58% and 1.73% of air-dry basis (4.17% and 4.56% of dietary protein) based on SGR and FCR respectively. Digestive enzymes activity results showed that fishes fed with diet 2.23% Phe, had significantly higher trypsin activity ($P < 0.05$). In conclusion, the current study has revealed that most of parameters that are related to growth performance, feed utilization and digestive enzyme activity were found to be linearly influenced by the dietary phenylalanine level in the diet.

Keywords: digestive enzyme; growth performance; *Cyprinus carpio*; phenylalanine

Introduction

Phenylalanine is an essential amino acid vital for various biological processes including cell signalling, energy metabolism, protein synthesis, and muscle growth (Wu, 2022). It is a precursor for tyrosine, which is important for neurotransmitter and hormone production (NRC, 2011). Phenylalanine is vital for neurological function, muscle growth, hormone production, and metamorphosis. Phe metabolite, phospholipids aid in neurological development while its product tyrosine is involved in protein phosphorylation and neurotransmitter production. Phenylalanine is necessary for the production of hormones like thyroxine and triiodothyronine which are crucial for metamorphosis (Zehra & Khan, 2014; Sharf & Khan, 2023; Schreiber & Specker, 1998; Li et al., 2007).

Inclusion of dietary phenylalanine in the aquatic animal's diet has proven to significantly affect growth performance and feed utilization. Studies on *Channa punctatus* (Sharf & Khan, 2023), *catla catla* (Zehra & Khan, 2014), swimming crabs (*Portunus trituberculatus*) (Guo et al., 2022) and Nile tilapia (*Oreochromis niloticus*) (Zehra & Yousif, 2021) have shown significant improvement on growth performance and feed utilization. Appropriate levels of dietary phenylalanine promote better growth in fish, whereas both insufficient and excessive intake may cause negative impacts (Benakappa & Varghese, 2004). It helps control the transcription of growth hormone (GH) and insulin-like growth factor-I (IGF-1) in fish, both of which play vital roles in growth (Yang et al., 2023).

Phenylalanine in the diet significantly affects fish digestive and absorptive functions by modulating enzyme activity, shaping gut structure, and supporting overall intestinal health (S. Zhang et al., 2023). This vital amino acid is fundamental for enhancing digestion and improving nutrient utilization in fish (W. Li et al., 2015). Including phenylalanine in the diet enhances intestinal health by supporting gut structural integrity and boosting

its immune function (Ahmed, 2022). Moreover, it is essential for efficient nutrient absorption and metabolism (Yuan, 2000). The efficiency of fish digestion largely depends on the interaction between dietary nutrients and digestive enzymes, while phenylalanine playing a supportive role in enhancing these processes (Z. Ma et al., 2010). Furthermore, dietary phenylalanine can improve protein metabolism and overall feed utilization (Yi et al., 2024). This study aimed on investigating the effect of various dietary phenylalanine levels in the diet on growth performance, and digestive enzymes activity in *Cyprinus carpio*.

Material and methods

Experimental diets

The experimental diets were formulated based on the nutritional requirements of fish outlined in the (NRC, 2011) guidelines, using common feed ingredients. These included fishmeal, soybean meal, rapeseed meal, shrimp meal, squid paste, wheat meal, fish oil, and soy oil. Additional components consisted of granulesten, cholesterol, monocalcium phosphate (MCP), a premix, vitamin C, choline chloride, ecdysone, and dimethyl-beta-propantheline (DMPT). Six diets were prepared to be isonitrogenous (37.92% crude protein) and isolipidic (7.14% crude lipid). The control diet contained 0.38% phenylalanine, while the experimental diets were supplemented with phenylalanine at levels of 0.94%, 1.32%, 1.75%, 2.23%, and 2.70% (Table 1). All ingredients were finely ground, sieved, and weighed, after which the dry materials were mixed thoroughly before adding water and oil. The blend was processed using a twin-screw extruder (Guangzhou Huagong Optical Mechanical and Electrical Technology Co. Ltd., Guangzhou, China) to produce 1.0 mm extruded pellets. The pellets were then air-dried at room temperature, packaged, and stored in a freezer at – 15 °C until the feeding trial began

Experimental setup and feeding trials

The feeding trial experiment was conducted in an indoor re-circulating aquaculture system at the Freshwater Fisheries Research Center. 1200 fishes were divided into 24 cylindrical polypropylene tanks (800L of water was filled) with 50 individuals in each tank. Six formulated experimental feeds in four replicates were randomly assigned to the 24 tanks.

Cyprinus carpio were acquired from Dapu fish hatchery station of the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences (Wuxi, China). The fishes were acclimatized for 14 days while fed with commercial feed. At the beginning of the experiment, fishes' individual initial weight was 0.20 ± 0.07 g. fishes were fed by hand, to satiation three times a day (7.30am-8.00am, 12.30pm-1.00pm, 5.30pm-6.00pm). A daily feed amount was approximately 5–6% of tank biomass. The uneaten feeds were removed 1hr after feeding by siphoning the residual feeds. Water temperature ranged from 25 to 31°C, pH was maintained at 7.5–

8.0, ammonia nitrogen levels were below 0.02 mg/L, nitrite nitrogen ranged between 0.005 to 0.01 mg/L, DO levels were higher or equal to 6.0 mg/L, and water exchange were done one third of the tanks depending on the elevated levels of nitrite and ammonia every 7 to 10 days.

Sample collection

Sampling procedure was followed as the fishes were starved for 24 hours before collection of the samples. We counted the weight of all fishes from each tank. Nine fishes were selected from each tank for morphometric measurements of individual weight and length of the selected fishes were recorded for body indices analysis. 20 fishes from each tank were chosen for haemolymph collection, hepatopancreas extraction and guts collection. The guts and hepatopancreas of the fishes were promptly removed and collected into 2 mls cryogenic vial tubes and frozen in liquid nitrogen before being stored at -80 °C for other analytic assays.

Ingredients (%)	Dietary Phenylalanine Level (%)					
	0.38	0.94	1.32	1.76	2.23	2.70
Amino acid mix ^b	26.57	26.57	26.57	26.57	26.57	26.57
Fishmeal ^a	10.00	10.00	10.00	10.00	10.00	10.00
Soy protein concentrate ^a	2.00	2.00	2.00	2.00	2.00	2.00
α-starch ^a	25.38	25.38	25.38	25.38	25.38	25.38
Microcrystalline cellulose ^a	20.00	20.00	20.00	20.00	20.00	20.00
Fish oil: soybean oil (1:1). ^a	4.00	4.00	4.00	4.00	4.00	4.00
Soy phospholipids (oil)	2.00	2.00	2.00	2.00	2.00	2.00
Monocalcium phosphate ^b	3.00	3.00	3.00	3.00	3.00	3.00
Squid paste ^a	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix ^b	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin C ^b	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix ^b	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (50%) ^b	0.25	0.25	0.25	0.25	0.25	0.25
Glycine	2.50	2.00	1.50	1.00	0.50	0.00
Phenylalanine	0.00	0.50	1.00	1.50	2.00	2.50
DMPT ^c	1.00	1.00	1.00	1.00	1.00	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition (% Air dry basis)						
Crude protein	37.54	38.10	37.85	38.10	37.97	37.99
Crude fat	7.17	7.26	7.05	7.22	7.08	7.06
Ash content	5.17	5.11	5.20	5.21	5.14	5.18
Phenylalanine	0.38	0.94	1.32	1.76	2.23	2.70
Phenylalanine in Protein	1.01	2.47	3.49	4.61	5.87	7.11

Note: ^a obtained from Tongwei Co., Ltd. (Wuxi, China). ^b Wuxi Hanove Animal Health Products Co., Ltd. (Wuxi, China). ^c DMPT, dimethyl-beta-propantheline.

Amino acid mix (g/100 g diet): L-histidine, 0.50; L-isoleucine, 1.22; L-leucine, 1.79; L-lysine, 2.09; D, L-methionine, 0.76; L-phenylalanine, 0.91; L-threonine, 0.76; L-valine, 1.08; L-tryptophan, 0.29; L-aspartic acid, 2.59; L-serine, 0.39; L-glycine, 1.70; L-alanine, 1.83; L-cystine, 0.30; L-tyrosine, 0.85; L-glutamic acid, 3.65; L-proline, 0.53.

Vitamin Premix (IU or mg/kg diet): vitamin A, 25,000 IU; vitamin D3, 20,000 IU; vitamin E, 200

mg; vitamin K3, 20 mg; thiamine, 40 mg; riboflavin, 50 mg; calcium pantothenate, 100 mg; pyridoxine HCl, 40 mg; cyanocobalamin, 0.2 mg; biotin, 6 mg; folic acid, 20 mg; niacin, 200 mg; inositol, 1000 mg; Vitamin C, 2000 mg; Choline, 2000 mg.

Mineral Premix (g/kg diet): calcium biposphate, 20; sodium chloride, 2.6; potassium chloride, 5; magnesium sulphate, 2; ferrous sulphate, 0.9; zinc sulphate, 0.06; cupric sulphate, 0.02; manganese sulphate, 0.03; sodium selenate, 0.02; cobalt chloride, 0.05; potassium iodide, 0.004.

Table -2 Amino acid contents in the feeds (g/100g in air dry basis)

Amino acid	% Dietary Phenylalanine Level					
	0.38	0.94	1.32	1.76	2.23	2.70
EAA^a						
Histidine	0.70	0.66	0.67	0.64	0.67	0.67
Isoleucine	1.71	1.65	1.72	1.68	1.74	1.72
Leucine	2.91	2.87	2.91	2.84	2.92	2.88
Lysine	2.69	2.64	2.61	2.60	2.66	2.62
Methionine	0.86	0.88	0.92	0.86	0.91	0.91
Phenylalanine	0.38	0.94	1.32	1.75	2.23	2.70
Threonine	1.31	1.25	1.33	1.25	1.31	1.30
Valine	1.78	1.72	1.80	1.75	1.81	1.79
NEAA^b						
Arginine	2.73	2.70	2.75	2.67	2.77	2.70
Glycine	4.76	3.08	3.83	3.28	2.85	2.29
Cystine	0.11	0.11	0.12	0.11	0.12	0.12
Tyrosine	0.64	0.54	0.62	0.62	0.68	0.66
Proline	1.35	1.26	1.40	1.37	1.36	1.41
Aspartic acid	4.39	4.25	4.38	4.31	4.39	4.29
Serine	0.91	0.92	0.94	0.91	0.94	0.93
Alanine	2.42	2.39	2.46	2.42	2.45	2.45
Glutamic acid	6.17	6.11	6.24	6.01	6.20	6.13

Note: Tryptophane was not determined because of its degradation during acid hydrolysis.

^a EAAs, Essential Amino Acids. ^b NEAAs, Non-essential Amino Acids.

Growth parameters

All fishes were weighed in bulk to obtain total biomass of the tank then counted to obtain their number in the tank for analysis of growth indices.

The growth performance was assessed using biomass of the tanks and amount of feed used during the feeding trial experiment. Parameters

such as weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR) and protein efficiency ratio (PER) were calculated based on the biomass of each tank.

$WGR (\%) = 100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight};$

$SGR (\%/d) = 100 \times (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days of the experiment}$

FCR = feed intake/biomass increase

SR (%) = $100 \times (\text{final number of prawn} / \text{Initial number of fish})$

PER (%) = $100 \times (\text{biomass increase} / \text{protein supplied})$

Digestive enzymes analysis

The hepatopancreas samples, weighing approximately 0.1g, was measured in triplicate from each group. then, 0.9 mL of sterile 0.9% saline water was added along with iron balls, and the mixture was placed in a homogenizer machine operating at 60Hz for 15 seconds, repeated 3 to 5 times to ensure proper homogeneity of the sample. The sample was then centrifuged at 4 °C at 4000 rpm for 10 minutes, and the supernatant was stored at -20 °C for antioxidant indices analysis. The protein content of the hepatopancreas was determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All analytical procedures were conducted on ice to maintain sample integrity.

Digestive enzymes activity indices on Hepatopancreas were analysed using commercial kit for digestive enzymes determination (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Enzyme activity of lipase, amylase and trypsin were following the instructions of the kit providers, then analysed using a spectrophotometer machine (Thermo Fisher Scientific, Multiskan Go, Finland) at 580nm, 595nm and 253 respectively to obtain results.

Data collection and statistical analysis

The statistical analysis was conducted using Statistical package for social sciences (SPSS) version 23. One-way analysis of variance (ANOVA) was utilized to assess the impact of dietary Phenylalanine levels on the observed responses. Duncan's test was then employed to identify any significant differences among the

treatments. A significance level of $P < 0.05$ was established. To identify the most appropriate regression model, additional analyses were performed, the orthogonal polynomial contrasts were adopted and determine whether the effect was linear, quadratic and/or cubic. SGR and FCR were chosen for determination of the optimal dietary phenylalanine requirement, the second-degree polynomial regression analysis was used to determine the optimal values on SGR and FCR.

Results and Discussion

Growth performance

The growth performance indices are presented in Table -3. WGR, SGR, FCR and PER exhibited a linear significant relationship while final weight showed quadratic significant relationship with the dietary levels of phenylalanine on orthogonal polynomial contrast analysis ($P < 0.05$). However, Initial weight and SR did not show significant relationship with dietary phenylalanine levels ($P > 0.05$). Final weight was significantly higher in groups fed with 2.7%, 2.23%, 1.76% and 1.32% than 0.38% group ($P < 0.05$). WGR and SGR was significantly higher in groups fed with 2.7%, 2.23%, 1.76%, 1.32%, and 0.94% than 0.38% group ($P < 0.05$). FCR was significantly lower in the groups fed with 2.23% and 1.76% than 0.38% group ($P < 0.05$). The group fed with 1.76% dietary Phe showed higher PER significance difference than 0.38% group ($P < 0.05$).

SGR and FCR, as indicators of growth performance were used to determine the optimal phenylalanine requirement of *Cyprinus carpio* using broken-line regression analysis. Based on SGR and FCR, the analysis determined the optimum phenylalanine requirement to be 1.58% and 1.73% of air-dry basis (equivalent to 4.17% and 4.56% of dietary protein) for SGR and FCR, respectively (Figure -1).

Table -3 Effect of dietary phenylalanine on growth performance of *Cyprinus carpio*

Parameter	Phenylalanine inclusion in a diet (%)					
	0.38	0.94	1.32	1.76	2.23	2.70
Initial Weight(g)	0.20	0.20	0.20	0.20	0.20	0.19
Final Weight(g)	0.60 ^b	0.66 ^{ab}	0.70 ^a	0.72 ^a	0.69 ^a	0.68 ^a
SR (%/d)	92.67	88.67	88.67	90.00	90.67	91.33
WGR (%)	209.70 ^b	239.48 ^a	256.73 ^a	260.42 ^a	249.68 ^a	251.81 ^a
SGR(%/d)	2.02 ^b	2.18 ^a	2.27 ^a	2.29 ^a	2.23 ^a	2.24 ^a
FCR	1.77 ^a	1.62 ^{ab}	1.52 ^{ab}	1.42 ^b	1.47 ^b	1.50 ^{ab}
PER (%)	30.06 ^b	32.61 ^{ab}	35.00 ^{ab}	37.35 ^a	35.87 ^{ab}	35.38 ^{ab}

Note: Significant differences are indicated with different superscript letters in the same row

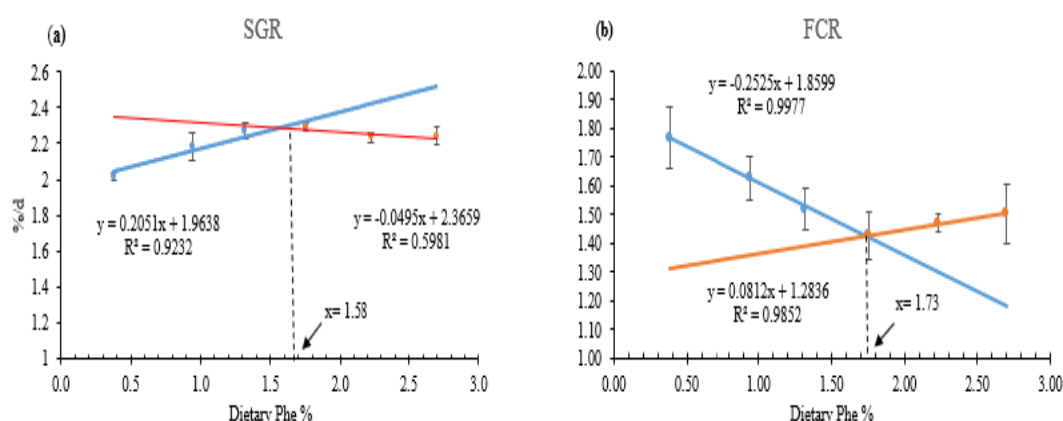


Figure -1 Optimal dietary phenylalanine requirement using SGR (a) and FCR (b) on broken-line regression analysis

Hepatopancrease digestive enzymes capacity

The effect of dietary phenylalanine levels on digestive enzyme activities in the hepatopancreas is detailed in Table -4. Through orthogonal polynomial contrast analysis, it was found that only trypsin exhibited a significant linear relationship

with varying dietary phenylalanine levels ($P < 0.05$). fishes that were fed a diet containing 1.32%, 1.76%, 2.23% and 2.7% phenylalanine displayed significantly higher trypsin activity compared to groups fed diets containing 0.38% and 0.94% phenylalanine ($P < 0.05$)

Parameter	Phenylalanine inclusion in a diet (%)					
	0.38	0.94	1.32	1.76	2.23	2.70
Amylase (U/gprot)	26.87	27.21	24.50	24.13	27.67	26.70
Lipase (U/gprot)	12.85	14.25	14.00	13.50	13.92	14.55
Trypsin (U/gprot)	38.87 ^b	41.25 ^b	58.49 ^a	60.27 ^a	64.49 ^a	57.84 ^a

Table -4 Effect of dietary phenylalanine on digestive enzyme activity of *Cyprinus carpio*

Note: Data are mean value and determined by Duncan's test, Values in

the same row with different superscripts are significantly ($P < 0.05$) different.

Discussion

Growth Performance and feed utilization

The study revealed that the quantity of phenylalanine in the diet had a significant effect on the growth performance and feed utilization of *Cyprinus carpio*. Groups that were fed higher levels of phenylalanine demonstrated higher performance compared to those with lower levels. Similar studies on blunt snout bream *Megalobrama amblycephala* (Ren et al., 2015) support these findings. However, inclusion level exceeding the optimal requirement led to a decrease in growth performance and feed utilization. Similar trends of results have been observed in studies on triploid rainbow trout (*Oncorhynchus mykiss*) (Zhang et al., 2023), Indian major carp: *Cirrhinus mrigala* (Hamilton)(Ahmed, 2009), Indian major carp *Labeo rohita* (Khan & Abidi, 2007) and silver perch *Bidyanus bidyanus* (Ngamsnae et al., 1999), hybrid grouper (Yang et al., 2023), largemouth bass *Micropterus salmoides* (Yi et al., 2022). Consumption of excessive amount of phenylalanine can have detrimental effects on growth and development. Elevated levels of phenylalanine in fish diets can result in increased energy expenditure to eliminate nitrogen, as surplus amino acids are broken down and excreted as ammonia so as energy is redirected from anabolic to catabolic processes (Campbell, 1991; Sayed & Ahmed, 2022). This process also can lead to the oxidation of substantial amounts of phenyl pyruvic acid stored in the body, ultimately producing toxic and potentially harmful effects (Zhu et al., 2017).

The variations in phenylalanine requirements mentioned may be attributed to differences in species, age, experimental conditions, breeding environments, or dietary protein sources (Zhang et al., 2023). These factors can significantly impact the amount of phenylalanine needed by individual specie. This study determined the optimal dietary phenylalanine requirement of *Cyprinus carpio* to be 1.58% and 1.73% for dietary protein levels of 4.17% and 4.56% based on SGR and FCR

respectively. Previous studies on phenylalanine requirements on fish and crustaceans indicated the same trend of requirement. Study on Kuruma shrimps indicated phenylalanine requirements to be 1.5% of 3% dietary protein (Teshima et al., 2002), pacific white shrimp Phe requirement was 1.58% of 3.86% dietary protein (Jin et al., 2019), Phe requirement of swimming crab was 1.19% of 2.64% dietary protein (Guo et al., 2022). In this study the optimal requirement of Phe of common carp is may be slightly higher but it is within the acceptable range with the mentioned studies, although there is a slight difference in percentage of dietary protein inclusion this may be attributed by stage of growth of common carp as juvenile requirement for protein is high for various metabolic processes and also type and source of the dietary protein and ingredients.

Digestive enzyme activity

The growth performance of aquatic animal is closely linked to their digestive and absorption capabilities (Zhang et al., 2022). Estimation of digestive enzyme activities can serve as indicators of nutrient utilization by fish (Sayed & Ahmed, 2022). This study revealed a significant linear relationship between the activity of trypsin and phenylalanine levels of in the diet. Trypsin activity was found to increase in correlation with higher dietary phenylalanine levels. These findings highlight the direct impact of dietary phenylalanine on hepatopancreatic trypsin activity. Trypsin is one of the mainly known digestive enzyme that is used in the synthesis of bioactive peptides. Peptides have been reported to exhibit a variety of bioactivities including antioxidant activity (Shahidi & Zhong, 2015) The presence of certain amino acids and their positioning within the peptide sequence significantly influences their antioxidant activity (Aluko, 2015). Specific amino acids such as tyrosine, histidine, tryptophan, phenylalanine, valine, leucine, and methionine are important for the antioxidant activity of peptides. (Akbarian et al., 2022). Studies have found that antioxidative peptides protects cells from damage

by ROS by activating specific genes (Sarmadi & Ismail, 2010). In this study it indicated that the higher levels of phenylalanine increased the trypsin activity which may influence the synthesis of bioactive peptides which ensure oxidation of ROS. In contrast to this study, research conducted on stinging catfish (*Heteropneustes fossilis*) demonstrated a significant increase in amylase activity, which indicated the potential conversion of excess phenylalanine into glucose through the process of gluconeogenesis (Sayed & Ahmed, 2022). These contrast results may be influenced by the type of specie, age. and source of dietary protein.

Conclusion

In conclusion, the current study has revealed that most of parameters that are related to growth performance, feed utilization and digestive enzymes activity were found to be linearly, quadratically, or cubically influenced by the dietary phenylalanine level in the diet. A broken-line regression analysis was used to determine the optimal phenylalanine requirement for *Cyprinus carpio*. Based on SGR and FCR optimal phenylalanine value were found to be 1.58% and 1.73% of the air-dry basis, which is equivalent to 4.17% and 4.56% of the dietary protein, respectively. These findings indicated that dietary phenylalanine is crucial for growth, development, digestion and overall health. Adequate intake of phenylalanine is necessary to support proper growth and metabolism of common carp. However, the results of the showed that dietary phenylalanine inclusion exceeding optimal threshold resulted in a decline in performance of almost all parameters. These findings offer valuable insights into the nutritional requirements of *Cyprinus carpio*, which can be utilized to enhance their growth and overall well-being in aquaculture environments. This study established a fundamental basis for inclusion of dietary phenylalanine in the diets of oriental river prawn and potential application in crustacean nutrition. The impact of this research on other

aquatic animals warrants further investigation and consideration.

References

- Ahmed, I. (2009). Dietary total aromatic amino acid requirement and tyrosine replacement value for phenylalanine in Indian major carp: *Cirrhinus mrigala* (Hamilton) fingerlings. *Journal of Applied Ichthyology*, 25(6), 719–727. <https://doi.org/10.1111/j.1439-0426.2009.01284.x>.
- Ahmed, I. (2022). Effect of dietary phenylalanine levels on growth, hemato-biochemical composition and tyrosine replacement value for phenylalanine in stinging catfish *Heteropneustes fossilis* –Bloch 1974 fingerling. *Animal Feed Science and Technology*, 288, 115294. <https://doi.org/https://doi.org/10.1016/j.anifeedsci.2022.115294>.
- Akbarian, M., Khani, A., Eghbaltpour, S., & Uversky, V. N. (2022). Bioactive Peptides: Synthesis, Sources, Applications, and Proposed Mechanisms of Action. *International Journal of Molecular Sciences*, 23(3). <https://doi.org/10.3390/ijms23031445>
- Aluko, R. E. (2015). Amino acids, peptides, and proteins as antioxidants for food preservation. In *Handbook of Antioxidants for Food Preservation*. Elsevier Ltd. <https://doi.org/10.1016/B978-1-78242-089-7.00005-1>
- Benakappa, S., & Varghese, T. J. (2004). *Total Aromatic Amino Acid Requirement Of The Indian Major Carp, Cirrhinus Mrigala (Hamilton-Buchanan)*. <https://api.semanticscholar.org/CorpusID:36132156>
- Campbell, J. W. (1991). Excretory nitrogen metabolism” in Environmental and Metabolic Animal Physiology. In C. L. Prosser (Ed.), *Comparative Animal Physiology* (4th ed., pp. 277–324). Wiley-Interscience.

- Guo, C., Zhang, X., Xie, S., Luo, J., Zhu, T., Yang, Y., Li, X., Huang, C., Dang, Y., Zhou, Q., & Jin, M. (2022). Dietary phenylalanine level could improve growth performance, glucose metabolism and insulin and mTOR signaling pathways of juvenile swimming crabs, *Portunus trituberculatus*. *Aquaculture Reports*, 27(October), 101395. <https://doi.org/10.1016/j.aqrep.2022.101395>
- Jin, Y., Liu, F.-J., Liu, Y.-J., & Tian, L.-X. (2019). Dietary Phenylalanine Requirement of the Juvenile Pacific White Shrimp *Litopenaeus vannamei* (Boone) Reared in Low-Salinity Water. *Journal of Shellfish Research*, 38(1), 35–41. <https://doi.org/https://doi.org/10.2983/035.038.0103>
- Khan, M. A., & Abidi, S. F. (2007). Total aromatic amino acid requirement of Indian major carp *Labeo rohita* (Hamilton) fry. *Aquaculture*, 267(1–4), 111–118. <https://doi.org/10.1016/j.aquaculture.2007.02.025>
- Li, P., Yin, Y., Li, D., Kim, S., & Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, 98, 237–252.
- Li, W., Feng, L., Liu, Y., Jiang, W., Kuang, S., Jiang, J., & Zhou, X. (2015). Effects of dietary phenylalanine on growth, digestive and brush border enzyme activities and antioxidant capacity in the hepatopancreas and intestine of young grass carp (*Ctenopharyngodon idella*). *Aquaculture Nutrition*, 21, 913–921. <https://doi.org/10.1111/anu.12223>
- Ma, Z., Zhu, X., Xie, S., Yang, Y., & Dong, H. (2010). DIETARY PHENYLALANINE REQUIREMENT OF JUVENILE GIBEL CARP. *Acta Hydrobiologica Sinica*. <https://api.semanticscholar.org/CorpusID:88321668>
- Ngamsnae, P., De Silva, S. S., & Gunasekera, R. M. (1999). Arginine and phenylalanine requirement of juvenile silver perch *Bidyanus bidyanus* and validation of the use of body amino acid composition for estimating individual amino acid requirements. *Aquaculture Nutrition*, 5(3), 173–180. <https://doi.org/10.1046/j.1365-2095.1999.00102.x>
- NRC. (2011). *Nutrient Requirements of Fish and Shrimp*. The National academies Press.
- Ren, M., Liu, B., Habte-Tsion, H. M., Ge, X., Xie, J., Zhou, Q., Liang, H., Zhao, Z., & Pan, L. (2015). Dietary phenylalanine requirement and tyrosine replacement value for phenylalanine of juvenile blunt snout bream, *Megalobrama amblycephala*. *Aquaculture*, 442, 51–57. <https://doi.org/10.1016/j.aquaculture.2015.02.029>
- Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides*, 31(10), 1949–1956. <https://doi.org/10.1016/j.peptides.2010.06.020>
- Sayed, S. F., & Ahmed, I. (2022). Effects of Dietary Phenylalanine: Tyrosine Ratio on Growth, DNA/RNA, Serum Biochemistry, Digestive Enzyme Activities and Physiological Responses of *Heteropneustes fossilis*. *Aquaculture Studies*, 23(1). <https://doi.org/10.4194/AQUAST859>
- Schreiber, A. M., & Specker, J. L. (1998). Metamorphosis in the summer flounder (*Paralichthys dentatus*): Stage-specific developmental response to altered thyroid status. *General and Comparative Endocrinology*, 111(2), 156–166. <https://doi.org/10.1006/gcen.1998.7095>
- Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, 18, 757–781. <https://doi.org/10.1016/j.jff.2015.01.047>
- Sharf, Y., & Khan, M. A. (2023). Dietary phenylalanine requirement and tyrosine replacement value for phenylalanine for fingerling *Channa punctatus* based on growth parameters, hematology and antioxidant status. *Aquaculture*, 572(August 2022), 739503. <https://doi.org/10.1016/j.aquaculture.2023.739503>
- Teshima, S., Alam, M. S., Koshio, S., Ishikawa, M., & Kanazawa, A. (2002). Assessment of

- requirement values for essential amino acids in the prawn, *Marsupenaeus japonicus* (Bate). *Aquaculture Research*, 33(6), 395–402. <https://doi.org/10.1046/j.1365-2109.2002.00684.x>
- Wu, G. (2022). Amino Acids: Biochemistry and Nutrition. In *Lippincott's Illustrated Reviews: Biochemistry* (2nd ed., Vol. 96, Issue 2005). CRC Press.
- Yang, P., Wang, H., Ma, L., Yin, H., Zhu, Z., Liu, C., Huang, W., Zhou, Z., Wu, X., & Taj, S. (2023). The Optimum Dietary Phenylalanine Requirement of Hybrid Grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) Juveniles : Effects on Growth Performance , Gut Micromorphology , and Antioxidation. *Aquaculture Nutrition*, 2023, 10. <https://doi.org/https://doi.org/10.1155/2023/9155290>
- Yi, C., Liang, H., Xu, G., Zhu, J., Wang, Y., Li, S., Ren, M., & Chen, X. (2022). Appropriate dietary phenylalanine improved growth, protein metabolism and lipid metabolism, and glycolysis in largemouth bass (*Micropterus salmoides*). *Fish Physiology and Biochemistry*, 50(1), 349–365. <https://doi.org/10.1007/s10695-022-01138-5>
- Yi, C., Liang, H., Xu, G., Zhu, J., Wang, Y., Li, S., Ren, M., & Chen, X. (2024). Appropriate dietary phenylalanine improved growth, protein metabolism and lipid metabolism, and glycolysis in largemouth bass (*Micropterus salmoides*). *Fish Physiology and Biochemistry*, 50(1), 349–365. <https://doi.org/10.1007/s10695-022-01138-5>
- Yuan, Y. H. (2000). *STUDY ON THE ABSORPTION OF L-LEUCINE AND L-PHENYLALANINE BY THE INTESTINE OF GRASS CARP (Ctenopharyngodon idellus)*. <https://api.semanticscholar.org/CorpusID:87824144>
- Zehra, S., & Khan, M. A. (2014). Dietary phenylalanine requirement and tyrosine replacement value for phenylalanine for fingerling *Catla catla* (Hamilton). *Aquaculture*, 433, 256–265. <https://doi.org/10.1016/j.aquaculture.2014.06.023>
- Zehra, S., & Yousif, R. A. (2021). Dietary total aromatic amino acid requirement and tyrosine replacement value for phenylalanine for fingerling *Oreochromis niloticus* (Linnaeus). *Aquaculture Nutrition*, 27(4), 1009–1018. <https://doi.org/10.1111/anu.13242>
- Zhang, C., Wang, X., Su, R., He, J., Liu, S., Huang, Q., Qin, C., Zhang, M., Qin, J., & Chen, L. (2022). Dietary gamma-aminobutyric acid (GABA) supplementation increases food intake, influences the expression of feeding-related genes and improves digestion and growth of Chinese mitten crab (*Eriocheir sinensis*). *Aquaculture*, 546(May 2021), 737332. <https://doi.org/10.1016/j.aquaculture.2021.737332>
- Zhang, L., Zhang, Y., Jia, S., Li, Y., Li, Q., Li, K., Hong, H., & Luo, Y. (2019). Stunning stress-induced textural softening in silver carp (*Hypophthalmichthys molitrix*) fillets and underlying mechanisms. *Food Chemistry*, 295(May), 520–529. <https://doi.org/10.1016/j.foodchem.2019.05.148>
- Zhang, S., Wang, C., Liu, S., Wang, Y., Lu, S., Han, S., Jiang, H., Liu, H., & Yang, Y. (2023). Effect of dietary phenylalanine on growth performance and intestinal health of triploid rainbow trout (*Oncorhynchus mykiss*) in low fishmeal diets. *Frontiers in Nutrition*, 10(March), 1–16. <https://doi.org/10.3389/fnut.2023.1008822>
- Zhu, L., Han, D., Zhu, X., Yang, Y., Jin, J., Liu, H., & Xie, S. (2017). Dietary selenium requirement for on-growing gibel carp (*Carassius auratus gibelio* var. CAS III). *Aquaculture Research*, 48(6), 2841–2851. <https://doi.org/10.1111/are.13118>

د Common carp پر وده او د غذايي موادو پر هضم باندي د تغذويي پينايل الانين اغيزي

پوهنيار سيف الله شريفی^۱، پوهنيار نقيب الله اعجاز^۲، پوهنيار عبدالولي همت^۳

^{۱,۲,۳}حيواني علوم خانگه، کرهني پوهنځی، هلمند پوهنتون

د مسؤل ايميل ادرس: saifullahsharifi1369@gmail.com

Abstract

دغه څېړنه د فینیل الانین د مناسب خوراکي اړتیاوو د ټاکلو لپاره ترسره سوه او همدارنگه د فینیل الانین د بېلابیلو مقدارو د اغیزو ارزونه وسوه، چې د *Cyprinus carpio* کبانو پر وده او هضم باندي څومره اغیزه لري. شپږ برابره نایتروجن لرونکي ($\pm 0.28\%$) 37.92 خام پروټین) او برابره غوړ لرونکي ($7.14 \pm 0.15\%$ خام لیپید) غذاګاني جوړي وي، چې پکښې د فینیل الانین زیاتېدونکي کچې 0.38% (کنټرول)، 0.94%، 1.32%، 1.75%، 2.23% او 2.7% اندازې اضافه کړل سوي. ټول 1200 کبان په 24 استوانوي پولیپروپیلین ټانکونو کې وویشل سول، چې په هر ټانک کې 50 کبان ځای پر ځای سول (د هر کب لومړنی وزن 0.07 ± 0.20 ګرامه) هر ګروپ خوراکه څلور ګروپه کبانو ته د 8 اونيو لپاره تغذیه سول. د پایلو له مخې وروستی وزن په هغو ډلو کې چې د 1.76% فینیل الانین رژیم یې ترلاسه کړی وو، په څرګند ډول د نورو ډلو په پرتله لوړ وو. ($P < 0.05$) د WGR او SGR ارزښتونه هم په 1.76% ډله کې په څرګنده توګه لوړ وو. ($P < 0.05$) په داسې حال کې چې د FCR کچه په 1.76% ډله کې د 0.38% ډلې په پرتله ټیټه وه. ($P < 0.05$) همدارنگه PER په 1.76% ډله کې د 0.38% ډلې په پرتله په څرګنده توګه لوړ وو. ($P < 0.05$). د فینیل الانین مناسبه اړتیا د SGR پر بنسټ 1.58% او د FCR پر بنسټ 1.73% د وچو خوړو پر بنسټ (چې د رژیمي پروټین 4.17% او 4.56% جوړوي) وټاکل سوه. د هاضمي انزایمونو د فعالیت پایلو څرګنده کړه، چې د 2.23% فینیل الانین رژیم ترلاسه کوونکو کبانو کې د تریپسین فعالیت په څرګنده توګه لوړ وو. ($P < 0.05$) په پایله کې دغه څېړنه څرګندوي، چې د ودي، د خوړو څخه ګټه اخیستنې او د هاضمي انزایمونو فعالیت اړوند زیاتره پارامترونه د فینیل الانین په کچه پورې په خطي ډول اغېزمن سول.

کلیدي کلیمې: هاضمي انزایم، وده، *Cyprinus carpio*، فینیل الانین.



BOST

Academic & Research National Journal



Volume: 4 Issue: 1

Year: 2025