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Effects of Dietary Phenylalanine on Antioxidant Activity and Muscle Quality of Common Carp (*Cyprinus carpio*)

Saifullah Sharifi ✉[1], Naqeebullah Ejaz[2], Abdul Wali Hemat[3]
[1]Department of Animal Science, Faculty of Agriculture, Helmand University, Helmand, Afghanistan
✉E-mail: saifullahsharifi1369@gmail.com (corresponding author)

ABSTRACT

This study was conducted to investigate the effects of dietary phenylalanine on antioxidant capacity and muscle quality of common carp (*Cyprinus carpio*). The study also examined how various inclusion levels of dietary phenylalanine affect flesh quality and the expression of genes related to protein utilization and muscle development in common carp (*Cyprinus carpio*). Six isonitrogenous (37.92 ± 0.28% crude protein) and isolipidic (7.14 ± 0.15% crude lipid) diets were formulated with incremental levels of phenylalanine (0.38%, 0.94%, 1.32%, 1.75%, 2.23%, and 2.7%). A total of 1200 fish were divided into 24 cylindrical polypropylene tanks, with 50 individuals in each tank (initial individual weight of fish was 0.20 ± 0.07 g), and were run in four replicates in a completely randomized design for 8 weeks. Antioxidant capacity results showed that superoxide dismutase (SOD) activity was significantly higher in the 2.23% group (P<0.05), while malondialdehyde (MDA) content in the 2.23% Phe group was significantly lower than in other groups (P<0.05). Total antioxidant capacity (T-AOC) activity was significantly lower in the first two groups (0.38% and 0.94% Phe) compared to other groups (P<0.05). Dietary phenylalanine levels led to improvements in muscle texture and the expression of genes associated with protein utilization and muscle development.

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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).

Antioxidants are compounds that prevent food spoilage and protect our bodies from diseases by inhibiting oxidation processes (Shahidi & Zhong, 2015). Antioxidants play a

crucial role in neutralizing the harmful effects of free radicals within cells, which can have detrimental impacts on organisms. Main antioxidant enzymes include Superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD is an enzyme that helps in counteracting the harmful effects of oxidative stress caused by free radicals in the body.(Munteanu & Apetrei, 2021). Phenylalanine supplementation has been shown to impact the antioxidant capacity of aquatic animals. Studies on hybrid tilapia (Xiao et al., 2019), *Chana punctatus* (Sharf & Khan, 2023), and grass carp (Li et al., 2015) indicated that a deficiency in dietary phenylalanine can lead to reduced antioxidant performance and impair the structural integrity of fish gills and decrease their antioxidant status.

Phenylalanine plays a vital role in protein synthesis and is crucial for the growth and development of organisms. Phenylalanine inclusion in the diet has been known to affect the quality of the flesh/ carcass of an aquatic animal. The texture of raw flesh plays a crucial role in determining the palatability and overall acceptability of fish products. (Zhang et al., 2019). The textural characteristics of fish, such as gumminess, springiness, cohesiveness, resilience, hardness, brittleness, adhesiveness, and chewiness, play a crucial role in the aquaculture industry(Cao et al., 2023). The textural attributes of fillets are influenced by a variety of physical factors, such as the species of fish, its age and size, seasonal variations, feeding habits, and conditions during slaughter. (Cheng et al., 2014). Due to the influence of phenylalanine on protein synthesis, growth, and other physiological metabolism in crustaceans, studies have implicated that phenylalanine can have a positive impact on the quality of shrimp flesh by enhancing its texture and taste and also maintaining the firmness and juiciness of shrimp, making it more desirable to consumers. Research conducted on *Oreochromis niloticus* indicated that dietary phenylalanine had a significant influence on the composition of carcass protein (Zehra & Yousif, 2021).

The common carp (Cyprinus carpio) is an important aquaculture species, widely cultivated in numerous Asian nations and certain parts of Europe (Rahman, 2015). Due to its broad popularity, humans have widely introduced it across the world, ranking it as the third most commonly introduced fish species worldwide (Wikimedia projects, n.d.). Common carp is also recognized as one of the world's most widely introduced and invasive fish species. The common carp is an omnivorous species, feeding on a wide variety of items. Its diet may include plant seeds, aquatic vegetation, insects, crustaceans, and even carrion such as dead fish (Vajargah* & Vatandoust, 2022). This study aimed;

To investigate the effect of various dietary phenylalanine levels on antioxidant capacity and muscle quality in *Cyprinus carpio*.

METHODS AND MATERIALS

Six isonitrogenous (37.92% crude protein) and isolipidic (7.14% crude lipid) experimental diets were formulated according to NRC (2011) using conventional ingredients, with phenylalanine levels ranging from 0.38% (control) to 2.70%. Diets were pelleted (1.0 mm), air-dried, and stored at −15 °C until use. A feeding trial was conducted in an indoor recirculating aquaculture

system, where 1,200 fish (initial weight 0.20 ± 0.07 g) were randomly distributed into 24 tanks (50 fish per tank) with four replicates per diet. Fish were acclimated for 14 days, then hand-fed to satiation three times daily for the trial period, with water quality maintained within optimal ranges throughout the experiment.

Experimental Diets

The experimental diets were formulated according to the nutritional needs of fish as described in the guidelines (NRC, 2011), using conventional ingredients. These ingredients included fishmeal, soya bean meal, rapeseed meal, shrimp meal, squid paste, wheat meal, fish oil, and soy oil. Other components of the feed include granulesten, cholesterol, monocalcium phosphate (MCP), premix, vitamin C, choline chloride, ecdysone, and dimethyl-beta-propantheline (DMPT). Six isonitrogenous (37.92% crude protein) and isolipidic (7.14% crude lipid) diets were formulated. The phenylalanine inclusion level in the control group diet was 0.38%. The experimental group included five treatments with phenylalanine inclusion levels at 0.94%, 1.32%, 1.75%,2.23% and 2.7% respectively (Table 1). The ingredients were crushed into a fine powder and sifted through a sieve. Then, they were weighed. The dry ingredients were thoroughly mixed before adding water and oil. The mixture was then passed through a twin extruder machine (Huagong Optical Mechanical and Electrical Technology Co. Ltd., Guangzhou, China) to produce extruded pellets of 1.0mm. Then, the pellets were air-dried at room temperature. The formulated feeds were packed into bags and stored in a cold freezer at -15°c, until feeding trials started.

Experimental Setup and Feeding Trials

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

The fish were acclimatized for 14 days while fed with commercial feed. At the beginning of the experiment, the fish's individual initial weight was 0.20 ± 0.07 g. Fish were fed by hand, to satiation, three times a day (7.30 am-8.00 am, 12.30 pm-1.00 pm, 5.30 pm-6.00 pm). A daily feed amount was approximately 5–6% of tank biomass. The uneaten feeds were removed 1 hour 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

The sampling procedure was followed as the fish were starved for 24 hours before the collection of the samples. We counted the weight of all fish from each tank. 20 fish from each tank were chosen for hepatopancreas extraction.

The muscle and hepatopancreas of the fish were promptly removed and collected into 2mls cryogenic vial tubes and frozen in liquid nitrogen before being stored at -80 ◦C for other analytic assays. The Flesh Muscle samples from five fish were collected into sealed plastic bags from each tank, then stored at room temperature at 4 °C to keep them fresh for immediate muscle texture analysis. Flesh muscle samples from 4 to 6 selected fishes from each tank were collected into 2 ml cryogenic vial tubes and promptly stored in Liquid nitrogen before being stored at -80 °C for gene expression analysis.

Table 1. *Feed formulation and proximate composition of experimental feeds on an air-dry basis*

	Dietary Phenylalanine Level (%)					
Ingredients %)	**0.38**	**0.94**	**1.32**	**1.76**	**2.23**	**2.70**
Amino acid mix	26.57	26.57	26.57	26.57	26.57	26.57
Fishmeal	10.00	10.00	10.00	10.00	10.00	10.00
Soy protein concentrate	2.00	2.00	2.00	2.00	2.00	2.00
α-starch	25.38	25.38	25.38	25.38	25.38	25.38
Microcrystalline cellulose	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	3.00	3.00	3.00	3.00	3.00	3.00
Squid paste	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin C	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride (50%)	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	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: 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-cysteine, 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 bisphosphate, 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 on air dry basis)*

Amino acid	% Dietary Phenylalanine Level					
	0.38	**0.94**	**1.32**	**1.76**	**2.23**	**2.70**
EAA						
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						
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
Cysteine	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: Tryptophan was not determined because of its degradation during acid hydrolysis. A EAAs, Essential Amino Acids. b NEAAs, Non-essential Amino Acids.

Antioxidant Indices Analysis

The hepatopancreas samples, weighing approximately 0.1g, were 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.

The antioxidative indices in the hepatopancreas, including superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAOC), were assessed using a spectrophotometer (Thermo Fisher Scientific, Multiskan Go, Finland) at 450 nm, 512 nm, and 660 nm, respectively following the procedures of the analysis kit provider (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Muscle Texture Profile Analysis

The texture parameters, including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience evaluated using a TA-XT plusC texture analyser from Stable Micro Systems (Surrey, United Kingdom). The analyser was equipped with a 50 mm flat-bottomed cylindrical probe P/50. The evaluation followed the method described by Ma et al. (2017) with slight modifications depending on the type of the sample and experimental

condition. All texture profile analyses and shear force tests were conducted at room temperature. Pieces of abdominal muscle tissue (3.113 ± 0.655 mm) from each selected fish were used. Cross-sectional area of 400mm and a constant sample width and length of 20mm, to determine muscle texture indices. The probe was pressed downward at a constant speed of 1 mm/s into the sample until it reached 60% of the sample height. Each sample was compressed once for each texture profile analysis test.

RT-PCR Analysis

Gene expression of protein utilization (CLP, SBDS, Rpl12) and muscle development (MSTN, MYF5) genes was analysed by RT-PCR. RNA was extracted from muscle tissue using the Trizol method, quantified (A260/A280 = 1.8–2.0), and adjusted to 400 ng/μL. cDNA was synthesized with the PrimeScript™ RT kit. Primers were synthesized commercially, and amplification was performed with TB Green® Premix Ex Taq™ II on a BIO-RAD CFX 96 system. PCR conditions included 95°C for 3 min, 40 cycles of 95°C for 10 s and 60°C for 30 s, followed by melt curve analysis. Relative expression was calculated using the 2^ΔΔCT method.

Table 1. *Primer sequences of target genes in real-time PCR analysis*

Gene	Primer Sequence Forward (5'-3')	Primer Sequence Reverse (5'-3')
CPL	CAGTCACTGAAGTAAAGGACCAG	AACTTAGAAGAGCAATCGACCA
Sbds	TTGGAGATTCTGGAAAAGG	TCATGGCAACAGTGTATGGG
Rpl12	TTGGTCCCCTTGGTCTATCT	AACACGAGCCTGACGGTTT
MSTN	AACTCCACCCGATATGACA	GCGTGAAGCGTAGATGTTA
MYF5	AGACCTGGCAAGGAAAC	CTGGAAGGCATCTGGAA

Note: *The nucleotide sequence is derived from the genome of common carp*

Data Collection and Statistical Analysis

The statistical analysis was conducted using the Statistical Package for the 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 determined whether the effect was linear, quadratic, and/or cubic.

FİNDİNGS

Dietary phenylalanine significantly influenced antioxidant capacity, muscle texture, and gene expression. SOD and T-AOC increased while MDA decreased with higher phenylalanine levels, with optimal antioxidant responses around 2.23%. Muscle hardness and springiness were unaffected, but gumminess showed a quadratic response, and moderate phenylalanine levels improved several texture parameters. Gene expression related to protein utilization and muscle growth was altered, with MYF5 increasing linearly and peaking at 2.23%, indicating its key role in phenylalanine-mediated muscle development.

Hepatopancreatic Antioxidant Indices

The effect of dietary phenylalanine level on antioxidant activity of SOD and T-AOC and contents of MDA are shown in Table 4. SOD, MDA, and T-AOC have a linear significant relationship with the dietary levels of Phenylalanine supplementation ($P<0.05$). SOD activity in a group fed with 2.23% Phe was significantly higher than other groups ($P<0.05$). MDA content on the groups fed with 0.38%, 0.94% and 1.32% was significantly higher than 1.76% 2.23% and 2.7% groups ($P<0.05$). T-AOC of groups fed with 1.32%, 1.76%, 2.23% and 2.7% was significantly higher than groups fed 0.38% and 0.94% Phenylalanine diet ($P<0.05$).

Table 4. *Effect of dietary phenylalanine on antioxidant activity of common carp*

Phenylalanine inclusion in a diet						
Parameter	0.38	0.94	1.32	1.76	2.23	2.70
SOD (U/ mg prot)	21.63^{c}	22.17^{c}	22.24^{c}	23.52^{bc}	27.77^{a}	26.39^{ab}
MDA (nmol/mgprot)	7.02^{a}	7.62^{a}	7.03^{a}	6.22^{b}	5.18^{c}	5.57^{bc}
T-AOC (nmol/mgprot)	0.07^{b}	0.09^{b}	0.14^{a}	0.14^{a}	0.15^{a}	0.15^{a}

Note: Data are means determined by Duncan's test. Values in the same row with different superscripts are significantly different ($P < 0.05$).

Muscle Texture Profile Analysis

Dietary phenylalanine did not affect hardness and springiness ($P>0.05$), but showed a quadratic effect on gumminess ($P<0.05$). At 2.23% phenylalanine, adhesiveness was higher than in the 1.32% group ($P<0.05$). The 1.32% group differed significantly in cohesiveness, chewiness, resilience, and gumminess compared to some lower-level groups (0.38%, 0.94%) but not from higher levels (1.76%, 2.23%, 2.7%) ($P>0.05$).

Table 5. *Effect of dietary phenylalanine levels on flesh texture of common carp*

Phenylalanine inclusion in a diet (%)						
Parameters	0.38	0.94	1.32	1.76	2.23	2.7
Hardness	714.15	744.64	794.23	802.39	815.03	734.63
Adhesiveness	-21.94^{ab}	-21.66^{ab}	-28.47^{b}	-24.28^{ab}	-20.97^{a}	-24.3^{ab}
Springiness	0.31	0.31	0.33	0.31	0.31	0.32
Cohesiveness	0.28^{b}	0.28^{b}	0.30^{a}	0.29^{ab}	0.28^{b}	0.29^{ab}
Gumminess	201.74^{b}	207.14^{b}	250.3^{a}	228.95^{ab}	229.56^{ab}	219.39^{ab}
Chewiness	63.83^{b}	66.74^{b}	83.92^{a}	75.06^{ab}	72.93^{ab}	72.86^{ab}
Resilience	$0.18a^{b}$	0.18^{b}	0.21^{a}	0.19^{ab}	0.18^{b}	0.2^{ab}

Note: *Data are determined by Duncan's test. Mean values with the same superscripts in the same row are insignificantly different (P > 0.05).*

Gene Expression

Dietary phenylalanine significantly affected gene expression related to protein utilization and muscle development. CLP and SBDS showed linear responses ($P<0.05$), while Rpl12 was unaffected ($P>0.05$). SBDS and CLP expression peaked at 0.94% phenylalanine, with the lowest at 2.23%. For muscle development, MYF5 expression increased linearly ($P<0.05$),

highest at 2.23%, while MSTN showed no effect (P>0.05). Overall, MYF5 appears to be key in mediating phenylalanine's role in muscle growth.

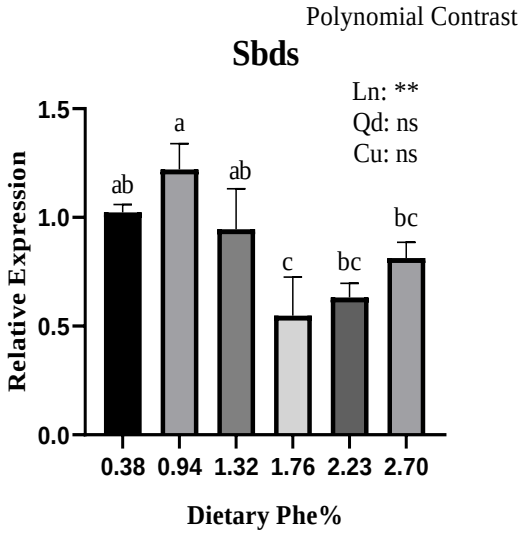

Figure 1. *SBDS; Ribosomal maturation protein*

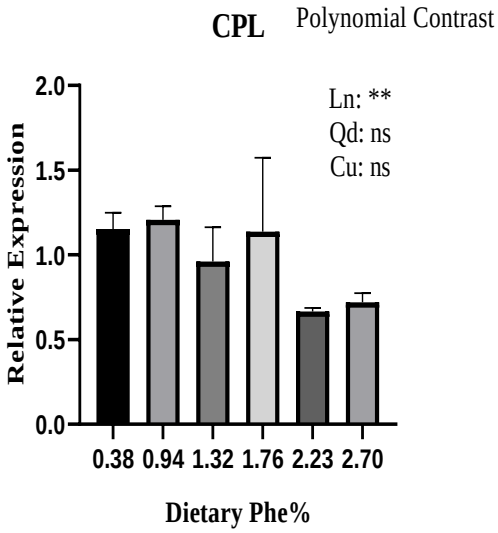

Figure 2. CLP; Cathepsin L

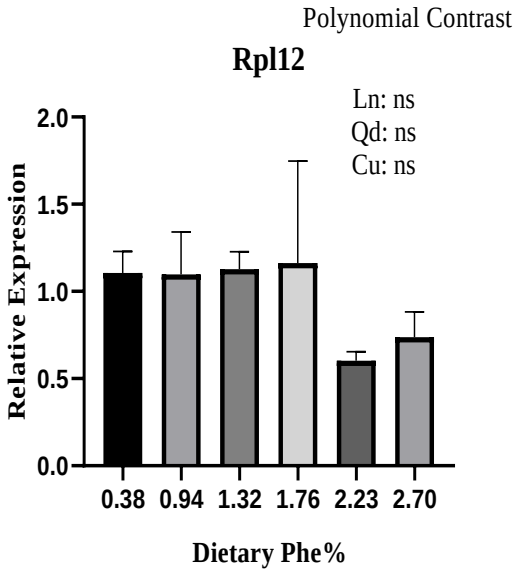

Figure 3. *Rpl12; 60S ribosomal protein L12*

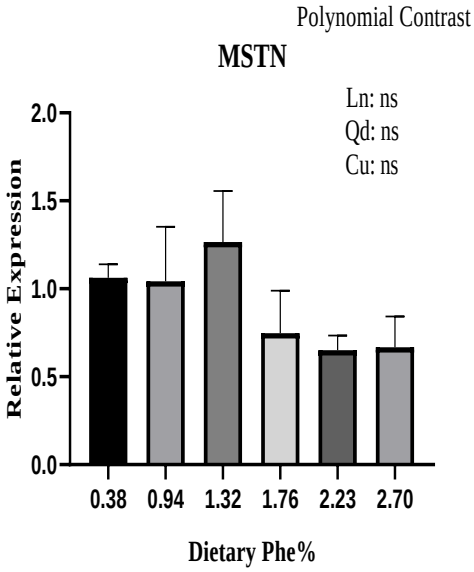

Figure 4. *Relative expression of Myostatin (MSTN)*

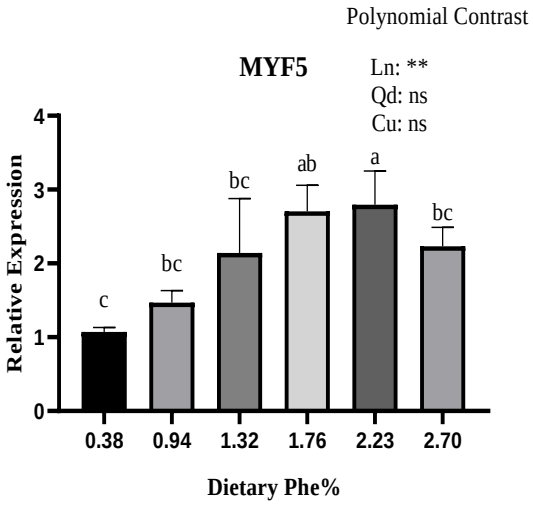

Figure 5. *Relative expression of Myogenic factor*

DISCUSSION

Aromatic amino acids, including phenylalanine, play a crucial role in converting free radicals into stable molecules by providing them with electrons (Elias et al., 2008). SOD and CAT are two independent enzymes that play vital roles in the antioxidative defence system of fish. SOD eliminates reactive oxygen species within the body while generating hydrogen peroxide as a by-product. CAT then efficiently neutralizes this by-product, ensuring the overall balance and effectiveness of the antioxidative defence system (Xiao et al., 2019; Wilhelm, 1996). MDA is a by-product of lipid peroxidation; it is a reliable indicator of oxidative damage (Dotan et al., 2004). The current study found a significant linear relationship between the activity SOD and T-AOC, as well as the content of MDA, with varying dietary levels of Phenylalanine. The activity of SOD and T-AOC has demonstrated a linear increase with increasing phenylalanine levels, while MDA content exhibited a linear decrease. However, a shift in this trend occurs when phenylalanine levels reach their optimal value. Several studies have shown increased activity of SOD with the increased level of phenylalanine supplementation in hybrid tilapia (Xiao et al., 2019), *Chana punctatus* (Sharf & Khan, 2023), and grass carp (Li et al., 2015). These result indicates that with the increasing level of dietary phenylalanine on a diet of *M. nipponense*, it increases the activity of SOD and T-AOC and lowers the content of MDA. These findings suggest that Phenylalanine supplementation may play a role in modulating antioxidant enzyme activity and lipid peroxidation levels in the body.

Texture properties of raw fish and prawns are mainly contributed by connective tissue and muscle fibers (Dunajski, 1980). The texture of fish varies depending on the species, biological condition, method of catch, and culinary treatments (Ginson et al., 2021). The study on dietary phenylalanine levels and muscle texture in bighead carp found that there was no significant relationship between phenylalanine levels and the hardness or springiness of the fish flesh. However, a quadratic significant relationship was seen with gumminess. The group fed with 2.23% phenylalanine had higher adhesiveness compared to the 1.32% group.

Cohesiveness and chewiness in the 1.32% group were significantly different from some groups but not others. Resilience in the 1.32% group was different from certain groups, while gumminess was significantly higher than the 0.38% group.

Meat quality traits are affected by genetics and environmental factors. Scientists study gene and protein levels to understand how genetic potential is influenced by the environment. This helps in identifying biomarkers that can predict meat quality (Picard et al., 2012). Biomarkers of gene expression are proving to be more accurate than genetic markers in understanding the biological processes that determine phenotypes. They are instrumental in explaining and predicting variations in meat quality (Te Pas et al., 2011). The process of protein deposition in organisms is guided by specific templates determined by the genetic and epigenetic codes of the animal. These specific targets are determined by both endogenous factors, such as genetics and life stage, as well as exogenous factors like the environment and diet (NRC, 2011). Proteins are the final product of an intricate process in which genes are expressed. Occasionally, variations of a protein can be produced from the same gene as a result of post-transcriptional modifications (Picard et al., 2012). This study demonstrates that the levels of phenylalanine in the diet can influence the expression of specific genes involved in protein synthesis and utilization. The genes CPL and Sbds exhibited significant effects in expression levels when exposed to different concentrations of dietary phenylalanine, whereas Rpl12 did not show significant changes. The Sbds gene produces a protein necessary for building ribosomes, which are vital for making proteins in cells. The Sbds protein is important for ensuring that ribosomes are formed correctly. Result indicated that Sbds was upregulated in the group fed 0.94% phenylalanine, and downregulated in the groups fed 1.76%, 2.23%, and 2.7% phenylalanine. CPL is a protein that helps control the degradation of specific proteins within cells, maintaining protein levels and cellular function. CPL was upregulated in the 0.94% group and downregulated in other groups. The gene RPL12 creates a protein that is part of the ribosome, the cellular machine that makes proteins. Rpl12 demonstrated upregulation in the 1.32% and 1.76% groups and downregulated in other groups. Previous studies on swimming crab, *Portunus trituberculatus,* showed that an increase in dietary phenylalanine level could influence upregulation of protein synthesis genes (Guo et al., 2022).

Protein synthesis is related to RNA amount in cell, which is directly involved in somatic growth (Sharf & Khan, 2023). The levels of dietary phenylalanine have been found to influence gene expression associated with muscle development, particularly with MYF5, showing a significant linear effect. Conversely, MSTN does not exhibit a significant relationship in this study. MSTN Myostatin is a gene that controls muscle growth by inhibiting its growth, so MSTN is a negative regulator of muscle growth (Ge et al., 2020). Upregulation of this gene can reduce muscle growth and strength by stopping muscle cells from growing and differentiating. Results from this study indicate that the MSTN was upregulated in the group fed with 1.32% dietary phenylalanine and downregulated in all other groups. Among the myogenic regulatory factors, MYF5 is the first to be expressed. In adult muscle, MYF5 is

the only myogenic regulatory factor found in truly quiescent satellite cells. Approximately 90% of quiescent satellite cells contain the MYF5 protein, although the levels may vary among cells (Francetic & Li, 2011; Zammit, 2017). The expression of MYF5 plays a crucial role in defining distinct muscle populations within the somite (Francetic & Li, 2011). The MYF5 gene is important for muscle growth in fish and other animals. It controls the development of muscle cells and is active during the early stages of development. The absence of MYF5 expression during specification could distinguish a subset of satellite cells as stem cells (Zammit, 2017). The MYF5 protein not only drives transcription, but it can also play a role in establishing an open chromatin structure at muscle-specific genes (Zammit, 2017). An increase in levels of dietary phenylalanine in the diet has been linked to upregulation of MYF5 in all groups, indicating an increase in muscle activity. This gives MYF5 a crucial role in muscle development. Phenylalanine has been found to positively impact the quality of shrimp flesh by improving texture, taste, and maintaining firmness and juiciness. Previous study on Oreochromis niloticus showed that inclusion of dietary phenylalanine significantly impacted the carcass protein composition (Zehra & Yousif, 2021) and hence quality. The study on triploid rainbow trout (*Oncorhynchus mykiss*) showed that the increased levels of Phenylalanine positively influenced the growth genes (Zhang et al., 2023); the same trend has been observed on Nile tilapia(*Oreochromis niloticus*) (Yamashiro et al., 2016).

CONCLUSION

In conclusion, the study has revealed a significant relationship between dietary phenylalanine levels of common carp with antioxidant capacity and flesh quality. The findings of the study revealed that levels of dietary phenylalanine had a significant effect on muscle texture and muscle water holding capacity. This study also showed a significant influence of dietary phenylalanine on genomic biomarkers of protein synthesis and utilization and muscle development in common carp. Expression of genes related to protein synthesis (CPL and Sbds), which indicated decreased protein degradation and increased protein synthesis, properly regulated growth patterns, and muscle development genes (MSTN and MYF5) indicated no inhibition of muscle growth and increased muscle activity and development.

The study emphasizes the crucial role of dietary phenylalanine requirement in improving antioxidant capacity, flesh quality, and muscle development in common carp. This study offers a foundational understanding of the inclusion of dietary phenylalanine in the feed and nutrition of common carp and highlighting its importance in promoting wellbeing and overall health in these aquatic species. These findings provide valuable insights into the complex interaction between diet, metabolic processes, genetics, and muscle development in *Cyprinus carpio*.

AUTHORS CONTRIBUTIONS

Hemat Abdul Wali conceptualized and supervised the study. Sharifi Saifullah investigated and analyzed data. Ejaz Naqeebullah wrote the manuscript with input from all authors. All authors reviewed and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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