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LIMITING AMINO ACIDS SUPPLEMENTATION IN LOW CRUDE PROTEIN DIETS AND THEIR IMPACTS ON GROWTH PERFORMANCE, BODY COMPOSITION, AMINO ACIDS PROFILE AND HEMATOLOGY OF *LABEO ROHITA* FRY

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ABSTRACT

The present study was planned on glass aquaria in triplicate groups to check the effect of limiting amino acid (lysine, threonine plus methionine) supplementation in low crude protein diets. Fish fry was served twice a day with five experimental diets designated as; Treatment 1 (35% CP with NRC recommended doses of amino acids) as control diet, Treatment 2 (34.36% CP with 5 % amino acids supplementation), Treatment 3 (34.36% CP with 10 % amino acid supplementation), Treatment 4 (33.60% CP with 10 % amino acids supplementation), and Treatment 5 (33.60% CP with 20 % amino acids supplementation).@ three percent of body weight. Growth results showed a significant increase in specific growth rate, weight gain and carcass protein (60.93±00.15a) in treatment served with T5. Moreover, the feed conversion ratio was improved in treatment administered with augmented levels of limiting amino acid supplementation. However, hematological assay of *Labeo rohita* fry showed non-significant results excluding thrombocytes (416.33±9.33a), which were increased significantly in treatment 4. Level of dispensable and indispensable amino acids including methionine (02.20±00.12ab), phenylalanine (03.33± 00.29a), lysine (04.34± 00.08a) and threonine (02.58±00.10a)were markedly increased in treatments served with an augmented level of amino acids.

Keywords: growth performance, amino acid, *Labeo rohita*, proximate composition, hematology.

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INTRODUCTION

Over the last few years, the price of fishmeal increases due to the rapid addition in the worldwide aquaculture. Fishmeal is the most important protein source for aquatic animals (Trushenski *et al.,* 2006). Consequently, the manufacturers of aquafeeds are concentrated on some other proteins like; herbal protein in order to decrease the cost of formulated diets (Ambardekar and Reigh, 2007). The incorporation of large quantity of herbal protein disturbs the essential amino acid level in the feed. Such feeds cause poor growth performance and feed utilization rate in fish (Abimorad *et al.,* 2009; Zhao *et al.,* 2010; Ngandzali *et al.,* 2011; Ye *et al.,* 2011; Zhou *et al.,* 2011). Many researchers reported that, these negative impacts could be eradicated by incorporating the limiting amino acids in fish feeds, as manifested in *Oreochromis niloticus, Pagrus major* (Takagi *et al.,* 2001) and *Oncorhynchus mykiss* (Gaylord and Barrows, 2009). In protein metabolism, the role of amino acids is vital and versatile (Wright & Fyhn, 2001; Wu *et al*., 2013). There are two kinds of amino acids, indispensable and dispensable amino acids. Generally, the most important and first limiting vital amino acid is lysine which is used in fish diets, particularly in herbal feedstuffs (Hauler & Carter, 2001; NRC, 2011). Lysine supplementation in fish feed results in reduced muscles fat (Berge *et al*., 1998; Nguyen *et al.,* 2013)along with increased retentivity of nitrogen (Cao *et al.,* 2012) & increase in weight (Khan and Abidi, 2011; Yang *et al*., 2011) in fish. Another important, indispensable amino acid which takes part in protein metabolism is methionine. Fish feeds formulated with soy protein are deficient in methionine, so supplementary methionine must be incorporate to the soy formulated protein diet to enhance the zootechnical performance of fish (Brosnan *et al*., 2007; Martinez *et al.,* 2017). Protein is a very costly component of fish diet and becomes essential to add protein ingredients having low cost in fish feed formulation. Threonine is the third very important, limiting indispensable amino acid used in the fish feeds based on plant protein (Bodin *et al*., 2008). Threonine takes part in the synthesis of protein. The destructive metabolism of threonine produces glycine, acetyl-CoA and pyruvate that use in metabolism process (Lemme, 2003). Sufficient addition of nutritional threonine, methionine and lysine is needed for the provision of maximum growth of fish being an important part of body muscle protein. As lysine requirement for carps is comparatively high i.e. 57 to 70 gram per kilo gram of protein (Satheesha & Murthy, 1999; Ahmed and Khan, 2004), so the carp's feed that based on soybean meal may also require lysine supplementation for maximum growth and fish health. Modern improvements in the functions of indispensable amino acids result in the supplementation of crystalline amino acid in the feed to check the limit of amino acids and to improve the efficiency of feed and fish growth (NRC, 2011) and also to increase body mass of fish (Wu, 2013). Therefore, present research work was planned to check the influence of threonine, methionine and lysine supplementation with low crude protein diets on growth, hemato-biochemical profiles, and amino acid profile and body composition of *Labeo rohita* fry.

MATERIALS AND METHODS

Diet preparation: Five practical diets were prepared by using maize, soybean-meal, corn-gluten 60%, fish meal, Di calcium phosphate (DCP), oil, threonine, lysine, methionine and vitamin-mineral premix (Table.1). Treatment 1 (35% CP with NRC recommended doses of amino acids) as control diet, Treatment 2 (34.36% CP with 5 % amino acids supplementation), Treatment 3 (34.36% CP with 10 % amino acid supplementation), Treatment 4 (33.60% CP with 10 % amino acids supplementation), and Treatment 5 (33.60% CP with 20 % amino acids supplementation). All feed ingredients were ground and then mixed well to form crumble feed. 0.5mm crumble size was used to feed the fish fry. Feed was sun-dried before use.

Table 1: Formulation of experimental fish diets.

Ingredients	T1	T2	T3	T4	T5
			Inclusion levels%		
Maize	38.75	39.76	39.76	41.50	41.75
Soybean meal	9.50	9.00	9.00	8.00	9.25
Corn gluten 60%	14.50	14.38	14.00	12.51	12.00
Fish meal	27.50	27.00	26.75	27.50	26.75
Di-calcium phosphate (DCP)	0.25	0.25	0.50	0.50	0.25
Oil	6.75	6.75	6.75	6.75	6.75
Threonine	0.60	0.63	0.66	0.66	0.72
Lysine	1.15	1.20	1.26	1.26	1.38
Methionine	0.75	0.78	0.82	0.82	0.90
Vitamin mineral premix	0.25	0.25	0.50	0.50	0.25
Total	100	100	100	100	100
		Calculated nutrients			
ME (Kcal/Kg)	3711	3715	3699	3713	3720
CP %	35.00	34.36	34.36	33.60	33.60
Lysine	2.26	2.27	2.29	2.29	2.34
Methionine	1.42	1.44	1.46	1.46	1.51
Threonine	1.76	1.77	1.78	1.78	1.80

T1= NRC requirements (Control Diet)
T2= low CP (34.36% of protein) with 5 % amino acid supplementation
T3= low CP (34.36% of protein) with 10 % amino acid supplementation
T4= low CP (33.60% of protein) with 10 % amino acid supplementation
T5=low CP (33.60% of protein) with 20 % amino acid supplementation

Experimental design: Four hundred and fifty *L.rohita* fry were supplied from the hatchery unit of UVAS, Ravi campus Pattoki, Pakistan in June - August 2018. Fish fry then transported in oxygen filled containers, and offered a prophylactic bath in the solution of Potassium per manganate (1:3000). Experiment was designed in completely randomized design in triplicate groups. Thirty fish fry with mean initial weight of 0.98gram was randomly placed in fifteen glass aquariums of equal size (4ft×2ft×2ft) with same water level of 435litre for ninety days and were served to satiation using practical feeds two times a day, at 10.00 and 16.00 o'clock at 3% of biomass. Fish were scaled fortnightly to check the weight gain by using (Anchor, SF-400C Mumbai) a top-loader balance. Fish were scaled every 15 day during the trial. Total feed quantity was readjusted according to weight gain. While on sampling day, no diet was served to *L.rohita* fry. Glass aquaria were cleaned and one third water was replaced with fresh water on daily basis. For the maintenance of dissolved oxygen continuous aeration was provided through air stones. Between 09:00 and 15:00 o'clock physicochemical parameters of water were monitored on regular basis. Dissolved oxygen (DO) meter (YSI, USA) was used to check DO and water temperature during the trial.

Samples collection: On termination of the ninety days dietary experiment, fry of *L.rohita* fish were weighed finally from every glass aquaria. Survival rate was hundred percent. Blood samples were collected at the end of the trial through cardiac puncture by injecting the sterilized needle. Then for hematological tests blood samples were transferred immediately into EDTA (Ethylenediamine tetra-acetic acid) vials to avoid blood coagulation. Complete blood count was performed by the procedure defined by (Blaxhall and Daisley,1973). However, on the completion of the trial fifteen fish fry per replicate were saved for further analysis, including carcass composition and amino acid profile.

Growth and feed utilization indices: Parameters given below were calculated to evaluate the growing performance of fish fry.

Calculations were made after the method described by (Hopkins,1992).

Weight gain (g) = Final body weight (g) - Initial body weight (g).

$$\text{Specific growth rate (SGR \%/day)} = \frac{\ln(\text{Final Wet Body Weight}) - \ln(\text{Initial Wet Body Weight})}{\text{No.of days}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Fish Survival: S = 100 X (LC/LS)

S = Stands for survival (%)

LC = Number of fish recovered at the end of the experiment.

LS = Number of fish stocked at the start of the experiment.

Chemical analysis: Proximate tests of fry fish meat were conducted in accordance with the techniques recommended by the Association of Official Analytical Chemist (AOAC, 2006) in the animal nutrition analysis laboratory at UVAS. Moisture in fish samples was checked by dehydrating the meat sample in an oven at a temperature of 105°C up to constant weight. Crude protein was estimated with Kjeldahl method (CP = Percent Nitrogen (N) x 06.25). Estimation was done by sample digestion with concentrated sulphuric acid in a semi-automated Kjeldahl system (Made by Technico Scientific Supply). For the determination of ash value, fish fry samples were burnt for twenty-four hours at a temperature of 105°C in a muffle furnace. The ether extraction method was adopted by using a Soxhlet apparatus for the estimation of crude lipid in samples

Amino acids analysis: Amino acid quantities in fry fish meat were calculated by the amino acid analyzer (Biochrom 30+, Biochrom Limited. Cambridge, UK) following the protocol of (Ullah *et al*., 2017) in amino acids analysis laboratory of UVAS. Samples were well crushed till five hundred microns & then oxidized with formic-acid for cysteine & methionine conservation. The process of this oxidation transformed methionine to methionine- sulfone & cysteine to cysteic-acid for their conservation. After this fish samples were hydrolyzed with six molar HCl/phenol for twenty-four hour and after that pH value was adjusted to the value of 2.2. Samples after filtration were transferred into small bottles for quantification of amino acids in a Biochrom 30+ amino acid analyzer by ion-exchange chromatography.

Hematobiochemical analysis: By using a Neubauer counting chamber, erythrocytes in fish fry blood samples were counted in fisheries analysis laboratory at UVAS. Samples were then diluted (01:200) by isotonic erythrocyte dilute solution. Then erythrocyte count was calculated by the formula: Number of red blood cells (millions/mm^3) = (No. of counted Red Blood Cells × dilution)/ (No. counted squares × Vol. of a square). Other hematological parameters like mean corpuscular hemoglobin concentration, erythrocytes, leucocyte, mean corpuscular volume thrombocyte, mean corpuscular hemoglobin, hemoglobin & hematocrit levels were determined by an automated hematology analyzer (Celltac MEK-6550, Japan).

Statistical analysis: Statistics were performed using Statistical Analysis Software version. 9.4 (SAS Institute Inc.), (Steel *et al.,* 1996). Repeated measures ANOVA was performed for growth parameters analysis. While data regarding carcass composition, hemato-biochemical analysis, amino acids analysis and physicochemical tests were analyzed using CRD one-way ANOVA. Where the analysis showed significant differences, the DMR post hoc tests were performed to check the comparison of means. Values are means ± standard error (SE) of three replicates and those do not share the same letter are significantly different (P<0.05).

RESULTS

Growth performance: Growth parameters of *Labeo rohita* fry served feed supplemented with augmented levels of limiting amino acids are shown in Table-2. In 2nd, 3rd ,4th and 5th fortnight final weight showed significant results among all the treatments. Weight gain showed significant results among all the treatments in 2nd and 4th fortnight. Significantly increased value of weight gain was recorded in T5. Feed conversion ratio showed significant results among all the treatments during 5th fortnight. Significantly improved value of feed conversion ratio was recorded in T5 followed by T3, T4 and T2 as compared to T1 (control). Specific growth rate showed significant results in 2nd and 5th fortnight. In 2nd fortnight significantly increased value of SGR was recorded in T5 while in 5th fortnight SGR value showed significant increase in T3 followed by T2, T4, T1 and T5.

Carcass composition: Carcass composition of *L. rohita* fry on completion of the nutritional trial is also shown in Table-3. In all the treatments, whole-body moisture content among carcass was significantly lowest (0.25±0.01), and significantly the highest value of moisture content (0.45± 0.02) in fish served with T4 (33.60% protein with 10 % amino acid supplementation) and T1 (35% CP and NRC recommended amino acid level) respectively. Fish served with T5 (33.60% protein with 20 % amino acid supplementation) showed the highest value of the body protein (60.93±0.15) significantly. Fish fry fed on T4 (33.60% protein with 10 % amino acid supplementation) showed highest value of body lipids (2.95±0.03) significantly. Ash content in carcass showed no significant difference among the fish of any treatment.

Hemato-biochemical profile: Hemato-biochemical profile of fry fish is shown in (Table 4). The Hb (Hemoglobin) content, HCT (Hematocrit) value, TEC (Total erythrocyte count), TLC (Total leucocyte count), MCV (Mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) depict non-significant results, among all the treatments while thrombocytes count were significantly lowest (244.67±82.27) in T2 (34.36% protein with 5 % amino acid supplementation) and significantly highest (416.33±9.33) in T4 (33.60% protein with 10 % amino acid supplementation).

Fish fry analysis of amino acids: Amino acid profile of fry fish served with diets supplemented with lysine; methionine & threonine are given in Table 5. Essential amino acids include histidine, isoleucine, leucine and valine exhibit non-significant results while phenylalanine, lysine, threonine and methionine showed significant results. Non-essential amino acids, excluding Tyrosine exhibit significant results.

Table 2 : Summary of results of repeated measures ANOVA on fortnight basis to evaluate the effects of limiting amino acids supplementation on growth performances of *Labeo rohita* fry fed crumble diet

		T1(Control)	**T2**	**T3**	**T4**	**T5**	**P-value**
Initial stocking group weight(g)		29.55±0.10	29.24±0.06	29.63 ±0.09	29.49±0.10	29.61±0.16	0.1433
Survival throughout the trial (%)		100	100	100	100	100	-----
Final weight(g)	1st Fortnight	83.48 ± 3.31	87.92 ± 1.35	88.46 ± 0.83	85.72±2.06	92.16±0.87	0.0816
	2nd Fortnight	122.32^{c} ± 3.92	131.41^{c} ± 6.45	147.95^{b} ± 2.88	134.21^{bc} ± 5.55	166.87^{a}±1.16	0.0003
	3rd Fortnight	181.57^{b} ± 7.4	183.73^{b} ± 5.74	200.4b ± 4.86	191.44^{b} ± 2.18	248.35^{a}±24.31	0.0147
	4th Fortnight	223.83^{b} ± 2.26	242.74^{b} ± 3.67	261.07b ± 3.86	247.04^{b} ± 1.67	328.69^{a}±24	0.0005
	5th Fortnight	275.67^{c}± 5.86	302.52^{bc} ± 4.34	346.36^{ab}±11.84	306.56^{bc} ± 7.46	397.18^{a}±32.64	0.0029
Weight gain (g/fortnight)	1st Fortnight	53.92 ± 3.22	58.68 ± 1.29	58.83 ± 0.75	56.24 ± 2.16	62.55±0.99	0.0818
	2nd Fortnight	38.85^{c} ± 4.31	43.49^{c} ± 5.15	59.48^{b} ± 2.27	48.49^{bc} ± 5.04	74.72^{a}±0.55	0.0005
	3rd Fortnight	59.24 ± 6.99	52.32 ± 0.72	52.45 ± 5.54	57.23 ± 3.47	81.47±23.16	0.384
	4th Fortnight	42.26^{b} ± 9.04	59.01^{ab} ± 6.11	60.67^{ab} ± 1.3	55.59^{b} ± 3.7	80.35^{a}±10.12	0.0355
	5th Fortnight	51.85 ± 5.9	59.79 ± 3.25	85.29 ± 8.1	59.53 ± 5.82	68.49±10.52	0.0618
FCR	1st Fortnight	0.97 ± 0.02	0.96 ± 0.02	0.94 ± 0.01	0.96 ± 0.001	0.93±0.001	0.1989
	2nd Fortnight	0.98 ± 0.03	0.96 ± 0.01	0.95 ± 0.02	0.97 ± 0.01	0.92±0.01	0.2859
	3rd Fortnight	1.09 ± 0.03	1.06 ± 0.07	1.04 ± 0.03	1.07 ± 0.04	0.95±0.03	0.2257
	4th Fortnight	1.13 ± 0.08	1.11 ± 0.01	1.07 ± 0.02	1.09 ± 0.04	0.98±0.01	0.1375
	5th Fortnight	1.17^{a} ± 0.02	1.15^{a} ± 0.04	1.11^{a} ± 0.01	1.14^{a} ± 0.01	1.00^{b}±0.001	0.0042
SGR (%/day)	1st Fortnight	7.41 ± 0.26	7.86 ± 0.09	7.87 ± 0.02	7.62 ± 0.19	8.11±0.10	0.0731
	2nd Fortnight	2.73^{c} ± 0.3	2.86^{c} ± 0.25	3.67^{ab} ± 0.10	3.19^{bc} ± 0.28	4.24^{a}±0.03	0.0034
	3rd Fortnight	2.82 ± 0.31	2.16 ± 0.22	2.40 ± 0.13	2.55 ± 0.22	2.77±0.66	0.6928
	4th Fortnight	1.50 ± 0.35	1.99 ± 0.23	1.99 ± 0.01	1.82 ± 0.12	2.03±0.34	0.5516
	5th Fortnight	1.49^{b} ± 0.16	1.57^{b} ± 0.08	2.01^{a} ± 0.14	1.54^{b} ± 0.13	1.34^{b}±0.14	0.0492

Superscripts on different means within row differ significantly at $P \leq 0.05$

Table 3: Carcass composition of *Labeo rohita* fry fed crumble diet supplemented with lysine, methionine & threonine.

Treatment	T1(Control)	T2	T3	T4	T5
Ash%	12.88± 00.28^{a}	13.11± 00.48^{a}	13.24± 00.10^{a}	13.84± 00.07^{a}	13.66± 00.44^{a}
Lipid%	02.57± 00.05^{b}	02.19± 00.07^{c}	02.56± 00.02^{b}	02.95±00.03^{a}	02.51± 00.02^{b}
Moisture%	00.45± 00.02^{a}	00.33±00.04^{b}	00.27±00.01^{b}	00.25±00.01^{b}	00.44± 00.02^{a}
Protein%	60.17± 00.05^{b}	60.23± 00.06^{b}	59.76± 00.31^{b}	59.13± 00.21^{c}	60.93± 00.15^{a}

Values exist mean ± SE of triplicate groups.
Means in the same row having different superscripts are significantly differ ($P < 0.05$).

Table 4: Hemato-biochemical profile of fish fry

Parameters	T1 (Control)	T2	T3	T4	T5
Hb (g/dl)	01.39± 00.03ᵃ	01.57± 00.03ᵃ	01.58± 00.11ᵃ	01.68± 00.12ᵃ	01.76± 00.38ᵃ
RBC (×10^6 ul)	00.54± 00.18ᵃ	00.69± 00.06ᵃ	00.81± 00.23ᵃ	00.71± 00.10ᵃ	00.56± 00.43ᵃ
WBC-TLC (×10^3 ul)	03.83± 00.38ᵃ	03.60± 00.31ᵃ	03.93±00.22ᵃ	03.53± 00.26ᵃ	03.70± 00.31ᵃ
Thromb-LT (10^3 ul)	328.66±26.14^{ab}	244.67±82.27^{b}	360.67±41.59^{ab}	416.33±9.33^{a}	407.67±13.04^{a}
PCV-HCT (%)	03.02± 01.46^{a}	03.27± 00.50^{a}	03.10± 00.83^{a}	04.39±00.59^{a}	03.29±01.46^{a}
MCV (Fl)	77.33± 3.28^{a}	83.00±0.58^{a}	84.66± 6.98^{a}	79.33± 3.67^{a}	79.67± 03.18^{a}
MCH (Pg)	19.23± 04.16^{a}	20.03± 05.06^{a}	19.97± 03.95^{a}	24.47± 02.20^{a}	25.43±02.17^{a}
MCHC (g/dl)	09.33± 02.82^{a}	08.07± 03.02^{a}	09.10 ±03.23^{a}	14.70± 01.45^{a}	13.23± 01.05^{a}

Values exist mean ± SE of the triplicate group.
Means in the same row having different superscripts are significantly differ (P < 0.05).

Table 5: Complete amino acid profile of fry fish meat

Essential amino acids	T1(Control)	T2	T3	T4	T5
Valine	02.31± 00.05^{a}	02.34±00.15^{a}	02.28±00.10^{a}	02.18±00.10^{a}	02.31±00.05^{a}
Iso leucine	02.34± 00.10^{a}	02.90± 00.01^{a}	02.77±00.08^{a}	02.50±00.35^{a}	02.62± 00.16^{a}
Leucine	04.32±00.06^{a}	04.70± 00.14^{a}	04.77± 00.08^{a}	04.42±00.28^{a}	04.71±00.11^{a}
Phenylalanine	02.54±00.12^{bc}	03.33± 00.29^{a}	02.90±00.05^{ab}	02.29±00.01^{c}	02.54±00.12^{bc}
Lysine	03.86± 00.03^{b}	04.34± 00.08^{a}	04.24±00.04^{a}	03.55±00.05^{c}	03.94± 00.06^{b}
Threonine	02.31±00.02^{b}	02.58±00.10^{a}	02.47±00.09^{ab}	01.07±00.07^{c}	02.53±00.06^{ab}
Methionine	01.62±00.08^{c}	02.20±00.12^{ab}	01.94±00.15^{b}	01.61±00.19^{d}	01.69±00.20^{a}
Histidine	01.56±00.28^{a}	01.14± 00.04^{a}	01.22±00.11^{a}	01.19±00.11^{a}	01.33±00.05^{a}
Non-essential amino acids					
GlutamicAcid+glutamine	08.59± 00.18^{c}	09.16±0.10^{b}	10.36±0.21^{a}	09.47± 0.15^{b}	09.63±0.11^{b}
Tyrosine	02.64±00.30^{a}	02.97±0.02^{a}	02.70± 0.20^{a}	02.04±0.04^{b}	02.97± 0.03^{a}
Cystein	00.43±00.02^{b}	00.55± 0.03^{a}	00.36±0.02^{ab}	00.24± 0.01^{c}	00.36±0.01^{ab}
Glycine	02.35± 00.07^{b}	03.09± 0.07^{a}	03.25± 0.37^{a}	02.35± 0.09^{b}	02.76±0.07^{ab}
Serine	01.80± 00.10^{c}	02.51±0.23^{ab}	02.24±0.06^{b}	01.01± 0.02^{d}	02.86± 0.04^{a}
Ornithine	00.23±00.02^{b}	00.18± 00.03^{b}	00.22±00.03^{b}	00.86 00.06^{a}	00.22±00.02^{b}
Alanine	03.10±00.06^{b}	03.38±0.12^{ab}	03.42±0.11^{ab}	03.69±0.24^{a}	03.24±0.09^{ab}
AsparticAcid+Asparagine	08.59± 00.18^{c}	09.16±0.10^{b}	10.36±0.21^{a}	09.47± 0.15^{b}	09.63±0.11^{b}
Arginine	03.87± 00.06^{c}	04.28±0.14^{bc}	04.72±0.10^{ab}	03.90± 0.35^{c}	04.93±0.03^{a}

Values exist mean ± SE of triplicate group. Means in same row having different superscripts are significantly differ (P < 0.05).

Water quality parameters: In the present study minimum and maximum ranges for dissolve oxygen and temperature were 4.92±0.23^{a} and 5.05± 0.26^{a}, 32.96± 0.36^{a} and 33.18± 0.11^{a} recorded respectively Table-6.

Table 6: Average water quality parameters recorded in different treatments during ninety days feeding trial

Treatment	Temperature (^{0}C)	Dissolve Oxygen (mg/L)
T1	32.96± 0.36^{a}	4.96± 0.21^{a}
T2	33.18± 0.11^{a}	4.92±0.23^{a}
T3	33.05± 0.36^{a}	4.96± 0.27^{a}
T4	33.12± 0.24^{a}	5.01±0.45^{a}
T5	33.19± 0.29^{a}	5.05± 0.26^{a}

Values exist mean ± SE of triplicate group.

DISCUSSION

The present research work elaborates the first corroboration in Pakistan to the best of our knowledge about the dietetic requirements of limiting amino acids in *L.rohita* fry fish. In formulated low crude protein diets, the supplementation of limiting amino acids is essential to enhance the growth factor in *L. rohita* fish fry. The results of this study clearly showed that the growth of *L.rohita* fish fry was excellent when served diets with increasing level of amino acid supplementation. The treatment served with 20% amino acids supplementation was more effective to boost up the growth performance and nutritive value and could satisfy the needs of *L. rohita* fish fry. Results of the present study are supported by (Khan and Jafri, 1993; NRC, 1993; Murthy and Varghese, 1998; Satheesha and Murthy, 1999; Ahmed *et al*., 2003; Ahmed and Khan, 2004; Sardar *et al*., 2009). In this study *L. rohita* fry served with increased level of limiting amino acids showed considerably improved feed conversion ratio, increase in the net gain in weight, and specific growth rate as compared to fish fry served diet with a lower level of limiting amino acids. In confirmation with this study similar results in *Lateolabrax japonicus* (Mai *et al*., 2006), in juvenile *Trachinotus blochii* (Ebeneezar *et al*., 2019), in Hybrid-Catfish (Zhao *et al.,* 2020), in rainbow trout (Lee *et al.,* 2020), and in *L.rohita* (Ayub *et al*.,2021) were reported, when these fishes served with increased level of threonine, lysine and methionine based feeds, exhibited good growing performance, increase gain in weight & improved feed conversion ratio. These results are similar with (Takagi *et al*., 2001) in which *Pargus major* showed good performance with supplemented methionine in diet & further enhanced by the addition of both lysine and methionine compared to treatments served with a diet without lysine or methionine supplementation. In this study, experimental fish showed a significant increase in weight gain, the net gain in the weight and improved feed conversion ratio in the treatment served with a maximum level of lysine, methionine plus threonine supplementation. Recently (Gao *et al*., 2019) conducted an experiment on *Scophthalmus maximus* and reported that an insufficient amount of methionine in the fish diet significantly decreases growth rate and feed utilization. Present trial results are parallel with results of (Zehra and Khan, 2016), for *Catla catla*, an Indian major carp which showed dramatically increased in weight gain & better feed conversion ratio when served threonine supplemented feed. More recently, it has been observed that the addition of lysine in the feed of gift tilapia showed better growth rate and utilization of feed (Prabu *et al*., 2020). In this study augmented level of lysine, threonine plus methionine supplementation showed better growth and good feed utilization in *L. rohita* fry. These results are confirmed by previously mentioned study results. Not long ago, another research trial was conducted by (Hua and Suwendi, 2019) in which *Oreochromis niloticus* showed maximum growth when served with lysine supplemented diet, and present study results are agreed with these results.

In the present experiment, whole body ash content showed non-significant results. Consistent to present study results, an experiment conducted by (Prabu *et al*., 2020) postulated that inclusion of lysine in fish feed showed non-considerable results in the carcass composition of gift tilapia. Fish product quality is considered to be good if it has high content of protein. In this study, meat protein showed the expressively high value in treatment served with 20% limiting amino acids while considerably lower lipid content was noted in the same treatment. It is due to the best consumption of protein with less fat deposition when lysine, methionine plus threonine are present in fish feed. Present trial results agree with the findings of (Robinson, 1991) in which channel catfish was served with the feed supplemented with and without lysine and showed no adverse effects. He observed that when protein content increased than fat content decreased. Same results are obtained in the present study. In a study (Webster *et al*., 1995) postulated that no considerable variations in carcass composition were noted when *Ictalurus furcatus* served with methionine supplemented diet. These results partially agree with the results of the present study. (Mukhopadhayay and Ray, 1999); Mukhopadhayay, 2000; Ayub *et al.,* 2021) reported, *L. rohita* served with lysine, methionine plus cysteine supplemented diet showed good fish quality results. These results support the present study results. Furthermore, in compliance with this study (Khan *et al*., 2003) concluded that *L. rohita* body composition could be improved by adding methionine in soybean meals diet and fortify with minerals. In this study, blood assay showed non-considerable results. Highest values of hemoglobin and mean corpuscular hemoglobin concentration were recorded in treatment served with 20% limiting amino acids. Mean corpuscular volume, white blood cell, thrombocyte, PCV, hematocrit and red blood-cells; the level was higher in treatment served with 10% limiting amino acids. Results of the present study are similar to the findings recorded in *Oreochromis niloticus* fry. The higher values of red blood cells and hemoglobin were recorded in the treatment fed with increasing level of amino acids. Parallel with this study the increased values of blood parameters were recorded in the treatment served with an increased level of limiting amino acids.(Rathore and Yusufzai, 2018). In the present study, high hematocrit level was recorded in the treatments served with an increased level of limiting amino acids. (ShuennDer *et al*., 2001) declared that high hematocrit level was found in *Mylopharyngodon piceus* when served with soybean meal feed added with both methionine and lysine. Same in the case of this study hematocrit level increased due to the supplementation of lysine, threonine and methionine.

Present study results reflect notable differences among essential and dispensable amino acids when served with an augmented level of limiting amino acids. Essential amino acids including phenylalanine, lysine, threonine and methionine were considerably increased with the increased level of limiting amino acid. In contrast, all dispensable amino acids reflect considerable differences among treatments. This level would provide more amino-acids for the production of protein and energy to *Labeo rohita* fry for growth improvement. Researches has been discovered that limiting amino acid methionine would be converted to some other sorts of amino acids by a chain of enzymes including adenosine-triphosphate, adenosyl-methionine, endoenzymes and transferase in the fish body (Kasper *et al*., 2000; Twibell *et al*., 2003). Present findings are in agreement with the results of this study. Another experiment conducted by (Espe *et al*., 2014) recorded increased level of lysine. These results were similar to this study. Highest methionine level was recorded in the treatment served with 5% of limiting amino acids. This specifies exogenous methionine would increase the growth of *L.rohita* fry by holding the constancy of amino acid in fish fry producing better participation of indispensable amino acid in production of the protein while producing dispensable amino acids as an energy source (Ogino, 1980; Ronnestad *et al.,* 2000). Present study results are parallel with (Ronnestad *et al.,* 2005) in which exogenous amino acids considerably improve the level of cysteine and glycine, which must be linked to biological features of methionine. Furthermore, in compliance with present study results, another research work has been declared that methionine being a sulphur-containing amino acid can synthesize cysteine with the help of endoenzymes. In this study, the level of cysteine was increased. So the results of this study are confirmed by previous mention results. Moreover, variations in the level of dispensable amino acid glycine would be accredited to enhance the level of threonine that could be divided to glycine and aldehyde on catalyzed with threonine aldolase (Ronnestad *et al.,* 2005). In present study water quality parameters including temperature and dissolve oxygen were maintained with in suitable ranges for maximum growth of fry fish. Results of the study are supported by the findings of (Ali *et al.,* 2000). Similar to the present study results findings were reported by (Noor *et al.,*2010).

Conclusion: It is concluded from the results that limiting amino acids including lysine, methionine and threonine in low crude protein diets showed excellent performance and were effectively utilized by *L.rohita* fry. Therefore limiting amino acids could be safely used in fish diet as feed supplement and will help in the formulation of cost-effective feed-in fisheries sector. Low crude protein diets supplemented with limiting amino acids in a balanced proportion is a good strategy to improve the apparent feed conversion ratio and to enhance the growth performance of *L.rohita.* The supplementation of 20% limiting amino acids (lysine, methionine and threonine) with 33.60% CP in crude protein is best to improve the growth performance of *L. rohita* fry. It is concluded from the present study that the *L. rohita* fry showed positive response in terms of every parameter including growth performance, hematological analysis, proximate analysis and amino acids analysis when limiting amino acids were incorporated in low crude protein diets.

REFERENCES

Ahmed, I., M.A. Khan and A.K. Jafri (2003). Dietary methionine requirement of fingerling Indian major carp (*Cirrhinus mrigala*) (Hamilton). Aquac. Int. 11(5): 449–462.

Ahmed, I. and M.A. Khan (2004). Dietary lysine requirement of fingerling Indian major carp (*Cirrhinus mrigala*) (Hamilton). Aquaculture. 235(1-4): 499–511.

AOAC. (2006). Official method of analysis. 18th Ed. (Washington). D.C: Association of Official Analytical Chemist.

Ali, M. A. Salam, A. Azeem , M. Shafiq and B.A. Khan (2000). Studies on the effect of seasonal variations on physical and chemical characteristics of mixed water from river ravi and Chenab at union site in Pakistan. J. Res.(Sci). 11:11-17.

Ambardekar, A.A and R.C.Reigh (2007). Sources and utilization of amino acids in channel catfish diets: a review. N. Am. J. Aquac. 69: 174-179.

Abimorad, E.G., G.C. Favero, D. Castellani., F. Garcia and D.J. Carneiro (2009). Dietary supplementation of lysine and/or methionine on performance, nitrogen retention and excretion in pacu (*Piaractus mesopotamicus*) reared in cages. Aquaculture. 295:266-270.

Ayub, A., F. Rasool, N. Khan, S.N. Qaisrani and S. Parveen (2021). Limiting amino acids supplementation in low crude protein diets and their impacts on growth performance, body composition, amino acids profile and hematology of *Labeo rohita* fingerlings. Pakistan J. Agric. Sci. 58(1): 151-160.

Berge, G. E., H. Sveier and E. Lied (1998). Nutrition of Atlantic salmon (*Salmo salar*) the requirement and metabolic effect of lysine. Comp. Biochem. Physiol. 120(3):477–485.

Bodin, N., M. Mambrini, J.B. Wauters, T. Abboudi, W. Ooghe, E.L. Boulenge, Y. Larondelle and X. Rollin (2008). Threonine requirements for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) at the fry stage are similar. Aquaculture. 274(2-4): 353–365.

Brosnan, J.T., M.E. Brosnan, R.F.P. Bertolo and J.A. Brunton (2007). Methionine: A metabolically unique amino acid. Life Sci. 112 (1-2): 2-7.

Blaxhall, P.C. and K.W. Daisley (1973). Routine haematological methods for use with fish blood. J. fish. Boil. 5(6): 771-81.

Cao, J. M., Y. Chen, X. Zhu, Y. H. Huang, H. X. Zhao, G. L. Li and Q. Pan (2012). A study on dietary L-lysine requirement of juvenile yellow catfish, *Pelteobagrus fulvidraco*. Aquac Nutr. 18(1): 35–45.

Espe, M., S.M. Andersen, E. Holen, I. Ronnestad, E. Veiseth-Kent, J.E. Zerrahn and A. Aksnes (2014). Methionine deficiency does not increase polyamine turnover through depletion of hepatic S-adenosylmethionine in juvenile Atlantic salmon. B. J. Nutr. 112(8): 1274-1285

Ebeneezar, S., P. Vijayagopal, P.P. Srivastava, S. Gupta, T. Varghese, D.L. Prabu, S. Chandrasekar, E. Varghese, P. Sayooj, C.S. Tejpal and L. Wilson (2019). Dietary lysine requirement of juvenile

Silver pompano (*Trachinotus blochii*) (Lacepede, 1801). Aquaculture. 511: 734234.

Gao, Z., X. Wang, C. Tan, H. Zhou, K. Mai and G. He (2019). Effect of dietary methionine levels on growth performance, amino acid metabolism and intestinal homeostasis in turbot (*Scophthalmus maximus* L.). Aquaculture. 498: 335-42.

Gaylord, T.G. and F.T. Barrows (2009).Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, Oncorhynchus mykiss, feeds. Aquaculture.287(1-2):180-4.

Hauler, R. C. and C. G. Carter (2001). Reevaluation of the quantitative dietary lysine requirements of fish. Rev. Fish. Sci. 9(3): 133– 63.

Hopkins, K.D. (1992). Reporting fish growth: A review of the Basics 1. J. World. Aquacult. Soc. 23(3): 173-179.

Hua, K., E. Suwendi and D. P. Bureau (2019). Effect of body weight on lysine utilization efficiency in Nile Tilapia (*Oreochromis niloticus*). Aquaculture. 505: 47–53.

Kasper, C. S., M. R. White and P. B. Brawn (2000). Choline is required by tilapia when methionine is not in excess. J. Nutr. 130(2): 238–244.

Khan, M.A. and A.K. Jafri (1993). Quantitative dietary requirements for some indispensable amino acids in the Indian major carp *Labeo rohita* fingerling. J. Aquac. Tropics. 8: 67–80.

Khan, M.A., A.K. Jafri, N.K. Chadha and N. Usmani (2003). Growth and body composition of rohu (*Labeo* rohita) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. Aquac. Nutr. 9(6): 391–401.

Khan, M. and S. Abidi (2011). Effect of dietary L-lysine levels on growth, feed conversion, lysine retention efficiency and haematological indices of *Heteropneustes fossilis* (Bloch) fry. Aquac Nutr. 17(2): 657–667.

Lemme, A. (2003). Reassessing amino acid levels for Pekin ducks. Poult. Int. 42(4): 18–24.

Lee, S., B.C. Small, B. Patro, K. Overturf and RW. Hardy (2020). The dietary lysine requirement for optimum protein retention differs with rainbow trout (*Oncorhynchus mykiss Walbaum*) strain. Aquac. 1:514-734483.

Martinez, Y., X. Li, G. Liu, P. Bin, W. Yan, D. Mas, M. Valdivie, C.A. Hu, W. Ren and Y. Yin (2017). The role of methionine on metabolism, oxidative stress, and diseases. J. Amino Acids. 49(12): 2091-2098.

Murthy, H.S. and C.P. Varghese (1998). Total sulphur amino acid requirement of the Indian major carp, *Labeo rohita* (Hamilton). Aquac. Nutr. 4: 61–71.

Mukhopadhayay, N. and A.K. Ray (1999). Improvement of quality of sal (Shorea robusta) seed meal protein with supplemental amino acids in feed for rohu , *Labeo rohita* (Hamilton), fingerlings. Acta. Ichthyol. Piscat. 1(29): 25–39.

Mukhopadhayay, N. (2000). Improvement of quality of copra (dried kernel of Cocos nucifera) seed meal protein with supplemental amino acids in feed for rohu (*Labeo rohita*) (Hamilton), fingerlings. Acta. Ichthyol. Piscat. 30(2): 21–34.

Mai, K.S., L. Zhang, Q.H. Ai, A.Y. Duan, C.X. Zhang, H.T. Li, J.L. Wan and Z.G. Liufu (2006). Dietary lysine requirement of juvenile Japanese seabass, (*Lateolabrax japonicus*). Aquaculture. 258:535-542

Nguyen, M.V., A.-E.O. Jordal, M. Espe, L. Buttle, H.V. Lai, I and Ronnestad (2013). Feed intake and brain neuropeptide Y (NPY) and cholecystokinin (CCK) gene expression in juvenile cobia fed plant-based protein diets with different lysine to arginine ratios. Comp. Biochem. Physiol. 165(3): 328–337.

Noor E.L., A.I.E. Deen and S.Z Mona (2010). Impact of climatic changes (oxygen and temperature) on growth and survival rate of Nile Tilapia (*Oreochromis niloticus*).Rep. Opinion.2:192-195.

National Research Council. (1993). Nutrient requirement of fish. National Academy Press, Washington, (D.C).

National Research Council. (2011). Nutrient Requirements of fish and shrimp. The National Academies Press, Washington (D.C).

Ngandzali, B.O., F. Zhou, W. Xiong, Q.J. Shao and J.Z. Xu (2011). Effect of dietary replacement of fish meal by soybean protein concentrate on growth performance and phosphorus discharging of juvenile black sea bream, *Acanthopagrus schlegelii*. Aquac Nutr. 17: 526–535.

Ogino, C (1980). Requirements of carp and rainbow trout for essential amino acids. Nippon. Suisan. Gakk. 46: 171–174.

Prabu, E., N. Felix, A. Uma and J. Praveenraj (2020). Effects of dietary L-lysine supplementation on growth, body composition and muscle-growth-related gene expression with an estimation of lysine requirement of GIFT tilapia. Aquac. Nutr. 26(2): 568-78.

Rathore, S. S. and S. I. Yusufzai (2018). Changes in haematological and serum biochemical indices of Nile tilapia (*Oreochromis niloticus*) fry fed dietary shrimp head meal. J. Entomol. Zool. Stud. 6: 663–667.

Robinson, E.H. (1991). Improvement of cotton seed meal protein with supplemental lysine in feeds for channel catfish. J. Appl. Aquac. 1(2): 1–14.

Ronnestad, I., L. E. C. Conceicao, C. Aragao and M. T. Dinis (2000). Free amino acids are absorbed faster and assimilated more efficiently than protein in post larval Senegal sole (*Solea senegalensis*). J. Nutr. 130(11): 2809.

Ronnestad, I. and Conceicao, L. E. C. (2005). Aspects of protein and amino acids digestion and utilization by marine fish larvae. Physiological and ecological adaptations to feeding in vertebrates. In J. M. Starck, & T. Wang (Eds.). Enfield, NH: Science Publishers.

Sardar, P., M. Abid, H.S. Randhawa and S.K. Prabhakar (2009). Effect of dietary lysine and methionine supplementation on growth, nutrient utilization, carcass compositions and haemato biochemical status in Indian Major Carp, Rohu (*Labeo rohita* H.) fed soy protein based diet. Aquac. Nutr. 15(4): 339-46.

Satheesha, S.K. and H.S. Murthy (1999). Dietary requirement of Indian major carp, *Catla catla* for the essential amino acid lysine. Indian. J. Anim. Sci. 69(4): 277–279.

ShuennDer, Y., L. TainSheng, L. ChyngHwa, C. YewHu, P. HungKuang and L. I. Chiu (2001). Partial substitution of white fish meal with soybean meal or lupin meal in diets for fingerling black carp (*Mylopharyngodon piceus*). Taipei. J. Fish. Soc. Taiwan. 28(4): 317–328.

Steel, R.G.D., J.H. Torrie and D.A.Dinkey. (1996). Principles and Procedures of Statistics, 2nd Ed; McGraw Hill Book Co (Singapore).

Takagi, S., S. Shimeno, H. Hosokawa and M. Ukawa (2001). Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream (*Pagrus*) major. Fish. Res. 67(6): 1088–1096.

Twibell, R. G., M. E. Griffin, B. Martin, J. Price and P. B. Brown (2003). Predicting dietary essential amino acid requirements for hybrid striped bass. Aquac. Nutr. 9(6): 373–381.

Trushenski, J.T., C.S. Kasper and C.C.Kohler (2006). Challenges and opportunities in finfish nutrition. N. Am. J. Aquac. 68: 122-140.

Ullah, Z., Z.U. Rehman, Y. Yin, H.H. Stein, Z. Hayat, G. Ahmed, M.U. Nisa, M. Akhtar and M. Sarwar (2017). Comparative ileal digestibility of amino acids in 00-rapeseed meal and rapeseed meal fed to growing male broilers. Poult. Sci. J. 96(8): 2736-42.

Webster, C.D., L.S. Goodgame-Tiu and X.H. Tidwell (1995). Total replacement of fishmeal by soyabean meal, with varius percentages of supplemental L-methionine in diets for blue catfish (*Ictalurus furcatus*) (Lesueur). Aquac. Res. 26(5): 299–306.

Wright, P. A. and H. J. Fyhn (2001). Ontogeny of nitrogen metabolism and excretion. In P. A. Wright, P. M. Anderson (Eds.), Nitrogen excretion, Fish physiol. San Diego: Academic Press.

Wu, G., Z. Wu, Z. Dai, Y. Yang, W. Wang, C. Liu and Y. Yin (2013). Dietary requirements of "nutritionally non-essential amino acids" by animals and humans. J. Amino. Acids. 44(4): 1107–1113.

Wu, G. (2013). Amino Acids Biochemistry and Nutrition. CRC Press, Boca Raton; FL (USA).

Yang, S.D., F.G. Liu and C.H. Liou (2011). Assessment of dietary lysine requirement for silver perch (*Bidyanus bidyanus)* juveniles. Aquaculture. 312(1-4): 102–108.

Ye,J.D., K.Wang, F.D. Li, Y.Z. Sun and X.H. Liu (2011). Incorporation of a mixture of meat and bone meal, poultry by-product meal, blood meal and corn gluten meal as a replacement for fish meal in practical diets of Pacific white shrimp *Litopenaeus vannamei at* two dietary protein levels. Aquac. Nutr. 17: 337.

Zhao, H., R. Jiang, M. Xue, S. Xie, X.Wu and L. Guo. (2010). Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) less than 2 g. Aquac. Nutr.16: 648-654.

Zhao, Y., Q. Jiang, X.Q. Zhou, S.X. Xu, L. Feng, Y. Liu, W.D. Jiang, P. Wu, J. Zhao and J. Jiang (2020). Effect of dietary threonine on growth performance and muscle growth, protein synthesis and antioxidant-related signaling pathways of hybrid catfish (*Pelteobagrus vachelli*♀) × (*Leiocassis longirostris*♂). Br. J. Nutr.123:121-134.

Zhou, F., W. Song, Q. Shao, X. Peng, J. Xiao, Y. Hua, B.N. Owari, B.N., T. Zhang and W.K. Ng (2011). Partial replacement of fish meal by fermented soybean meal in diets for black sea bream, *Acanthopagrus schlegelii*, juveniles. J. World. Aquac. Soc. 42, 184-197.

Zehra, S. and M.A. Khan (2016). Dietary threonine requirement of fingerling Indian major carp, *Catla catla* (Hamilton) estimated by growth, protein retention efficiency, threonine deposition, haematological parameters and carcass composition. Aquac. Res. 1(3): 253-65.