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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* FINGERLINGS

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The present study was conducted to evaluate the effects of limiting amino acids supplementation in low crude proteins diet on *Labeo rohita* fingerlings having an average weight of 4.340g. Fish were kept in n hapas and were fed two times a day @ 3% of body weight to obvious satiation at 10 AM and4:00 PM regularly for ninety days in triplicates on five diets designated as, D1 (30% CP and NRC recommended amino acid level) as control diet, D2 (with 2% low protein and 5% amino acid supplementation), D3 (with 2% low protein and 10 % amino acid supplementation), D4 (with 4% low protein and 10 % amino acid supplementation) and D5 (with 4% low protein and 20 % amino acid supplementation). Net increase in weight, specific growth rate and percent increase in weight were significantly increased while significantly lower value of feed conversion ratio was observed in regimes served with D5 diet. However, meat proximate composition showed increase in protein level with significant decreased in lipid content at 4% low protein and 20 % amino acid supplementation in diet. Furthermore, erythrocytes increased with the increased level of limiting amino acids supplementation in diet among treatments. Whole body essential and non-essential amino acids content, showed significant increase values with the addition of limiting amino acids among the treatments. Consequently, *Labeo rohita* fingerlings showed positive response to limiting amino acids supplemented diet in low crude protein. Therefore, such diets can be used safely in fish feed to decrease the cost of production in aquaculture.
Keywords: Limiting, growth, amino acid, *Labeo rohita*, proximate, hematology.

INTRODUCTION

Formulation of a well-proportioned and specific diet for a species is a big challenge in aquaculture, it should be completely balanced to meet the nutrient requirements necessary for cultural fish species. Insufficient information about nutritional requirements is the main factor which ultimately leads to deprived development and low efficacy in fish (Watanabe, 1995; Heilman and Spieler, 1999). To optimize food proteins level along with the increase of nutritive retention by fish would lessen nitrogen loading and surely influence the cost of production (Thoman *et al*., 1999). Aside from nutritional proteins, ideal amino acids content is also inescapable to increase growth and optimized production of fish. Presences of 10indispensable amino acid in diet are necessary for maximum growth in fish just like other animals (NRC, 2011). The requirement of all these amino acids is find out, for few numbers of culturable species of fish e.g. fry of chum-salmon fish (Akiyama and Arai, 1993), Common-carp (Nose, 1979), Milk Fish (Borlongan and Coloso, 1993) *C.catla* (Ravi and Devaraj, 1991) Chinook-salmon, channel-catfish, Japanese-eel (NRC, 1993), Nile-tilapia (Santiago and Lovell, 1988), Coho-salmon (Araiand Ogata, 1991). Insufficiency of essential amino acids will decrease the performance of growth and consumption of feed (Wilson and Halver, 1986). Subsequently this is necessary for the fulfillment of fish requirement of indispensable amino acid in balance feed. Generally, Lysine is first and most important limiting indispensable amino acid present in herbal feed components, particularly cereal utilized in fish feed of fish (Forster and Ogata, 1998; Small and Soares Jr, 2000; Mai *et al.*, 2006; Gatlin *et al.,* 2007), present abundantly in body of many fish (Wilson and Poe, 1985; NRC, 1993). Because of increased consumption of vegetal protein sources required inadequate amino acid, normally added in fish feed (Fournier *et al.,* 2003; NRC, 2011). To estimate other amino acids requirements, lysine must be utilized as a reference amino acid. Lysine is not present in endogenous synthesis and is entirely mandatory for the deposition of body muscles protein unlike sulfur amino acids (Baker and Han, 1994). Incorporation of one, two or more amino acid may accelerate the process of protein production and muscles development in animals (Wu *et al.,* 2009). Addition of lysine in fish feed increase gain in weight (Yang *et al*., 2011; Khan and Abidi, 2011) retentiveness of nitrogen (Cao *et al.,* 2012) also decrease fats in the body of fish (Berge *et al.*,1998; Nguyen *et al.,* 2013). In mitochondria, for β –oxidation lysine being carnitine precursor assists as transporter of fatty acid (Walton *et al*., 1984), to participate in the metabolism of lipid. Furthermore, lysine also takes part in single metabolic pathway directed for the purpose of muscles development (Valente *et al.,* 2013). Dietetic scarcity of Lysine results in delayed growth, anorexia, fin loss and ultimate death in fish (Ketola, 1983; Borlongan and Benitez, 1990; Mai *et al*., 2006). Many researchers highlighted the requirement of lysine for some common cultureable species of fish in a range between 3.20- 6.20 % nutritional proteins (Ogino, 1980; Robinson *et al*., 1980; Akiyama *et al*., 1985; Arai and Ogata, 1991; Griffin *et al.,* 1992; Tantikitti and Chimsung, 2001; Gurrea *et al.,* 2001; Wilson, 2002). Moreover, methionine is another indispensable limiting amino acid, deficiency of which has been shown to alter the gene expression, linked to growth by DNA methylation. (Roberts and Selker, 1995; Sadhu *et al.,* 2013). Apart from its role in protein production as a precursor, methionine the only sulfur containing indispensable amino acid and contribute to other metabolic reactions like synthesis of Sadenosyl-methionine, phospholipids cysteine, glutathione, phosphatidyl choline, homocysteine, and taurine etc (NRC, 2011). Subsequently threonine considered as third important limiting amino acids in experimental components after lysine and methionine (Ojano-Diranin and Waldroup, 2002). Threonine takes part in production of protein while its destructive metabolism produces several other components which are essential in the process of metabolism including pyruvate, glycine and acetyl Coenzyme A and served being a precursor in the synthesis of serine and glycine. Moreover, it exists in the process of immune response and required for the production of gastricmucin (Lemme, 2003). Sufficient addition of nutritional threonine is necessary to assist maximum aquaculture progress as it acts as an essential constituent of body muscle protein. Dietetic threonine demand have been studied for *Cirrhinus mrigala* fingerlings, *Labeo rohita* fingerlings, *Cyprinus carpio* fry, *Heteropneustes fossilis* fingerling, *Ictalurus punctatus* fingerling, *Sciaeno psocellatus* juvenile, *Oncorhynchus mykiss* fingerling, *Oreochromis niloticus* fingerling fry and *Salmo salar* fry (NRC, 2011).Due to importance of amino acids supplementation in growth &FCR betterment in variety of fish, this research was planned to see the impacts on growth, proximate composition, amino acids profile and hematology of *Labeo rohita* fingerlings when limiting amino acid supplementation in low crude protein diet was provided.

MATERIALS AND METHODS

***Preparation of Diets*:** Five experimental feeds were formulated by adding essential amino acids (Table 1). Maize, soybean meals, corn gluten 60%, fish meal, Dicalcium Phosphate (DCP) oil, threonine, lysine, methionine and vitamin mineral premix were used. Diet 1 (30% CP and NRC recommended amino acid level) as control diet, Diet 2 (2% low of protein with 5 % amino acid supplementation), Diet 3 (2% of low protein with 10 % amino acid supplementation), Diet 4 (4% low of protein with 10 % amino acid supplementation), and Diet 5 (4% low of protein with 20 % amino acid supplementation). All ingredients were grind well then mixed to form dough to make pellets of 3mm size which were sun dried serving to fish.

***Fish, Experimental Design and Condition*:** Fingerlings of *Labeo rohita* for the trial were procured from nursery unit of UVAS Lahore, Pakistan. Fish was transported in polythene containers, and were offered a prophylactic bath in the solution of $KMnO_4$ (1:3000), then transferred in fiber reinforced hapas for 90 days, during this period fingerlings were served to satiations using experimental feeds two times a dayat10:00AM and 04:00PM. Experimental fish (average primary weight about 4.340g) with similar size were evaluated and randomly stocked into fifteen square hapas@ 30 fingerlings per replica. The triplicate group of fish was used to test every investigational diet. On the day of sampling no diet was served to fingerling. Fortnightly gain in weight of the experimental fish was measured by weighing individuals with the help of a top-loader balance (SF-400C). Feed was readjusted according to gain in weight by experimental fish. In all hapas facility of constant aeration and continuous water flow was provided. The parameters of water value were checked between 9^{th} and 15^{th}h on daily basis.

***Samples collection*:** On the completion of90-days nutritional investigation, fingerlings of all hapas were weighed individually. Ten fingerlings were used from every hapas for carcass and amino acids analysis. Blood samples were drawn into EDTA vials by caudal puncture of six fingerlings from every hapa for blood analysis by adopting technique narrate by Blaxhall and Daisley (1973), Kocabatmaz and Ekingen (1984).

***Growth and nutrient utilization parameter*s:** To check growth performance, Net weight. Gain (NWG), Specific. Growth Rate (SGR) and Percent Weight Gain (PWG) parameters were selected. Feed conversion ratio, (FCR) parameter was used as indicator for feed utilization.

The calculations for the variables were done according to Hopkins (1992) as follows:

Percent weight gain = 100 × (Final Body Weight - Initial Body Weight) / Initial body weight

Net weight gain = Average Final Weight (g) - Average Initial Weight (g)

Table 1. Formulation of experimental fish diets.

Ingredients	D1[a]	D2[b]	D3[c]	D4[d]	D5[e]
			Inclusion levels%		
Maize	50.650	52.760	53.100	54.000	52.500
Soybean Meal	7	7	6	5	5
Corn Gluten 60%	6	6	5	5	5
Fish meal	27.00	25.50	27.00	26.75	26.50
DCP	0.250	0.250	0.250	0.250	0.500
Oil	5.750	5.000	5.000	5.350	6.000
Threonine	0.750	0.780	0.820	0.820	0.900
Lysine	1.450	1.520	1.590	1.590	1.740
Methionine	0.900	0.940	0.990	0.990	1.080
Vitamin mineral premix	0.250	0.250	0.250	0.250	0.250
Total	100.0	100.0	100.0	100.0	100.0
			Calculated Nutrients		
ME	3704	3700	3696	3702	3711
CP	30.00	29.40	29.40	28.80	28.80
Lysine	2.29	2.29	2.33	2.29	2.36
Methionine	1.43	1.43	1.49	1.48	1.56
Threonine	1.70	1.71	1.73	1.71	1.77

[a]NRC requirements; [b]low CP (2% of protein) with 5 % amino acids supplementation; [c]low CP (2% of protein) with 10 % amino acids supplementation; [d]low CP (4% of protein) with 10 % amino acids supplementation; [e]low CP (4% of protein) with 20 % amino acids supplementation

Specific growth rate = 100 × (In Final Weight –In Initial Weight)/days of the experiment

Feed conversion ratio = Feed Fed (g, Dry Weight) / Body weight gain (g).

Chemical analysis**:** The determination of protein, moisture, lipids, and ash in fish meat were calculated by standard techniques of (AOAC, 2006). Moisture values in samples were observed at a temp. of 105°C through oven-drying until constant mass. The determination of crude protein was calculated with Kjeldhal methodology (N × 6.25), by acid-digestion with semi-automated Kjeldhal apparatus (Made by Technico Scientific Supply). Crude lipids were determined with the help of Soxhlet apparatus by Ether-extraction method**.** The determination of ash quantity was calculated by burning meat-sample at 550°C in Muffle-furnace for a time period of 24 h.

Amino acids analysis**:** Quantities of amino acids in carcass of fingerlings were estimated through an amino acids analyzer (Biochrom 30$^+$, Biochrom Limited. Cambridge, UK) by following Ullah *et al.* (2017) protocol. Samples were finely crushed till 500 microns and were oxidized with formic acid for the conservation of cysteine and methionine. This oxidation transforms methionine to methionine-sulfone and cysteine to the cysteic-acid for their conservation. Hydrolyzed all samples with 6M HCl/phenol for 24hour. Thereafter pH was adjusted up to 2.2. Samples after filtration were transferred in bottles for amino acid quantification in the Biochrom 30+ amino acid analyzer with the help of Ion exchange chromatography.

Hematobiochemical analysis**:** Neubauer counting chamber was used to count Erythrocytes after diluting blood samples (1:200) to use an isotonic erythrocyte dilution solution. Red blood cells counts were calculated by formula: Number of red blood cells (millions/mm3) = (number of counted RBCs × dilution)/ (number of counted squares × volume of square). Erythrocytes, leucocyte, hemoglobin and hematocrit levels were also determined by an automated hematology analyzer (Celltac MEK-6550).

Data analysis**:** Data file was subjected to one-way ANOVA to evaluate significance among experimental treatments. For growth performances , whole body meat protein , moisture, fat, ash, erythrocyte count, indispensable amino acid including valine, iso-leucine, leucine, lysine, phenylalanine, methionine, threonine, and histidine while dispensable amino acids including arginine, glutamic acid + glutamine, cystein, glycine, ornithine, serine, alanine and aspartic acid +asparagine by using SAS software 9.4 version (SAS Institute Inc., Steel *et al*., 1997). When with ANOVA, considerable treatments influence was noticed then a post-hoc test, (Duncan's multiple range tests) was performed in-order to compare treatments mean values.

RESULTS

Growth performance**:** On ninety days completion of the trial, *Labeo rohita* fingerlings growth, showed clear effect of limiting amino-acids supplementation, in low crude protein diets with significant differences (Table.2).Net-gain in weight and % gain in weight showed similar significant differences

Table 2. Growth performance, feed utilization and meat proximate composition

Parameter	D1	D2	D3	D4	D5
FCR	1.31±.030a	1.06± .030ab	.96± .170b	1.00 ±.030b	.94± .020b
SGR	713.06±44.670c	1292.26±77.420b	1539.63±242.830ab	1587.45±99.570ab	1993.79±159.540a
NWG	26.40± 1.650c	38.76± 2.320bc	46.18 ± 7.280ab	47.62 ± 2.980ab	59.81± 4.780a
PWG	607.74±37.830c	890.79±53.220bc	1061.88±167.410ab	1094.55±68.520ab	1376.06±108.020a
Ash	18.05± .360bc	17.20±.160c	18.31± .030b	20.48±.030a	15.70± .460d
Lipid	5.60± .010b	5.30±.110c	5.72± .020b	6.26±.080a	5.11± .010c
Moisture	0.40± .020a	0.06±.010b	0.36±.020a	0.12± .020b	0.34± .020a
Protein	75.24±.09a	75.48± .11a	74.76± .050b	72.37± .090c	75.52± .190a

The average values, obtained from triplicate & expressed as: Mean ± Standard Error; Values with different superscripts in same row differ significantly (p<0.05).

Table 3. Hematology of *Labeo rohita* fingerlings

Parameter	D1	D2	D3	D4	D5
Hb	3.93± 0.46a	3.13±0.08a	3.83± 0.41a	4.66±0.43a	3.93± 0.74a
RBC	0.78±0.21ab	0.63± 0.23b	0.80± 0.16ab	1.32± 0.16a	1.03± 0.12ab
WBC-TLC	19.03±8.14a	14.90± 2.15a	16.63±1.90a	28.06± 7.17a	18.30± 5.67a
Thromb-PLT	334.66±123.33a	281.33±92.88a	489.66±20.69a	450.33±54.50a	256.00±52.81a
PCV-HCT	11.63±3.72a	8.23±3.67a	8.76± 2.95a	18.13± 2.51a	13.80±1.95a
MCV	145.33±10.17a	125.00±13.31a	81.33± 39.29a	137.00±9.53a	133.33± 8.66a
MCH	37.36± 2.42a	37.30±2.66a	37.00±2.30a	35.63±2.29a	37.66± 5.55a
MCHC	26.23±1.41a	31.70±5.07a	28.13±2.43a	26.13±1.45a	29.16±6.47a

The average values, obtained from triplicate & expressed as: Mean ± Standard Error; Values with different superscripts in same row differ significantly (p<0.05).

among all treatments, as the highest net-gain in weight and %gain in weight was observed in D5 (20 %limiting amino acids with 4%low crude protein diet) while D4 and D3 showed similar weigh gain but lower from D5. In D2 weight gain was higher from D1 but lower from D5, D4 and D3. Specific growth rate showed significant results between the treatments provided with high level and low level of limiting amino acids (Table.2). Feed conversion ratio exhibited significant results. Lowest value in feed conversion ratio was recorded in D5 while highest value was recorded in D1 (Table 2).

Proximate Composition**:** Whole body meat crude protein showed significant variations among treatments served with increased level of limiting amino acid (Table.2). Highest body meat crude protein level was noted in D5 treatment whereas lowest level was noted in D3 and D4 treatment, respectively. Body lipids showed variations among all treatments with significant differences. Lowest lipid level was recorded in D5 treatment which was fed with 20%limiting amino acids while the highest lipid level was recorded in D4 treatment (Table.2). Whole body moisture showed inverse relationship with limiting amino acids in all treatments except in D3, where slight increase in moisture was recorded. Significant increase in moisture level; was recorded in D3, D1 and D5, respectively while in D4 and D2 significant decreased in moisture level was recorded (Table 2).

Hematological Parameters**:** Effect of limiting amino acids supplementation, on hematology in fingerlings of *Labeo rohita* showed in the Table 3. Results in all hematological parameters were non-significant while RBCs showed significant results. Significant high values of RBCs were observed in D4 while significant low values were observed in D2 (Table 3).

Amino Acids analysis in Labeo rohita fingerlings: Amino acids content in meat of experimental fingerlings served with various quantity of limiting amino-acids supplementation, in low crude protein diets showed significant differences in indispensable and dispensable amino-acids (Table 4). Indispensable amino acids including valine, iso leucine, phenylalanine, leucine, threonine, lysine, methionine and histidine showed significant differences among treatment however dispensable amino acids including arginine, glutamic acid + glutamine, cystein, glycine, ornithine, serine, alanine and aspartic acid +asparagine showed significant difference Tyrosine, the only amino acid which revealed non-significant difference between treatments (Table 4).

DISCUSSION

This study provides first evidence in Pakistan, to our knowledge, about dietary requirement for limiting amino acid in *Labeo rohita* fingerling. Supplementation of limiting amino acids in formulated low crude protein diets is necessary to increase growth in fingerlings of *Labeo rohita*. In present study, growing performance of fingerlings *Labeo rohita*, showed positive response towards various levels of lysine,

Table 4. Amino Acids analysis in meat of *Labeo rohita* fingerlings

Essential Amino Acids	D1	D2	D3	D4	D5
Valine	3.28±.030b	3.39±.020a	3.36±.010a	3.14±.010c	3.28±.010b
Iso leucine	3.47±.020c	3.86±.010a	3.82±.010a	3.17±.010d	3.56±.010b
Leucine	5.51±.020c	5.87±.010a	5.82±.020a	5.24±.020d	5.62±.010b
Phenylalanine	3.60±.020d	4.03±.020a	3.96±.010b	3.42±.010e	3.71±.010c
Lysine	4.86± .020c	5.24±.030a	5.18±.010a	4.35±.010d	4.97±.010b
Threonine	3.41±.020c	3.87±.020a	3.83±.020a	1.60±.010d	3.63±.020b
Methionine	2.55±.010a	3.04±.020a	2.90±.010b	1.97±.010e	2.74±.010c
Histidine	2.04±.010c	2.32±.030a	2.27±.010a	1.53±.030d	2.16±010b
Non-Essential Amino Acids					
Arginine	4.77±.020a	4.34±.970a	5.30±.010a	2.87±.020b	5.08±.010a
Glutamic Acid +glutamine	10.85±.020c	11.03±.020b	11.69±.00a	10.49±.010e	10.62±.010d
Tryosine	2.01±1.000a	3.28±.030a	3.29±.010a	2.74±.020a	3.10±.010a
Cystein	0.63±.010b	0.80±.020a	0.66±.010a	0.40±.020c	0.66±.010b
Glycine	3.72±.020c	4.01±.020a	4.02±.010a	3.55±.010d	3.91±.010b
Serine	2.76±.020b	3.05±.020a	3.06±.010a	1.13±.010c	3.09±.100a
Ornithine	0.15±.010b	0.20±.020b	0.16± .010b	0.87±.010a	0.12±.010b
Alanine	4.07±.020c	4.31±.020a	4.31±.020a	4.03±.010c	4.20±.040b
Aspartic Acid +Asparagine	5.35±.020b	5.73±.020a	5.74±.010a	4.45±.020c	5.35±.020b

The average values, obtained from triplicate & expressed as: Mean ± Standard Error; Values with different superscripts in same row differ significantly (p<0.05).

methionine and threonine as diet supplements, showing significant improvement compared to NRC recommended levels of lysine, threonine and methionine as control diet. Evaluated results indicate that lysine, threonine and methionine are important for better growth performance and other zootechnical parameters of *Labeo rohita* fingerlings. During this trial increased net gain and specific growth rates were recorded. These findings are supported by recent similar consequences in Japanese seabass (6.0%) (Mai *et al.*, 2006), in Hybrid-Catfish (Zhao *et al.*, 2020), *Oncorhynchus mykiss* (1558 ± 28.3a) (Lee *et al*., 2020), and in juvenile Silver pompano (214.17 ± 1.77) (Ebeneezar *et al*., 2019). Furthermore the results of this study in weight gain are assisted by parallel findings in carp (6%) (Nose, 1979), red-drum (6.0%) (Brown *et al.*, 1988), yellow croaker (6.0%) (Zhang *et al*., 2008), African-Catfish (6%) (Fagbenro *et al.*, 1998), and for juveniles of *L. rohita* (6%) (Murthy and Varghese, 1997); *O.mykiss* fish (4.0%) (Kim *et al.*, 1992), fingerlings of nile-tilapia (5.10%) (Santiago and Lovell, 1988), Coho-salmon (4.0%) (Arai and Ogata, 1991), *M. asiaticus* (6%) (Lin *et al*., 2013) *H. fossilis* (5.30%) (Khan, 2013) juveniles of Cobia (5.40%) (Zhou *et al.*, 2007), *C. catla* (6.20%) (Ravi and Devaraj, 1991), *B. bidyanus* (6.0%), (Yang *et al*., 2011).and *T.ovatus* (7%) (Du *et al*., 2011). Parallel to our studies (Abimorad *et al*.,2009) reported, average values for gain in weight of *Pacu* fish when served with 23% plant based protein feed, supplemented either with lysine plus methionine or with methionine only showed good results as compared to the feed contained 30% nutritional protein. Present study confirms by previous results. Viola *et al*. (1992) postulated that 30% protein feed for *Cyprinus carpio* may be decreased up-to twenty five percent by altering soybean-meal with the addition of lysine, 0.50 percent and methionine, 0.30 percent without affecting the development of fish. Moreover, experiment conducted by Viola and Lahav (1991) in which, 0.5% supplementation of lysine with 25% protein showed similar growth results as compared with feed with 30% protein. Present study findings are supported by previous results.

In the this study, amino acids supplementation was used with low crude protein diet and obtained distinct weight gain and feed utilization as our results are analogous to those of Gan *et al*. (2012) in which it was listed that addition of methionine and lysine into plant based feed can improve utilization of feed and development of C. *idella* and the nutritional crude protein could be reduced from 32% - 30% by balanced profile of dietetic amino acid. These results support this experiment. Improvement in growth is due to sufficient consumption of indispensable amino acid to assist more deposition of protein. Hence, to increase growing performance, feed must consist of calculated amount of balanced indispensable amino acid profile. Lysine, threonine &Methionine are the limiting and indispensable amino-acids, and during this experiment growing performance of *Labeo rohita* fingerlings enhanced due to supplementation of these amino acids, therefore confirming its essentiality for this fish. In present experimental study highest net gain in weight was observed in the group, supplemented with 20% amino acids and lower in the treatment supplemented with NRC recommended levels of amino acids while poor feed utilization was recorded in the treatments served with low level of limiting amino acid. Result of the study are confirmed by recording poor feed utilization at lower amino acids supplementation level in *O. keta* (Akiyama *et al.,* 1985), and *Oncorhynchus mykiss* (Rodehutscord *et al.,* 1995), whereas anorexia condition along with weight loss was recorded in common carp fingerlings (Nose, 1979) and in *C. catla* (Ravi and Devaraj, 1991) responded to experimental regimes with deficient threonine level although, covering the satisfactory levels of all nutrients. Protein content is very important factor because it is linked with product quality, and results explore that protein contents in fish meat were increased with the increased amino acids supplementation. Present results are similar to those of Akpinar *et al.* (2012). Moreover, experiment conducted by Ravi and Devaraj (1991) demonstrated that, whole body moisture of *Labe rohita* fingerlings was not affected considerably by nutritional protein level with amino acids addition in diet results are similar with present study results whole body moisture was not considerably affected in control and high level amino acids supplemented treatment. Present study results of proximate analysis also have been discussed by some researches (Bechara *et al*., 2005; Li and Robinson, 1998). In this research whole body lipids content was reduced while body protein content was improved with the increased amino acids supplementation level, similar findings have been demonstrated in *Acanthopagruss chlegelii* by Zhou *et al.* (2011*)*.As present study revealed the variations in ash and moisture contents, reduced lipids content and improved in protein contents these results are supported by the work done by Furuya *et al*. (2004) on *Oreochromis niloticus*, further the previous described outcomes of trial are supported by the study done on *O. mykiss* (Cheng *et al.*, 2003; Sardar *et al.*, 2009) on *I. punctatus* (Robinson, 2001) on *Ctenopharyngodon idella* (Gan *et al*., 2012). Keeping in view the results of present discussing trial, decreased lipid content of *Labeo rohita* fingerlings might be linked to boost up the protein consumption known to be effective when complete essential amino acid are exist together at production site due to amino acids as feed supplement. Limiting amino acids including lysine and methionine, serve as precursor for the synthesis of L-carnitine. To produce energy from β-oxidation since they transport fatty acid from cytosol to mitochondria (Li *et al*., 2009; Wilson, 2002). No data are available on hematological values on consuming limiting amino acid on *Labeo rohita*, but fundamental hematological profile i.e., packed cells volume, hemoglobin, leukocytes and erythrocytes in fish are important to observe health rank of fish (Emre *et al*., 2016). Previous coated statement supports these findings, as present results revealed positive variations among hematological status of *Labeo rohita* fingerlings. In this study increased values blood parameters agreed with good growth, specifically in treatment fed with D4. However, in comparison to D4 treatment, lower hematocrit, erythrocytes and hemoglobin values were recorded in fish fed D1 treatment. An experiment conducted by Pohlenz and Gatlin (2014) declared that nutritional manipulations directly affected with fish safety and health. Results of present study confirmed by the previous declared results, as the hematological parameters showed variations among all the treatments due to different levels of amino acids supplementation in low crude protein diets. During this trail, there was no considerable changes were noted in hemoglobin and hematocrit, PLT, MCV and MCHC but considerable differences were observed in erythrocyte for *Labeo rohita* fingerlings. In treatment served with D4 depict high level of erythrocyte and lower in the treatment served with D2. Similar results were demonstrated by Daniels and Gallagher (2000) for *Paralichthys dentatus*in which fish showed increased level of erythrocyte and hematocrit as compared to control treatment. An experiment conducted by Abdel-Tawwab *et al*. (2010) on *O. niloticus* explore that various level of dietary protein showed variations among hematological parameters of fish. Results of this study are line as, among all treatments variations in hematological parameters was recorded. Outcomes of this research postulated that, variations between the parameters of blood of *Labeo rohita* fingerlings was recorded while in erythrocytes considerable difference was recorded in the treatment discussed earlier. Results of this study are supported by similar findings on hematological parameters by Abdel-Tawwab *et al*. (2010) in *Oreochromis niloticus* (Khan, 2014; Khan and Abidi, 2011a, b) on *Heteropneustes fossilis* further findings of this research are linked with those of Habte-Tsion*et al*. (2013) in fish *Megalobramaambly cephala.* In a study Zhou *et al*. (2010) declared, no considerable variation has been noticed in leukocytes, similar case in this trial no considerable variations were recorded in leukocytes in *Labeo rohita* fingerlings. Series of experiments conducted by Zehra and Khan (2015, 2016) on *Catla catla* declared that hematological parameters in fish increased with histidine supplementation and highest erythrocyte value have been recorded in the treatment supplemented with higher level of amino acid as compared on control. Similarly, in this study erythrocyte values were higher in the treatment supplemented with higher level of amino acids and lower in control treatment, previous discussed results are support to this work. On provision of limiting amino acid incorporated diet of *Labeo rohita* fingerlings, showed notable differences among indispensable amino-acids profile excluding tryptophan and dispensable amino-acids profile. All indispensable and dispensable amino acid level was increased markedly except in tyrosine when compared to the control treatment. Results of present study, are similar with those of Zhou *et al*. (2010) which explored, when juveniles of *Sparus macrocephalus* served with various level of lysine supplemented diet an increase in the essential amino acids level were recorded excluding tryptophan. Another experiment conducted by Lin *et al.* (2013) concluded, when Chinese sucker fish served with different amount of lysine, noticeable differences were observed among essential amino acids. Results are matched with these findings as obvious differences were observed in essential amino acids when served with high level of limiting amino acids in *Labeo rohita* fingerlings.

Akiyama *et al.* (1997) persuaded on efficacy of greater proportion of dispensable amino acid to evaluate nutritional protein. Furthermore, (Marcouli *et al*., 2004) retention of protein in *Gilthead* juveniles was considerably enhanced due to increased level of nutritional indispensable or dispensable amino acids, which were in agreement with this study that indispensable or dispensable amino acids index revealed, tendency of accretion in body protein.

Conclusion**:** It is concluded from results that limiting amino acids supplementation including Lysine, Methionine and Threonine can safely be used in fish diet with low CP without showing any negative or harmful effect, which will ultimately decrease the cost of production in aquaculture.

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