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Dietary Lysine Requirement Of Fingerling Hybrid Clarias (Clarias Gariepinus X Clarias Macrocephalus)

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Abstract

The dietary lysine requirement of fingerling hybrid Clarias (Clarias gariepinus×Clarias macrocephalus) (4.2+0.03 cm, 0.056+0.04 g) was determined by feeding six isonitrogenous (400 g kg-1 crude protein) and isocaloric (17.9 kj g−1) amino acid test diets containing casein, gelatin and L-crystalline amino acids with graded levels of lysine (10.0, 12.5, 15.0, 17.5, 20.0 and 22.5 g kg−1) for 4 weeks to triplicate groups. Diets were fed twice a day at 09:00 and 16:00 hours at 8% body weight−1. Maximum weight gain (523%), best feed conversion ratio (FCR, 1.41), Protein efficiency ratio (1.78) and specific growth rate (6.53%) were recorded in fish fed the diet containing lysine at 20.0gkg−1 of the diet. Second-degree polynomial regression analysis of live weight gain and FCR values indicated the dietary lysine requirement at 17.8 and 20.0 g kg−1 of dry diet respectively. Significantly higher carcass protein and protein deposition values were recorded at the requirement level (20.0 g kg−1). Higher fat and lower moisture values were obtained in carcass of fish fed the diet with 15.0g kg−1 lysine. The maximum carcass ash value was noticed in the fish fed at 20.0 g kg−1 dietary lysine. We recommend that the diet for hybrid Clarias (C.gariepinus× C.macrocephaluskg) should contain lysine in the range of 17.8–20.0 g kg−1 of the dry diet, corresponding to 44.5 and 50 g kg−1 of dietary protein respectively.

Keywords: - Lysine, fish, hybrid Clarias, dietary requirement, aquaculture

Introduction

In intensive culture where contribution of natural organisms is not significant with respect to stocking density, formulated feeds are used as the only source of dietary nutrients satisfying the nutritional needs of the concerned species. Formulation of such balanced feeds requires complete knowledge of the nutritional requirements of the cultured species. The major constraint in formulating cost-effective diets is the lack of information on nutritional requirements of fish and the digestibility of suitable feed ingredients (Tacon 1994, McGoogan & Reigh 1996). If the amount of lysine is increase in the feed according to the needs of the fish, the intestine become more digestible so that nutrients to be more absorbed quickly,the growth leads to high, and increase in feed efficiency.(Rachmawati, D., et al., 2022)

Dietary protein constitutes one of the primary nutrient costs of the feed and is the initial source of nitrogen waste products entering a culture system. Optimization of dietary protein levels along with increasing nutrient retention by the fish could reduce nitrogen loading and positively influence production costs (Thoman, Davis & Arnold 1999).African catfish was able to digest protein very effectively in almost all tested ingredients with, Apparent digestibility coefficients ADC values ranging from 85.6 to 105.1% across feeding periods.(Elesho, F. E., et al., 2021)

Protein is essential for growth and development. It provides the body with energy and is needed for the synthesis of hormones, antibodies, enzymes and tissues. It is usually considered to be the most important

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nutrient in fish feeds (Keembiyehetty & Gatlin 1992). Fish generally have a higher protein requirement than land animals (Lovell 1989).

The gross dietary protein requirement is directly influenced by the amino acid composition of the diet. Ten amino acids have been found to be essential for all fish studied to date, and lysine is of particular concern because it is the essential amino acid found in the highest concentration in the carcass of many species of fish (Wilson & Cowey 1985, Wilson & Poe 1985, Kim & Lall, 2000). Also, lysine is the most limiting amino acid in plant protein meal such as cereal grains, which are important feedstuffs locally available for formulating fish diets (Robinson, Wilson & Poe 1980, Forster & Ogata 1998, Small & Soares 2000, Murillo-Gurrea, Coloso, Borlongan & Serrano 2001, Tantikitti & Chimsung 2001). Amino alkanoic acid profiles of the diets generally reflect the rise in protein levels in fish body tissues of the tested diets. (Kari, Z. A.,et al.,2022)

Precise estimations of the lysine requirement are needed for evaluating the quality of the dietary proteins supplied. This is tremendously important for aquaculture where fish meal, which makes up the bulk of the dietary protein supply, has and needs to be replaced by plant proteins which often show deficiencies in some indispensable amino acids (IAA) (Hardy & Barrows 2002, Wilson 2002).

Lysine is considered to be indispensable to animals due to its inability to undergo transamination reactions. To be biologically available, the ε-amino group of lysine must be free and reactive, otherwise the utilization of lysine will be reduced (de Man 1990).

An indispensable amino acid deficiency may cause reduced growth and poor feed conversion (Wilson & Halver 1986). In severe cases, deficiency reduces the ability to resist diseases and lowers the effectiveness of the immune response mechanism.

Thus, establishment of the dietary lysine requirement is essential for developing lysine balanced diet of hybrid Clarias. Immunological responses and antioxidant status of C. gariepinus were not affected when they consumed a diet with FM (fish meal) replaced by up to 50% with PP (soybean and sunflower meal) with methionine and lysine supplementation, but total globulin, NO (nitric oxide), and cumulative mortality were impaired with a diet containing a 100% FM replacement.(Reda, R. M., et al.,2021)

The complete 10 essential amino acid requirements have been worked out only for a limited number of cultured fish species such as Chinook salmon, channel catfish, Japanese eel (NRC, 1993), coho salmon (Arai & Ogata 1993), chum salmon (Akiyama& Arai 1993), common carp (Nose 1979), Nile tilapia (Santiago & Lovell 1988), Indian major carp, catla (Ravi & Devaraj 1991), and milkfish (Borlongan & Coloso 1993).

10 EAA requirements (expressed as %crude protein, CP) of the fish were: arginine (Arg) 5.0 (±0.14), histidine (His) 2.0 (±0.11), iso-leucine (Ile) 3.3 (±0.16), leucine (Leu) 4.9 (±0.24), valine (Val) 3.8 (±0.11), lysine (Lys) 5.2(±0.12), sulfur amino acids (Met + Cys) 3.5 (±0.18), total aromatic amino acids (Phe + Tyr)6.2 (±0.12), threonine (Thr) 3.5 (±0.18) and tryptophan (Trp) 0.9 (±0.08). (Xing, S.,et al., (2024).

Dietary lysine requirements have been estimated for several species of fish, including African catfish (Fagbenro, Balogan, Nwanna, Fasakin & Bello-Olusoji 1998), Atlantic salmon (Anderson Lall, Anderson, & McNiven 1993; Berge 1999; Berge et al. 2002; Abboudi, Mambrini, Ooghe, Larondelle & Rollin 2006), Asian sea bass (Gurrea M., Coloso R.M., Borlongan I. G. & Serrano 2001), blue tilapia (Liou 1989), catla (Ravi & Devaraj 1991), channel catfish (Wilson, Harding, & Garling 1977; Robinson et al. 1980), chinook salmon (Halver, Delong, D.C. & Mertz 1958), chum salmon (Akiyama & Arai 1993), coho salmon (Arai & Ogata 1993), common carp (Nose 1979; Ogino 1980), European sea bass (Tibaldi & Lanari 1991), gilthead

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seabream (Marcouli, Alexis, Andriopoulou & Iliopoulou-Georgudaki 2006), grass carp (Wang et al 2005), grouper (Luo, Liu , Mai, Tian, Tan, Yang, Liang & Liu 2006), hybrid striped bass (Griffin, Brown & Grant 1992; Keembiyehetty & Gatlin 1992), Japanese eel (NRC 1993), Japanese flounder (Forster & Ogata 1998), Japanese seabass (Mai, Wan, Ai, Xu, Liufu, Zhang, Zhang, & Li, 2006), milkfish (Borlongan & Benitez 1990), Mozambique tilapia (Jackson & Capper 1982), Nile tilapia (Santiago & Lovell 1988; Furuya et al 2006), rainbow trout (Kim, Kayes & Amundson 1992; Walton Cowey & Adron 1984; Pfeffer, Al-Sabty, & Haverkamp 1992; Ogino 1980; Ketola 1983; Rodehutscord 2000; Rodehutscord, Borchet, Gregus, Pack, & Pfeffer 2000), red drum (Craig & Gatlin 1992; Brown, Neill & Robinson 1988; Moon & Gatlin 1991), red sea drum (Forster & Ogata 1998), rohu (Khan & Jafri 1993; Murthy &Varghese 1997), sea bass (Tibaldi & Lanari 1991), South African abalone (Shipton et al. 2002), striped bass (Small & Soares 2000), tilapia (Wu et al. 2000), white sturgeon (Ng and Hung 1995) and yellow tail (Ruchimat et al. 1997).

Among culturable finfish species, catfish culture contributes a lot to this practice. Catfishes belonging to Ictaluridae, Claridae, Pangasidae and Siluridae families are widely distributed in different parts of the world, and their culture has been a traditional practice in some parts of South Asia. Their hardy nature and ability to remain alive out of water for long periods have been of special value in tropical countries and there is a specialized trade in 'live fish' in eastern India as well. One of the important culturable catfish species is hybrid Clarias (Clarias gariepinus x Clarias macrocephalus). The ability to adapt wide variations in salinity with the low oxygen content and to grow under generally poor environmental conditions makes this species extremely valuable for culture.

Since, no published information is available on dietary lysine requirement of hybrid Clarias (Clarias gariepinus x Clarias macrocephalus), present study was, therefore, undertaken to determine the dietary lysine requirement of this fish.

Experimental diet

Six isonitrogenous (40% CP) and isocaloric (4.28 kcal/g, GE) amino acid test diets containing graded levels of lysine were formulated using casein, gelatin and L-crystalline amino acid premix (Table 1). The dietary protein level was fixed at 40% CP, reported optimum (Hashim, Ali & Matsaat 1992) for the growth of hybrid catfish *(Clarias gariepinus x C.macrocephalus)*. L-crystalline amino acid premix in the test diets simulated the amino acid profile to that of 40% whole chicken egg protein, excluding the test amino acid lysine. The levels of lysine were in increment of 0.25g/100g of dry diet. A casein-gelatin ratio contributing the minimum quantity of the lysine and maximum quantities of other amino acids was maintained. The quantity of lysine was increased at the expense of glycine so as to make the diets isonitrogenous. The diets were made isoenergetic by adding α-Cellulose. The dietary levels of lysine in the amino acid test diets were fixed on the basis of information available for channel catfish, *Ictalurus punctatus* (NRC 1993).Pre-weighed quantities of L-crystalline amino acids and salt mixture (Halver 2002) were thoroughly stirred in hot water (80 ^{o}C) in a steel bowl attached to a Hobart electric mixer. . The pH of the resulting mixture was adjusted to neutral with 6N NaOH solution (Nose, Arai, Lee & Hashimoto 1974). Gelatin powder was dissolved separately in a volume of water with constant heating and stirring and then transferred to the above mixture. The mixer bowl was removed from heating and dextrin was added. Other dry ingredients and oil premix, except carboxymethyl cellulose, were added to the lukewarm bowl (40 ^{o}C) one by one with constant mixing. Carboxymethyl cellulose was added last and the speed of the blender was gradually increased as the diet started to harden. The final diet, with the consistency of bread dough, was poured into a teflon-coated pan and placed into

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refrigerator to gel. The prepared diet was in the form of moist cake (50% dry matter) from which cubes were cut and stored at -20 ^{o}C in sealed polythene bags until used.

Experimental design and Feeding trial

Fingerlings of hybrid *Clarias (Clarias gariepinus x Clarias macrocephalus*) produced by induced bred fingerlings were procured from the Kolkata fish hatchery. The fingerlings were transported to the wet laboratory in oxygen filled polythene bags, given the prophylactic dip in $KMnO_4$ solution (1:3000), and stocked in circular aluminum plastic lining (Plastic Crafts Corpn., Mumbai, India, 4ft x 3ft x 3ft) fish tank (water volume 600L) for a fortnight. During this period, the fish were fed to satiation a mixture of fish meal, soybean meal, mustard oil cake, rice bran and wheat bran in the form of moist cake twice a day at 0900 and 1600 hours. These fish were then acclimatized for one week with a casein-gelatin based (400 g Kg^{-1} CP) H-440 diet (Halver 2002).

Fingerling (4.33±0.04 cm; 0.770±0.32g) hybrid *Clarias (C. gariepinus x C. macrocephalus*) were then randomly stocked in triplicate groups in 70 L circular polyvinyl troughs (water volume 55L) fitted with continuous water flow-through (1-1.5L/min) system at the rate of 10 fish per trough for each dietary treatment level. The fish were fed diet in the form of moist cake at a rate of 8% of their body weight (divided in two equal halves) per day at 0900 and 1600 h. No feed was offered to the fish on the day when weekly measurements were taken. Initial and weekly weights were recorded on a top loading balance (Precisa 120A) and feed allowances adjusted accordingly. The feeding trial was conducted for four weeks. Fecal matter and unconsumed feed, if any, were siphoned off before feeding. The average water temperature, dissolved oxygen, free carbondioxide, pH and total alkalinity over the four weeks feeding trial, based on daily measurements, were 26-28 ^{0} C, 6.6-7.5 ppm, 5.5-10 mg/l, 7.2-7.8 and 60-80mg/l, respectively. These values were noted following the standard methods (APHA 1992).

Chemical analysis

Proximate composition of casein, gelatin, experimental diets, and initial and final carcass was estimated using standard (AOAC 1995) methods for dry matter (oven drying at 105±1 ^{0}C for 24 h), crude protein (N-Kjeldhal x 6.25), crude fat (solvent extraction with petroleum ether B.P. 40-60 ^{0}C for 12-14 h) and ash (oven incineration at 600 ^{0}C for 2-4 h). Gross energy content was determined on a Gallenkamp ballistic bomb calorimeter (Loughborough, U.K.). Desired number of fish were randomly sacrificed before the commencement of the feeding trial and pooled sample in triplicate were taken for determining the initial carcass composition. All 10 fishes were sacrificed for whole body composition analysis. Amino acid analysis of casein and gelatin was made with the help of a Beckman System Gold HPLC unit, as detailed earlier (Ahmed & Khan 2004). The carcass protein deposition values for the fish fed graded levels of lysine were calculated by using following method:

$$\text{Protein deposition} = \frac{(BW_f \times BCP_f) - (BW_i \times BCP_i)}{TF \times CP} \text{ x100}$$

BW_f & BWi = mean final and initial body weight

BCP_f & BCPi = mean final and initial percentage of carcass protein

TF = Total amount of diet consumed

CP = Percentage of crude protein in diet

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Statistical analysis

Responses of fingerling hybrid *Clarias* (*C. gariepinus x C. macrocephalus*) fed graded levels of test amino acids were measured by live weight gain (%), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and by analyzing the carcass composition. These response variables were subjected to one-way analysis of variance (ANOVA) (Snedecor & Cochran 1967; Sokal & Rohlf 1981). To determine significant differences ($P<0.05$) among the treatment means, Duncan's Multiple Range Test (Duncan 1955) was employed. The break-point for optimum dietary requirement for lysine was estimated using quadratic regression analysis (Zeitoun, Ullrey, Magee, Gill & Bergen 1976).

RESULTS

Significant differences in live weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and body protein deposition (BPD) values of hybrid *Clarias (Clarias garipinus x Clarias macrocephalus*) fed diets containing graded levels of lysine were recorded (Table 2). Maximum weight gain (536%), SGR (6.60), PER (1.49%), FCR (1.68) and BPD (21.2) were evident in fish fed diet containing 2.5% of lysine (Diet IV). Poor growth and conversion efficiencies in fish fed diets containing 1.75% (Diet I), 2.0% (Diet II) and 2.25% (Diet III) lysine were evident. Weight gain and conversion efficiencies of fish improved significantly ($P<0.05$) as dietary lysine levels increased from 1.75 to 2.50% of the dry diet whereas at higher levels a significant fall in growth parameters was registered.

On subjecting the live weight gain data to quadratic regression analysis (Zeitoun et al. 1976), a break-point was evident at 2.64% lysine of the dry diet, corresponding to 6.6% of the dietary protein (Fig. 1). The mathematical relationship being;

$$Y=911.688\ X^2 + 4261.553\ X - 4460.08 \quad (r=0.878,\ P<0.05)$$

The FCR in fish fed 2.5% of lysine differed significantly ($P<0.05$) from the other dietary inclusion. The FCR (Y) to dietary levels of lysine (X) relationship was estimated by the following second-degree polynomial regression equation. The relationship being;

$$Y= 1.394\ X^2 - 7.005\ X + 10.581 \quad (r = 0.970,\ P<0.05)$$

Based on the above equation, the best FCR occurred at 2.51% of dietary lysine, corresponding to 6.27% of dietary protein (Fig. 2).

Similarly, PER of the fingerling fed 2.50% lysine diet differed significantly from the other levels of lysine inclusion. The PER (Y) to dietary concentrations of lysine (X) relationship was estimated by the following second-degree polynomial regression equation:

$$Y=-0.9000X^2+4.5081X-4.2362\ (r=0.959)$$

Based on the above equation, the estimated PER occurred at a dietary lysine concentration of approximately 2.67 % of dry diet, corresponding to 6.68 % of dietary protein (Fig 3).

The SGR of fingerling fed 2.50% lysine diet differed significantly from the other levels of lysine inclusion. The SGR (Y) to dietary concentrations of lysine (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=-2.3549X^2+12.4672X-10.54\ (r=0.950)$$

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Based on the equation, the estimated SGR occurred at a dietary lysine concentration of approximately 2.64% of dry diet, corresponding to 6.6% of dietary protein

On the basis of above polynomial equations, the maximum live weight gain percent, best FCR, PER and highest occurred at lysine levels of approximately 2.64, 2.51, 2.67, and 2.64 %of dry diet, corresponding to 6.6, 6.27, 6.68 and 6.6 % of dietary protein, respectively.

Body composition of fish fed diets containing graded levels of lysine is given in Table 3. Body protein and body protein deposition values were found to be significantly ($P<0.05$) higher in fish fed diet containing 2.5% lysine (Diet IV) compared to other dietary treatments. Significantly ($P<0.05$) lower body fat values were recorded in fish fed Diet I, V and VI containing 1.75, 2.75 and 3.0% of dietary lysine compared to that fed Diet II, III and IV with 2.0, 2.25, and 2.5% of lysine where significantly higher body fat content were noted. However, no significant ($P>0.05$) differences in body fat content were evident among fish fed Diet II, III and IV. Minimum body moisture was recorded in fish fed diet containing 2.5% of lysine which is significantly ($P<0.05$) different from those fed diets with 2.0, 2.75 and 3.0% lysine. Ash content was found to be decrease significantly with increasing dietary lysine concentration upto 2.5% of the diet (Diet IV)beyond which a significant increase in ash content was evident (Table 3).

DISCUSSION

All fish species studied so far require the same 10 indispensable amino acids in their diets for maximum growth (Wilson 1985). If any of the essential amino acid is not present in sufficient amount or present in excessive amounts relative to other amino acid, proteins synthesis will not be supported. Under these circumstances, labile body proteins will be catabolized to provide the amino acids so that the protein synthesis may continue (Abidi & Khan 2007). A basic principle of amino acid nutrition is the constant relationship of the amino acid requirements with protein intake up to the level of maximum growth.

This principle is the basis for expressing the requirement data as the percentage of protein (NRC 1983). Inclusion of an optimum amount of lysine is a prerequisite to the formulation of nutritionally adequate cost-effective diets for fish culture. Since lysine is the limiting amino acid in most of the ingredients used for making fish diets, inclusion of its optimum amount is a prerequisite to the formulation of nutritionally adequate cost-effective diets for fish culture. This fish registered significantly high weight gain when fed on a diet with 2.5% of lysine. Nutrient rich protein that is protein bound methionine and lysine composition. After feeding body weight gain 5% comparatively initial weight of catfish (fingerling). (Hamid, S. N. I. N., & Abdullah, R. et., al. 2017).

Best FCR, PER and SGR were also noted at the above dietary lysine level. This value is higher than the value reported for African catfish 5.7% (Fagbenro et al. 1998), Atlantic salmon 4% (Anderson *et al*. 1993); 3.2-3.6% (Berge 1999); 6.1% (Rollin 1999), catla 6.2% (Ravi and Devaraj 1991), channel catfish 5.1% (Wilson *et al.* 1977); 5% (Robinson et al.1980), chinook salmon 5% (Halver et al. 1958), chum salmon 5% (Akiyama and Arai 1993), *Clarias* hybrid 4.8% (Unprasert 1994), coho salmon 3.8% (Arai and Ogata1993), common carp 5.7% (Nose 1979) 5.3% (Ogino 1980), gilthead seabream 5.04% (Marcouli et al 2006), grass carp 5.89% (Wang et al 2005), grouper 5.56% (Luo et al 2006), hybrid striped bass 4% (Griffin et al. 1992); 4% (Keembiyehethy and Gatlin 1992), Japanese flounder 4.6% (Forster and Ogata 1998), Japanese seabass 5.8% (Mai et al. 2006), , milkfish 4% (Borlongan and Benitez 1990), Mozambique tilapia 4.1% (Jackson and Capper 1982), Nile tilapia 5.1% (Santiago and Lovell 1988) 5.23% (Furuya et al 2006), rainbow trout 3.7% (Kim et al. 1992); 4.2% (Walton et al. 1984); 4.2% (Pfeffer et al. 1992); 5.3% (Ogino 1980); 6.1% (Ketola 1983), rohu

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5.9% (Khan and Jafri 1993); 5.7% (Murthy and Varghese 1997), red drum 4.4% (Craig and Gatlin 1992); 5.7% (Brown et al. 1998) and striped bass 4.9% (Small and Soares 2000). Supplementation of lysine-deficient diets with lysine improved weight gain in channel catfish (Robinson et al., 1980; Robinson, 1991; Robinson and Li, 1994; Zarate and Lovell, 1997) and common carp (Viola and Lahav, 1991) raised in ponds or aquaria.

Control diet (CD) without amino acid supplementation or SPB inclusion contained 40% of fish meal (FM) and 20% of soybean meal (SBM), four isonitrogenous and isocaloric diets formulated to represent two levels of SPB (50% and 75%) with two levels of L-lysine supplementation (0.0 and 1.0%), SPB50%0.0, SPB50%1.0, SPB75%0.0 and SPB75%1.0, where the subscripts and superscripts refer to the replacement level of FM with SPB and L-lysine supplementation level respectively.(El-Husseiny., et., al 2018)

The quantitative lysine requirements of several fish species appear to vary according to many aspects. Kim et al. (1992) pointed out that large variation in the values for lysine requirements may be due to differences in the composition of basal diet used for different experiments and also suggested that the wide variability and the reliability of lysine requirements of fish may be affected by fish size and age, feeding regime, feed allowance, adequate levels of other nutrients, water temperature, flow rate, stock density, and environmental as well as other culture conditions adopted in different laboratories. Digestibility, amino acid profile and energy content may also bring about variable effects in amino acid requirement studies (Simmons, Moccia, Bureau, Sivak & Herbert 1999; De Silva, Gunasekera & Gooley 2000). Because of different levels of dietary proteins used by several workers in amino acid test diets, the results of amino acid requirement studies of fish have been reported as a percentage of dietary protein. Increasing lysine in the diet improved the growth performance and feed utilization upto requirement level, which confirmed results from other reports (Walton et al. 1984; Kim et al. 1992; Pfeffer et al. 1992; Rodehutscord et al. 1997; Berge et al. 1998; Rodehutscord et al. 2000a,b; Murillo-Gurrea et al. 2001). However, further increase in the dietary lysine level did not result in additional improvement in growth and feed conversion ratio as shown in other studies (Walton et al. 1984, Lab ref----------). Higher concentrations of dietary lysine were reported to cause depressed growth and lower efficiency of feed utilization in salmonids (Chiu et al. 1987; Anderson et al. 1992), Indian major carp (Murthy & Varghese 1997) and mrigal (Ahmed & Khan 2004). In the present study feeding lysine beyond requirement resulted in depressed growth. This reduction in growth fed higher concentration of lysine (Diet VI) was similar to results reported by other workers (Ahmed & Khan 2004, Walton 1985; Choo et al. 1991; Murthy and Varghese, 1997). As evident in the growth curve, no clear sign of antagonism between lysine and arginine was observed in the present study beyond the dietary lysine requirement level (Diet IV). However, reduction in growth of fish fed still higher amount of lysine (diet VI) may be due to the negative effects (lysine–arginine interaction) of excessive amount of free lysine at this level (Ahmed & Khan 2004). An quantity of dietary lysine increase with protein concentration have shown in improved fish growth.(Langi, S.,et.,al 2024)

Survival of hybrid *Clarias (C. gariepinus x C macrocephalus*) in present study was found to be 100%. Ketola (1983) observed a very high rate of mortality and incidence of caudal fin erosion in rainbow trout fed with lysine deficient diet. Lysine deficiency caused low feed utilization efficiency and depressed growth, as shown in juvenile grouper (Luo et al 2006), milkfish (Borlongan & Benitez 1990; Borlongan & Coloso 1993), Atlantic salmon (Anderson et al. 1992), rainbow trout (Kim et al. 1992), rohu (Khan & Jafri 1993) and C. mrigala (Ahmed & Khan 2004). However, neither mortality nor such pathological symptoms were evident in present study on hybrid *Clarias* (*C. gariepinus x C. macrocephalus*).

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Dietary lysine requirement of hybrid *Clarias* (*C. gariepinus x C. macrocephalus*) was worked out to be 2.51% of the dry diet, corresponding to 6.27% of protein. Data generated during the present study would be useful in developing lysine balanced diets for the intensive culture of this fish.

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