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Dietary Lysine Requirements and Gustation of Hybrid Clariid Catfish (*Heterobranchus bidorsalis* ♂ x *Clarias gariepinus* ♀) (“Heteroclarias”)

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ABSTRACT

The study aims to evaluate the effect of supplementation of Lysine on growth, feed utilization, and preference response in hybrids catfishes (*Heterobranchus bidorsalis* ♂ x *Clarias gariepinus* ♀). Five diets isonitrogenous with 40% CP were formulated with different levels of crystalline L-lysine (0.00%, 0.74%, 1.04%, 1.34%, and 1.64%), whereas the level of methionine was constant at 0.30 g/100 g for all diets and referred as D1-D5 diets respectively. A total of 300 juveniles of heteroclarias fish, weighing 7.60 ± 0.20 g, were randomly allocated to 15 plastic aquaria ($70 \times 45 \times 45$ cm³) with 10 fishes per tank. The fish was fed at 5% of the body weight for 90 days. The findings revealed significant differences ($P < 0.05$) in growth performance parameters including weight gain, specific growth rate, survival rate, protein efficiency ratio, feed conversion ratio, and gustation between fish fed the experimental diets and those on the control diet. Diet D3 recorded the best performance, with the most efficient feed conversion ratio (1.03 ± 0.10) and the highest specific growth rate (0.76 ± 0.10). Feed intake also differed significantly ($P < 0.05$) among treatments, with the highest value observed in D4 (67.68 ± 1.88). Furthermore, carcass protein and lipid contents increased as dietary L-lysine levels increased, while fibre content decreased with higher lysine inclusion. Gustation responses also varied significantly ($P < 0.05$), with the shortest grasping time (0.75 seconds) recorded in D3 (20% CP + 1.04 g/100 g lysine). Thus, the result suggested that diet supplemented with lysine at 20%CP+1.04g/100g (D3) inclusion level optimally enhance the growth performance, nutrient utilization and gustation of heteroclarias fingerlings.

Keywords: Growth response; nutrient utilization; Lysine supplement; Gustation; isonitrogenous.

Introduction

Proteins are complex macromolecules made up of carbon, hydrogen, oxygen, nitrogen, and occasionally sulfur. Unlike fats and carbohydrates, proteins are distinguished by their nitrogen content and serve as fundamental components of tissues and organs. They are

present in all living cells and constitute a major part of fish body structure. In aquaculture, fish require dietary protein to supply essential amino acids needed for maintaining existing tissues and supporting the development of new ones for growth and reproduction. If protein supply is inadequate, development can be hampered, and fish may experience a reduction in weight due to protein being used up by less essential organs to maintain physiological processes (Martinez

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et al., 2017). As reported by the Food and Agriculture Organization FAO in (2020), protein is crucial for growth, repair, and maintenance because no other nutrient can serve as an alternative to its function in body cell formation. Proteins are involved in muscle contraction and are essential components of enzymes, hormones, and antibodies. Proteins can react with other substances like hemoglobin, carbohydrates, fats, and nucleic acids. Protein is the second-largest class of chemical compounds in the animal kingdom after water (NRC, 2011).

Fish need ten essential amino acids (EAAs) for proper growth and survival, and these are used in protein construction (Cao et al., 2012). The amino acids cannot be produced by fish; hence they must be supplied through the diets (Wilson, 2003). Lysine is a limiting amino acid in fish feed, and this is necessary for normal growth and physiological development of fish. Lysine is also involved in the formation of carnitine, which is found in almost all cells of organisms (Khan, 2011). Lysine becomes necessary in cases where the protein content of the feed is from plants instead of fishmeal. According to Ahmed and Khan (2004), the skeletons of fish are rich in lysine. Fish feeding behaviors are complicated; however, the nutrient content and digestibility of the feed are crucial variables influencing the feed's nutritional value (Bureau et al., 2002). Lysine plays an important role in various physiological processes such as muscle growth, strong bones, repair of injured tissues, hormone regulation, enzymes, antibodies, and possibly antiviral actions (Cao et al., 2012). The heteroclaris is a fish that is produced through the mating of *Clarias gariepinus* and *Heterobranchius bidorsalis*, which possess all the desirable qualities of each parent fish (Bartley et al., 2000). The heteroclaris is highly sought after in aquaculture because of its disease and environmental resistance, high survival rate, and fast growth rate (Okeke, 2014). The heteroclaris feed on fish and

insects and are omnivorous fish. Their diet and feeding behavior can be influenced by their body size, age, and season. This is true for most fish species regardless of whether they are located in tropical or temperate zones. Hybridization is considered one of the best ways of genetically improving the stock of fish in aquaculture (Khaleg, 2000). Due to increasing costs and scarcity of fishmeal, it has become increasingly important to find alternative sources of proteins for fish feed. Most of these sources contain insufficient amounts of essential amino acids such as lysine, which requires supplementation. This study was therefore conducted to assess the effects of lysine supplemented diets on the growth performance, nutrient utilization, and feeding response of heteroclaris fingerlings.

Materials and Methods

Experimental set up

This study was carried out at the Department of Fisheries and Aquaculture Technology Fish Farm, Federal University of Technology Akure, Ondo State Nigeria.

Samples Collection

Feedstuffs which include fishmeal, groundnut cake, yellow corn, vitamin premix, binder were purchased from K₂ Akure and L-lysine and L-methionine in crystalline forms, was also purchased from animal care feeds, Akure.

Experimental Fish

A total of 500 *Heteroclaris* fingerlings (7.50 ± 0.05 g) were obtained from a reputable fish farm in Akure, Ondo State. The fish were maintained in plastic tanks (70 cm × 45 cm × 45 cm) at the research farm of the Department of Fisheries and Aquaculture

Technology, Akure, where they were acclimated and fed a commercial diet containing 35% crude protein for two weeks prior to the feeding trial. To ensure uniformity in weight and clear the digestive tract of residual feed, the fish were deprived of food for 24 hours before the commencement of the experiment.

Preparation of Experimental Diets

Five isonitrogenous experimental diets containing 40% crude protein (D1–D5) were formulated using groundnut cake and fishmeal, with graded levels of crystalline lysine (0.00%, 0.74%, 1.04%, 1.34%, and 1.64% of dry diets). The lysine supplied by groundnut

cake and fishmeal in the basal diet ranged from 0.49% to 2.5% of dietary protein, depending on species requirements. A constant methionine level of 0.3% was also included in all diets (D1 to D5), as presented in Table I. All ingredients were milled using a hammer mill, thoroughly mixed to ensure uniformity, and then pelleted using a Hobart pelleting machine (Model A. 200T, CAUSA) to produce uniform 2.0 mm pellets. The pellets were sun-dried to achieve a moisture content of less than 10% and stored in a cool, dry environment to prevent contamination. Proximate composition analysis of the diets was conducted following AOAC (2010) standard methods.

Table 1: Gross Composition of Experimental Diets (g/100g) Containing Graded Levels of Lysine with constant Methionine Supplements.

Ingredients	Diet1 (D1)	Diet2 (D2)	Diet3(D 3)	Diet4 (D4)	Diet5 (D5)
Fish meal (72%CP)	20.00	20.00	20.00	20.00	20.00
Groundnut cake (48%CP)	50.00	50.00	50.00	50.00	50.00
Yellow maize (10%CP)	18.00	18.00	18.00	18.00	18.00
Fish oil	6.00	6.00	6.00	6.00	6.00
Carboxymethyl cellulose	4.00	2.96	2.66	2.36	2.06
Lysine	0.00	0.74	1.04	1.34	1.64
Methionine	0.30	0.30	0.30	0.30	0.30
Vit/min premix	2.00	2.00	2.00	2.00	2.00
Analyzed Composition of the Experimental diets (per kg on as fed basis) dry matter fed to Heteroclaris.					
Moisture	7.36±0.35 ^a	8.00±0.67 ^{ab}	9.50±0.31 ^c	9.16±1.04 ^{bc}	9.18±0.86 ^{bc}
Ash	12.20±0.26 ^a	12.06±0.96 ^a	11.49±0.47 ^a	11.43±0.39 ^a	11.76±0.76 ^a
Lipid	16.76±0.25 ^a	20.01±2.01 ^{cd}	21.59±0.63 ^d	19.57±0.35 ^{bc}	18.12±0.00 ^{ab}
Protein	39.86±0.10 ^a	40.31±0.02 ^a	40.37±0.02 ^a	40.38±0.04 ^a	40.42±0.30 ^a
Nfe	18.44±0.51 ^a	16.53±2.32 ^b	13.75±0.43 ^d	15.14±0.30 ^c	16.15±0.95 ^b
Fibre	5.38±0.59 ^c	3.09±0.01 ^a	3.30±0.02 ^a	4.32±0.01 ^b	4.37±0.46 ^b

Mean ±S.D. bearing distinct superscript letters within the same row differ significantly (P < 0.05). Premix content as supplied by Dotan feed mill, plot 1 and 2 block J, Abiola layout, Ondo road Akure.

Experimental Tanks and Fish Feeding Trial

Apparently healthy (300) heteroclaris fingerlings with mean body weight (7.50 ± 0.05 g) were randomly distributed into 15 plastic tanks ($65 \times 45 \times 45$)cm³ filled with 60 litres of water. Fish were randomly distributed into tanks at a stocking density of ten fish per tank. Five groups of fish were assigned to each experimental diet, with three replicate per treatment, over a 90-day feeding trial. All fish were fed twice daily, and each group received its designated diet at a consistent rate of 5% of body weight per day. To monitor growth performance and ensure accurate feeding adjustments, the fish were batch-weighed every two weeks. Feeding rates were then modified based on the updated body weights to maintain appropriate dietary intake throughout the experimental period.

Water Quality Monitoring

Dissolved Oxygen Concentration

The dissolved oxygen concentration was monitored every three days at 0800h using Hanna 40SE,

Water Temperature

Water temperature were monitored every three days and measured using Knick portable meter (Model 912) Around 0800h.

Hydrogen Ion Concentration (pH)

Water pH were monitored and measured using Knick portable pH meter (Model 912) around 08:00h.

Evaluation of Growth and Nutrient Retention

Assessment of the growth indices was conducted in accordance with the protocols described by Fasakin *et al.* (2003).

Weight Gain (WG) $WG = W_2 - W_1$;

Where W₂ denotes the terminal fish, mass recorded upon completing the feeding period. and

W₁ = the baseline mass of the fish in each tank measured before the trial commenced.

N is the number of weeks of feeding trials

Mean Weight Gain (g/week) $MWG = \frac{W_2 - W_1}{N}$

Daily weight gain percentage

$$SGR (\%/day) = \frac{100 (\ln W_2 - \ln W_1)}{T (day)}$$

Where the baseline mass and terminal mass of the fish are denoted by W₁ and W₂, respectively, and the experimental timeframe is represented by T. InW₁ and InW₂ were the natural log of weight at the beginning and the end of the feeding trial.

Feeds Conversion Ratio (FCR)

$$FCR = \frac{\text{Feed consume (g)}}{\text{Weight gain by fish (g)}}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{\text{Live body weight gain (g)}}{\text{Protein intake (g)}} \times \text{Survival (\%)} =$$

$$\frac{\text{No. of fish at the end of the experiment}}{\text{No. of fish at the onset of the experimental fish}} \times 100$$

Gustatory Food Evaluation in Fish

Feed pellets were added to the tanks one at a time at intervals of 10–15 minutes. For each treatment, data were collected on several parameters, including the experimental unit, frequency of grasping per fish, whether the pellet was swallowed or rejected, and the time taken to grasp the pellet. If a fish did not attempt to grasp the pellet within two minutes, the trial was stopped and not recorded. However, when the pellet was successfully grasped and swallowed, the recorded duration was taken as the time of grasping. This procedure was done as described by Sveinsson and Hara (1990) with modifications from Gbadamosi and Salako (2015) for the fish reared in the recirculatory system. The present study was done to strictly determine the gustatory range of *Heteroclarias* in other to know the palatability and acceptability of formulated diets supplemented with graded levels of lysine with fixed Methionine level of (0.30g/100g). Result obtained from these practices were represented in bar chart as shown in Figure 1.

Analytical procedure

Proximate analyses of the diets and fish were performed according to the procedure of the AOAC (2000), at

the Centre laboratory of the Federal University of Technology, Akure.

Statistical Evaluation

The Complete Randomized Design (CRD) was employed to structure the experiment and analyzed the resulting data via Analysis of Variance (ANOVA). Differences among treatment means were considered significant at $p < 0.05$ using SPSS version 25.0, particularly for growth performance and nutrient utilization across the various treatments. Where significant differences were detected, mean separation was performed using Duncan’s Multiple Range Test and Tukey’s test.

Results

Water Quality Parameters

The water quality parameters in tanks are presented in Table 2. There were no significant differences ($P>0.05$) in temperature; dissolved oxygen; ranged from 5.70 ± 0.05 mg/l to 5.94 ± 0.05 , and the hydrogen ion concentration (pH); has it lowest value of 6.12 ± 0.05 in control treatment D1 and highest values 7.18 ± 0.05 in treatment D2, The values were within the recommended ranges for *C. gariepinus* as recommended by Omitoyin, (2000); Ajani *et al.*, 2015)

Table 2: Physicochemical Parameters of the Experimental Unit

Parameters	Diet1 (D1)	Diet2 (D2)	Diet3 (D3)	Diet4 (D4)	Diet5 (D5)
Temperature (°C)	26.70±0.12 ^a	26.65±0.10 ^a	26.58±0.09 ^a	26.60±0.09 ^a	26.65±0.07 ^a
DO ₂ (mg/l)	5.80±0.06 ^a	5.80±0.13 ^a	5.80±0.11 ^a	5.80±0.08 ^a	5.90±0.12 ^a
Conductivity(S/m)	20.65±0.11 ^a	20.65.09 ^a	20.65±0.12 ^a	20.65±0.08 ^a	20.65±0.10 ^a
pH	6.12±0.06 ^a	6.18±0.10 ^a	6.15±0.08 ^a	6.15±0.13 ^a	6.16±0.05 ^a

Mean ±S.D. bearing distinct superscript letters within the same row differ significantly ($P < 0.05$).

Growth Performance and Nutrient Utilization of Heteroclarias Fed Supplemented Diet Containing Varied Levels of L-Lysine with Constant Methionine (0.30g/100g)

The results presented in Table 3 indicate that final weight, weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio of fish fed diets containing varying levels of lysine and a constant level of methionine differed significantly ($p < 0.05$) after the 90-day feeding trial. These performance indices were influenced by the dietary lysine levels.

Overall, the treatment groups (T2–T5) showed better results compared to the control group (T1). The highest weight gain (72.97 ± 10.63) was recorded in T3, followed by T5 (62.10 ± 9.90), while the lowest value (36.08 ± 7.14) was observed in the control (T1). Improvements were also observed in specific growth rate (SGR), feed efficiency ratio (FER), and protein efficiency ratio (PER), with peak values of 0.76 ± 0.10 , 1.08 ± 0.12 , and 1.75 ± 0.26 , respectively. In addition, feed conversion ratio varied significantly, with the best (lowest) value recorded in T3 (1.03 ± 0.10).

Table 3: Growth Performance and Nutrient Utilization of Heteroclarias fed Lysine Supplemented Diets with Constant Methionine (0.30g/100g)

Parameters	Diet1 (D1)	Diet2 (D2)	Diet3 (D3)	Diet4 (D4)	Diet5 (D5)
IW (g)	73.55±2.45 ^a	73.56±0.88 ^a	73.64±1.89 ^a	73.67±1.39 ^a	73.68±1.60 ^a
FW (g)	109.63±5.21 ^a	128.44±2.72 ^{ab}	146.71±8.81 ^b	131.27±24.10 ^{ab}	136.35±8.42 ^b
WG(g)	36.08±7.14 ^a	55.02±2.16 ^{ab}	72.97±10.63 ^b	57.57±22.98 ^{ab}	62.10±9.90 ^{ab}
SGR (g/day)	0.48±0.08 ^a	0.62±0.02 ^a	0.76±0.10 ^{ab}	0.64±0.22 ^a	0.68±0.09 ^a
FI(g)	65.37±0.71 ^a	65.77±1.86 ^a	67.28±2.71 ^a	67.68±1.88 ^a	66.65±1.46 ^b
FCR	1.75±0.29 ^b	1.19±0.04 ^{ab}	1.03±0.10 ^a	1.34±0.54 ^{ab}	1.21±0.21 ^{ab}
FER	0.58±0.11 ^a	0.84±0.03 ^{ab}	1.08±0.12 ^b	0.82±0.31 ^{ab}	0.84±0.16 ^{ab}
PI (g)	27.01±0.49 ^a	27.37±0.76 ^a	28.04±1.14 ^a	28.25±2.02 ^a	30.59±0.54 ^b
PER	0.95±0.18 ^a	1.33±0.06 ^{ab}	1.75±0.26 ^b	1.36±0.55 ^{ab}	1.45±0.23 ^{ab}
SVP (%)	100.00±0.00 ^a	96.67±5.77 ^a	100.00±0.00 ^a	93.33±5.77 ^a	96.67±5.77 ^a

Mean ±S.D. bearing distinct superscript letters within the same row differ significantly ($P < 0.05$).

Carcass Profile of Fish Heteroclarias Both Initial and Final Fed Experimental Diets Containing Graded Levels of Lysine with Fixed Methionine Level (0.30g/100g);

Table 4 outlines the proximate profile of Heteroclarias, where statistical analysis revealed distinct variations ($P < 0.05$) among the treatment means. Fluctuations in moisture content were notably dependent on the specific lysine levels supplied in the feed, but a constant methionine level, with the highest value recorded in

T1 (8.89 ± 0.13) and the lowest in T2 (7.52 ± 0.47). Ash content also differed significantly ($p < 0.05$), with T1 having the highest value (17.40 ± 0.47) while T5 recorded the lowest (10.05 ± 0.54). Similarly, crude lipid content marked distinctions were detected ($p < 0.05$), with the highest level observed in T1 (13.48 ± 0.44) and the lowest in T5 (10.61 ± 0.12). Crude protein content marked distinctions were detected ($p < 0.05$), starting at 54.30 ± 0.48 in T1 and rising to 67.41 ± 0.51 in T5. Generally, the trend on crude protein showed that the protein levels appreciate from treatment 1 to 5.

Thus, treatment 5 had the highest crude protein level of 1.64g/100g feed yielded the highest crude protein in *Heteroclarias* carcass. Thus, Lysine inclusion levels level in the *Heteroclarias* carcass.

Table 4: Proximate Composition of (*Heteroclarias*) fed graded levels of lysine with fixed Methionine (0.30g/100g dry weight)

Parameter	Initial	Diet1 (D1)	Diet2 (D2)	Diet3 (D3)	Diet4 (D4)	Diet5 (D5)
MOISTURE	3.45±0.13 ^a	8.80±0.13 ^c	7.52±0.47 ^b	8.03±0.08 ^b	8.14±0.24 ^b	7.88±0.07 ^{bc}
ASH	10.05±0.54 ^a	17.40±0.41 ^d	15.28±0.37 ^c	14.70±0.45 ^c	13.10±0.30 ^b	11.39±0.36 ^{ab}
LIPID	20.02±0.85 ^d	13.48±0.44 ^c	12.21±0.19 ^b	12.13±0.21 ^b	12.14±0.24 ^b	10.61±0.27 ^a
PROTEIN	53.86±0.63 ^a	54.30±0.48 ^{ab}	60.36±0.16 ^c	69.27±0.52 ^b	61.71±0.84 ^d	67.41±0.51 ^c
NFE	12.62±0.05 ^d	4.85±0.11 ^{bc}	4.33±0.41 ^b	5.87±1.03 ^c	4.91±1.19 ^{bc}	2.74±0.60 ^a

Mean ±S.D. bearing distinct superscript letters within the same row differ significantly (P < 0.05).

Gustation of *Heteroclarias* Fed Graded Levels of Lysine with Fixed Methionine (0.30g/100g)

The apparent gustation coefficients represented in bar chart Figure 1: shows, time of grasp and treatment

tanks of *Heteroclarias* fed Lysine supplemented diet, was plot against each other’s, the best result was obtained in D3 with the lowest time of grasp (0.75) sec, marked distinctions were detected (p < 0.05), compared with the remaining diets.

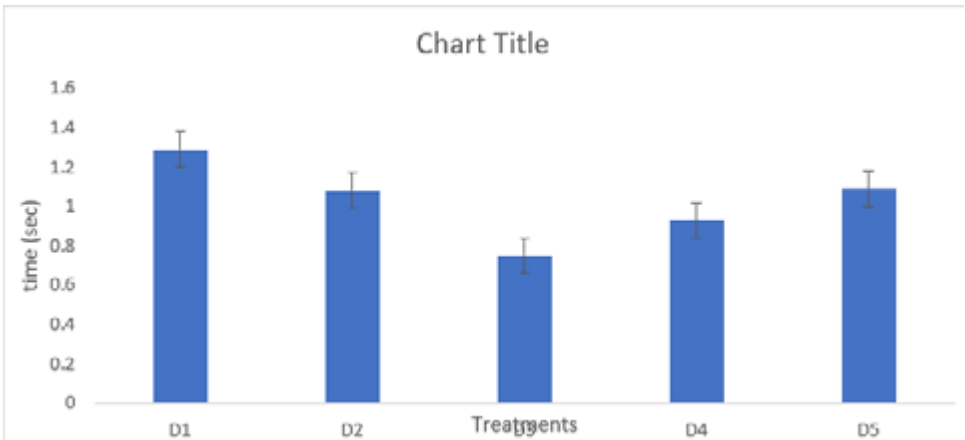


Figure 1: Gustation in fish fed lysine supplemented diet with fixed methionine level (0.30g/100g)

Discussion

Discussion of the Water Quality

All water parameters (temperature, Dissolved oxygen, pH and conductivity) in all cultured tanks were not

significantly different. There was no adverse effect of the water quality parameters of the cultured tanks. All physicochemical water parameters remained within acceptable limits as reported by Omitoyin, (2000); Ajani *et al.*, 2015) for Clariid fishes.

Nutritional Profile of the Formulated Feeds

The proximate analysis of the formulated diets revealed no significant difference in crude protein content; however, significant variations were observed in lipid, ash, crude fibre, and nitrogen-free extract (NFE) levels. Overall, the lysine-supplemented diets showed higher values of lipid, ash, crude fibre, and NFE compared to the control diet. These findings showed that lysine supplemented diet can enhance the nutritional quality of practical diet when properly formulated as stated by Faturoti and Ajiboye, 2011 and Rachmawati *et al.*, (2022). The overall nutrient profile of the diets indicated that lysine is a promising essential amino acid capable of meeting the nutritional requirements of *Heteroclaris* fingerlings.

Evaluation of Growth and Nutrient Retention

In the current investigation, Supplementing lysine allows for the reduction of expensive, high-quality, animal-based proteins (fishmeal) up to (20%) in favor of cheaper alternative plant proteins without significantly reducing the growth of the fish, Lysine supplementation enhances the fish's ability to retain nutrients, increases muscle deposition, and helps in the reduction of nitrogenous waste in the water. Biomass accumulation and development velocity are typically regarded as the primary indicators of production efficiency (Bilal *et al.*, 2020). The study indicated that *Heteroclaris* exhibited positive responses to all lysine-supplemented diets, as shown by improved growth and nutrient utilization outcomes. Growth performance measures, including mean final weight (MFW), mean weight gain (MWG), specific growth rate (SGR), and feed intake (FI), were higher in fish fed lysine-enriched diets than in those on the control diet (0.00 g/100 g). Among the treatments, fish fed

1.04 g/100 g lysine (D3) demonstrated the best overall performance in MFW, MWG, SGR, and FI. These results agree with previous studies (Ng *et al.*, 2000; Marimuthu *et al.*, 2011; Andem, 2013; Ashley-Dejo *et al.*, 2014; Odedeyi and Ademeso, 2015), which reported improved growth in *Clarias gariepinus* as dietary lysine levels increased. However, the present study also observed a decline in growth performance indices in diets D4 and D5, where lysine inclusion levels were higher. A similar pattern was seen regarding nutritional metrics, specifically feed efficiency ratio (FER), protein efficiency ratio (PER), and total protein intake (PI). This agrees with Rachmawati *et al.* (2022), who reported that lysine supplementation improves growth and feed utilization only up to an optimal level (about 1–2%), beyond which performance may decline. The slight reduction observed in D4 and D5 supports this trend. Overall, the results indicate that the optimal lysine inclusion level for *Heteroclaris* was 1.04 g/100 g (20% CP + 1.04 g/100 g lysine), which enhanced growth performance without negative effects.

Gustation of Experimental Fish

The present finding showed in the gustation of *Heteroclaris* fed practical diets, the best result was obtained in D3 (20%CP + 1.04g/100g) with the lowest time of grasp (0.75)sec., compared with the remaining diets. This demonstrated that the *Heteroclaris* was able to detect and recognized the practical diet on time, this agreed with the research conducted by (Kasumyan and Doving, 2003; Hansen and Reutter, 2004), who illustrate that *Cyprinus carpio* was able to detect and grasp diet in the cultured tanks. This is to established the palatability and acceptability of formulated diet supplemented with graded levels of lysine fed to *Heteroclaris* fingerlings as represented in the apparent gustation coefficients in bar chart showed in Figure 1 within the short period of introducing palletted diets into the cultured tanks. Thus, D3 with

supplementation level of (20%Cp +1.34g/100g protein diet) has the lowest time of grasp hence it was accepted more than other diets.

Conclusion

This finding of this study established lysine and plant-based protein such as ground nut cake can be use at minimal level in the diets of *Heteroclarialis*. Extensive research is still needed to identify areas for further research, such as exploring other alternative protein sources, optimising replacement levels of Lysine and evaluating the long-term effects on *Heteroclarialis* health and growth. This will make significant contributions to food security and national production figures.

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