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The effects of protein-bound methionine and lysine on the growth rate, feed utilization and digestibility for African catfish (Clarias gariepinus) fingerlings

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ABSTRACT

This paper reports the effects of protein-bound methionine and lysine on the growth rate, feed utilization and digestibility of African catfish (Clarias gariepinus) fingerlings. Three types of diets (LyMet A, LyMet B and LyMet C) were formulated by using locally available feed ingredients; earthworm powder, fish meal, soybean wastes, Leucaena leucocephala leaves, and rice bran. Each of the diets was analyzed for their limiting amino acids, protein-bound methionine and lysine. The commercial fish feeds act as a control diet. LyMet A with combination of 25.00 g, 20.00 g, 25.00 g, 10.00 g and 14.00 g of earthworm powder, fish meal, soybean wastes, Leucaena leucocephala leaves and rice bran respectively, gave the highest protein-bound methionine and lysine composition (2.06 and 8.11 % of amino acids in dietary protein respectively) among all diets. Feeding trial was conducted for 12 weeks to determine the utilization of formulated fish feeds towards African catfish fingerlings. The fingerlings, with a mean initial weight of 3.10±0.10 g were fed twice a day at 5 % of their body weight. LyMet A showed the highest weight gain (1676.18±13.60 %), specific growth rate (3.20±0.01 %/day) and methionine and lysine digestibility (92.33±0.11 and 96.39±0.01%, respectively). The results indicate that LyMet A has an adequate amount of limiting amino acids (methionine and lysine) to fulfill African catfish fingerling diets without supplementation of synthetic amino acids.

Keywords:
African catfish, Methionine, Lysine, Fish feed

1. Introduction

African catfish (*Clarias gariepinus*) is one of the highly demanding aquaculture cultivar species in Malaysia. The delicious taste of African catfish and high market demand from local food sector

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rendered it as one of the most profitable commercial aquaculture species in Malaysia and Southeast Asia Region [1]. Fish meal is commonly used as major ingredient in catfish's feeds [2]. It is utilized mainly due to high protein content and has a complete essential amino acids required in diets [3]. Sole use of fish meal in commercial feeds rendered it expensive. Thus, there is a need to explore the alternative protein ingredients that show the same quality as fish meal or better with lower cost and fulfilling the needs.

There are ten essential amino acids required by fish and other animals for growth [4]. It is compulsory to formulate fish diet by satisfying the essential amino acids required by fish in order to obtain a balance diet. Robinson and Li have reported that, if lysine requirement of catfish diet is met by the supplied feed ingredients, the other amino acids are deemed to be sufficient or exceeding their requirements [5]. Among all amino acids, methionine and lysine are the most essential amino acids which often act as limiting amino acids in commercial feeds [6, 7]. This has motivated researchers to conduct research to explore the importance of amino acids especially the limiting amino acid towards catfish. Determining the proper amount of amino acids will enable the cost reduction during the formulation of fish feed by selecting the correct protein source specially to provide proper amount of methionine and lysine needed for fish growth.

As far to our knowledge, there are limited studies on the limiting amino acids requirements for *Clarias gariepinus* species. Therefore, in this study, the available feed ingredients were identified and evaluated for their amino acids content. Four main ingredients with a vigorous amino acid profiles were selected to be used as a fish feeds for African catfish fingerlings. Earthworm powder and fish meal is the main protein sources with high limiting amino acid composition. Soybean wastes, *Leucaena leucocephala* leaves and rice bran were also incorporated to achieve a balanced diet for fish. The formulated fish feeds were analyzed for their methionine and lysine composition. The combination of the ingredient at different composition results in different methionine and lysine composition in feeds. The suitable formulated fish feeds were selected for feeding trial. The growth performances of African catfish fingerlings were determined with different level of protein-bound methionine and lysine incorporated in the fish feeds. In addition, the formulated fish feeds were evaluated in terms of feed utilization and digestibility and subsequently were compared with the commercial fish feeds used in fish farms.

2. Materials and methods

2.1. Materials

Earthworm powder was purchased from Solo Agrofarm, Indonesia while fish meal was produced from trash fish obtained from local market in Arau, Perlis, Malaysia. Soybean wastes and *Leucaena leucocephala* leaves were obtained from individual person in Arau, Perlis, Malaysia. Rice bran was obtained from Bernas Rice Mill Factory Perlis, Malaysia. Tapioca flour was obtained from local market in Perlis, Malaysia. Calcium dihydrogen phosphate, petroleum ether, sulfuric acid (H_2SO_4), D-glucose, phenol solution and hydrochloric acid (HCl) was purchased from Sigma-Aldrich, Malaysia and vitamin and mineral premix were purchased from Y.S.P Industries Sdn. Bhd. Malaysia. Commercial fish feeds obtained from local fish hatchery in Kedah, Malaysia will act as a control diet. Amino acid analysis kit was purchased from Phenomenex, LT Resources (M) Sdn. Bhd., Malaysia.

2.2. Proximate analysis of feed ingredients

Proximate analyses in each feed ingredients were performed in order to measure the biochemical (i.e. amino acid profile, crude protein, crude lipid and carbohydrate content) and elemental

properties (i.e. moisture and ash content). Amino acid profile of feed ingredients was obtained using High Performance Liquid Chromatography (HPLC) (Waters 2475) after acid hydrolysis using 6 N HCl for 24 hours at 110 ^{o}C. Hydrolysate was produced after acid hydrolysis process. The hydrolysate was added with α-aminobutyric acid (AABA) as internal standard and water to make 100mL solution. The solution was filtered twice which are using filter paper and syringe filter to remove any impurities. 10 µL of solution was taken for derivatisation and added with AccQ Fluor reagent before inject to HPLC column. Figure 1 simplified the sample preparation steps during HPLC analysis.

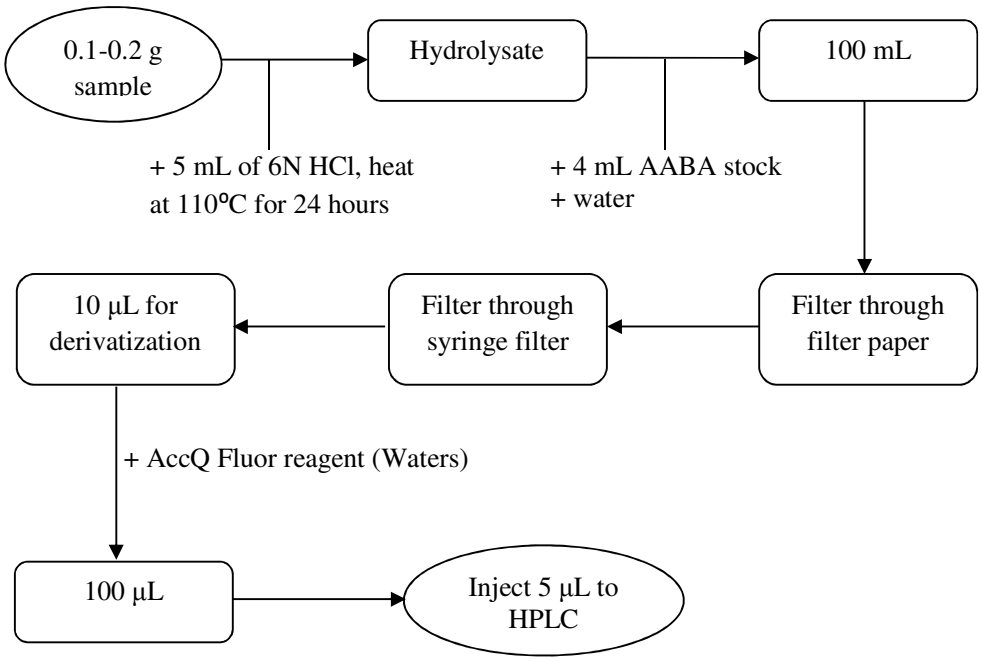

Fig. 1. Sample preparation during HPLC analysis

Crude protein analysis of feed ingredients was performed by using Kjehdahl nitrogen assay based on method proposed by Lynch [8]. Crude lipid was analyzed using Matsler method with petroleum ether used as solvent [9]. Carbohydrate content was analyzed by using Phenol-Sulfuric method as proposed by Suzanne [10]. The moisture content was analyzed using Foh method [11] whilst ash by combustion at 500-600^{o}C using Sluiter method [12].

2.3. Diet preparation

Three diets were formulated in this experiment namely LyMet A, LyMet B and LyMet C which vary in percentages of feed ingredients inclusion and their limiting amino acid composition (Table 1). The range for each feed ingredient was chosen based on previous study [13-17]. 100 g of LyMet A diet was prepared by mixing 25.00 g of earthworm powder, 20.00 g of fish meal, 10.00 g of *Leucaena* leaves, 25.00 g of soybean waste, 14.00 g of rice bran and the rest 6.00 g were vitamin mineral premix, calcium diphosphate and tapioca flour. All dry feed ingredients (earthworm powder, fish meal, soybean wastes, *Leucaena leucocephala* leaves, rice bran and tapioca flour) were mixed using dough mixer while hot water was slowly added to accomplish agglutination and to form a dough-like mixture. Vitamin mineral premix and calcium diphosphate were added into the dough mixture. The dough was passed through pelletizer to obtain pelletized feed and oven dried at 60 ^{o}C for 4 hours. The fish feeds were stored in air-tight container and kept in refrigerator until further used.

Table 1
Composition of the experimental fish feeds

Ingredients (g)	LyMet A fish feeds	LyMet B fish feeds	LyMet C fish feeds
Earthworm powder	25.00	20.00	15.00
Fish meal	20.00	10.00	0.00
Soybean wastes	25.00	20.00	25.00
Leucaena leucocephala leaves	10.00	7.50	10.00
Rice bran	14.00	36.50	44.00
Tapioca flour	5.00	5.00	5.00
Vitamin and Mineral	0.50	0.50	0.50
Calcium diphosphate	0.50	0.50	0.50

2.4. Methionine and lysine analysis

The methionine and lysine composition in fish diet was determined using Phenomenex EZ Faast amino acid analysis kit coupled with gas chromatography (GC) (GC-2010 Shimadzu) after underwent acid hydrolysis using 6 N HCl for 24 hours at 110 ^{o}C. The sample preparation method during acid hydrolysis was similar to that in Figure 1 except that Norvaline was used instead of AABA stock solution.

2.5. Feeding trial

African catfish (*Clarias gariepinus*) were obtained from local hatchery in Kedah, Malaysia. Prior to the start of feeding trial, all fish were acclimatized for 48 hours. There are four types of fish feeds (LyMet A, LyMet B, LyMet C and commercial fish feeds) tested during feeding trial. All fish feeds were tested in triplicate and took place for 12 weeks. 120 fish fingerlings with initial mean weight 3.10±0.10 g were distributed in 12 fish tanks equipped with resin-filtered and low pressure aerated blower with 10 fish per tank. Fish were manually fed twice a day at and the daily feed intake and fish mortality were observed. Uneaten pellets were collected 30 minutes after feeding and weighed. Water exchange was performed in weekly basis. The temperature and pH of the water were monitored and adjusted to ensure that they were consistently fall within range of 27.0 ^{o}C to 30.5 ^{o}C [18] and 6.5 to 7.5 [19], respectively.

2.6. Data calculation and analysis
2.6.1. Growth rate

All fish fingerlings were weighed individually once a week to observe their growth rate and subsequently, adjust their feeding levels. All collected data were calculated for their growth rate, feed utilization and methionine and lysine digestibility. The final weight gain, specific growth rate and survival rate were calculated by using Eq. 1, Eq. 2 and Eq. 3, respectively.

$$\text{Fish Weight Gain (WG)}(\%)=100\times\left(\frac{(\text{final mean weight-initial mean weight})}{\text{initial mean weight}}\right) \quad (1)$$

$$\text{Specific Growth Rate (SGR)(\% body weight/day)} = 100\text{-}\left(\frac{\ln(\text{final mean weight})\text{-}\ln(\text{initial mean weight})}{\text{day}}\right) \quad (2)$$

$$\text{Survival Rate (SR)(\%)} = 100\times\left(\frac{\text{final number of fish}}{\text{initial number of fish}}\right) \quad (3)$$

2.6.2. Feed utilization

The total fish feeds consumed by fish were recorded daily. Uneaten fish feeds were collected and weighed if present. Eq. 4 shows calculation for feed conversion ratio (FCR).

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{individual feeds intake}}{\text{individual weight gain}} \quad (4)$$

2.6.3. Methionine and lysine digestibility

Three weeks before termination of the experiment, the feces were collected daily using stripping method and oven dried at 60 ^{o}C for 24 hours for digestibility analysis. The lysine and methionine content in the feces were determined by using Phenomenex EZ Faast amino acid analysis kit coupled with GC (GC-2010 Shimadzu) after the feeds have underwent acid hydrolysis. The calculation for both methionine and lysine digestibility were shown in Eq. 5 and Eq. 6.

$$\text{Apparent Digestibility Coefficients } (ADC_{methionine}) = 100\text{-}\left(\frac{\text{marker concentration in diet}}{\text{marker concentration in feces}}\times\frac{\text{methionine concentration in diet}}{\text{methionine concentration in feces}}\right) \quad (5)$$

$$\text{Apparent Digestibility Coefficients } (ADC_{lysine}) = 100\text{-}\left(\frac{\text{marker concentration in diet}}{\text{marker concentration in feces}}\times\frac{\text{lysine concentration in diet}}{\text{lysine concentration in feces}}\right) \quad (6)$$

3. Results and discussion

3.1. Proximate analysis of feed ingredients

Proximate analysis was mainly used during experimental works to provide nutritional content of selected feed ingredients thus, showing the quality of the feed ingredients chosen. Table 2 shows the proximate analysis of each feed ingredients used in this study. Earthworm powder has the highest crude protein and ash content. The high crude protein in earthworm powder making it as the main protein sources during formulation of fish feeds along with fish meal. Rice bran which consist only 13.86 % of crude protein will act as filler rather than protein source. Soybean wastes gave the highest lipid content among all feed ingredients. High lipid content is not really necessary because lipid is required in small amount especially in omnivorous fish [20]. Fish meal has the highest carbohydrate and moisture content. However, carbohydrate are alternate source of energy and not necessary for fish growth [21].

Table 2
Proximate analysis of each feed ingredients

Ingredients	Crude Protein (%)	Crude Lipid (%)	Carbohydrate (%)	Moisture Content (%)	Ash Content (%)	NFE* (%)
Earthworm powder	60.34	3.67	2.40	9.18	14.66	9.75
Fish meal	46.00	1.67	8.39	11.28	14.17	18.49
Leucaena leucocephala leaves	26.41	1.33	2.06	6.44	4.99	58.77
Soybean wastes	33.25	5.67	3.00	5.53	3.92	48.63
Rice bran	13.86	2.89	5.00	5.50	9.51	58.35
Commercial fish feed	32.52	4.67	6.08	8.17	8.87	36.69

*Nitrogen-free extract = 100 – (crude protein – crude lipid – carbohydrate – moisture – ash)

Table 3 shows the amino acid profile on each feed ingredients. Earthworm powder which consisted high protein contents also gives high methionine and lysine content compared to the other feed ingredients. Previous study of earthworm species in Nigeria gives 2.08-2.24% and 4.95-5.70% of methionine and lysine respectively [22]. Earthworm powder of *Eudrillus euginae* gives 2.53% and 6.04% of methionine and lysine respectively [23]. Methionine and lysine are often limiting amino acids in ingredients used during production of fish feeds and the deficient of them may limit the protein synthesis process [24, 25]. In most studies, synthetic methionine and lysine were added in fish diet to prevent amino acids deficiencies.

Table 3
Amino acid profile of each feed ingredients

Amino Acid	%w/w				
	Earthworm powder	Soybean waste	Fish meal	*Leucaena leucocephala* leaves	Rice bran
Aspartime	6.66	2.36	4.55	2.54	1.66
Serine	3.83	3.89	3.14	2.54	1.05
Glutamin acid	10.01	4.22	8.25	3.50	2.72
Glycine	8.54	10.25	6.78	4.17	0.89
Histidine	4.76	4.49	4.19	2.81	1.30
Arginine	5.63	6.88	4.99	2.84	1.61
Threonine	0.74	3.14	3.12	2.39	0.89
Alanine	2.68	1.00	1.97	1.41	0.86
Proline	1.78	0.63	1.93	1.32	0.68
Thyrosine	3.49	4.22	2.02	1.88	0.60
Valine	3.53	2.31	2.56	2.10	1.01
Methionine	1.97	0.45	1.50	0.78	0.35
Lysine	4.48	0.73	3.60	2.01	1.00
Isoleucine	2.47	2.43	1.74	1.23	0.50
Leucine	6.14	4.42	4.42	3.48	1.44
Phenylalanine	4.91	7.68	3.07	2.80	0.93

In this study, earthworm powder gave the highest methionine and lysine content making it as high quality feed ingredients. The finding from previous study also shows the potential of earthworm powder as the major methionine and lysine sources in fish feeds. Moreover, it contains high

composition of all individual amino acid required in catfish diet. In this study, feed ingredients with satisfactory amino acid profiles will be incorporated during production of African catfish fingerlings fish feeds in order to promote optimal growth of fish. Plant ingredients usually are low methionine and lysine. However, among all feed ingredients analyzed, *Leucaena leucocephala* leaves have the highest methionine content. Therefore, it is expected that the proper combination of selected feeds ingredients will enhance the limiting amino acids (methionine and lysine) in fish feeds without addition of synthetic methionine and lysine.

3.2. Methionine and lysine analysis

There are wide variations for methionine and lysine requirement for fish depending on fish species, diet formulation, size and age of fish, feeding practice and rearing condition [6]. Table 4 shows the methionine and lysine composition in prepared diets. LyMet A diet has the highest methionine and lysine composition among all formulated diets and control diet due to the higher percentages of earthworm powder incorporated into the diet compare to the other formulated diets. The greater amount of methionine and lysine in earthworm powder rendered it as the greatest contributor during formulation of the diets. LyMet C diet contained deficient methionine and lysine composition because there is no fish meal incorporated. The methionine and lysine in LyMet C are contributed only by the earthworm powder, *Leucaena leucocephala* leaves and soybean wastes. Plant-based ingredient contained low composition of methionine and lysine making them the smallest contributor in fish diet. The effect of methionine and lysine composition in fish feeds were expressed with fish performances.

Table 4
Methionine and lysine composition in fish feeds

	Types of fish feeds			
	LyMet A	**LyMet B**	**LyMet C**	**Commercial**
Methionine (% in protein)	2.06	0.91	0.37	0.80
Lysine (% in protein)	8.11	1.59	0.27	3.64

3.3. Growth rate analysis

Growth rate of fish is important since it can be used to predict changes in size-specific rates such as mass and length of fish and even the production cost [26]. The formulated fish feeds were well accepted by the reared catfish fingerlings. Table 5 shows the weight gain, specific growth rate and survival rate of African catfish fingerlings fed with different diets.

Table 5
Weight gain, specific growth rate and survival rate of African catfish fingerlings fed with different fish feeds

	LyMet A	**LyMet B**	**LyMet C**	**Commercial**
Initial Weight (g)	3.17±0.11	3.00±0.02	3.02±0.12	3.21±0.16
Final Weight (g)	56.31±0.43	49.13±0.52	24.64±1.56	46.42±0.97
Wet weight gain (g)	53.14±0.43	46.13±0.52	21.62±1.56	43.21±0.97
WG (%)	1676.18±13.60	1537.50±17.21	715.73±51.75	1345.95±30.18
SGR (%/day)	3.20±0.01	3.07±0.06	2.33±0.07	2.97±0.02
SR (%)	95.00±7.07	95.00±7.07	70.00±0.00	75.00±7.07

Values are given as mean ± standard deviation.

LyMet A which consist the greater composition of methionine and lysine gained the highest weight gain compared to the other diet formulations. The results obtained from this study revealed the encouraging potential for the growth performance of African catfish fingerlings fed with LyMet A diet which consist high level of protein-bound methionine and lysine was higher than the fish fed with control diet (commercial feeds). This suggests that without supplementation of methionine and lysine in fish diet was enough to fulfill a balance dietary amino acid profile of African catfish fingerlings. This is due to the WG and SGR achieved by African catfish fed with LyMet A diet was the highest among all diets, suggesting that African catfish fingerlings are able to utilize protein-bound methionine and lysine. Although the formulated fish feed were incorporated with food wastes (soybean wastes and Leucaena leucocephala leaves), it is safe for fish consumption and even better in comparison with fish fed with commercial fish feed [27] and it was proven by positive results obtained from LyMet A fish feed.

During feeding trial, only small differences were recorded for their survival rate. Some of experimental fish were died due to cannibalism among them. African catfish was known for their cannibalism especially when there is a difference in size among them [28]. Fish fed with deficient methionine and lysine showed the lowest WG and SGR indicating that methionine and lysine is essential for growth of African catfish fingerlings. Methionine is important in fish as primary constituents of structural proteins and metabolic function for production of antibodies [29]. Methionine also serves as a start signal to initiate protein synthesis [30]. This enables muscle buildup in catfish fingerlings fed with high level of methionine and lysine. However, excess methionine will accumulate S-adenosylmethionine in the liver and become toxic to the fish [29]. Lysine is the least toxic amino acids. However, excessive amount of lysine in fish diets may cause growth depression in several fish species as reported by Farhat and Khan [31]. In this study, the formulated feeds with highest methionine and lysine content do not shows impaired growth which indicates that the methionine and lysine level in diets is adequate and not in excessive amounts.

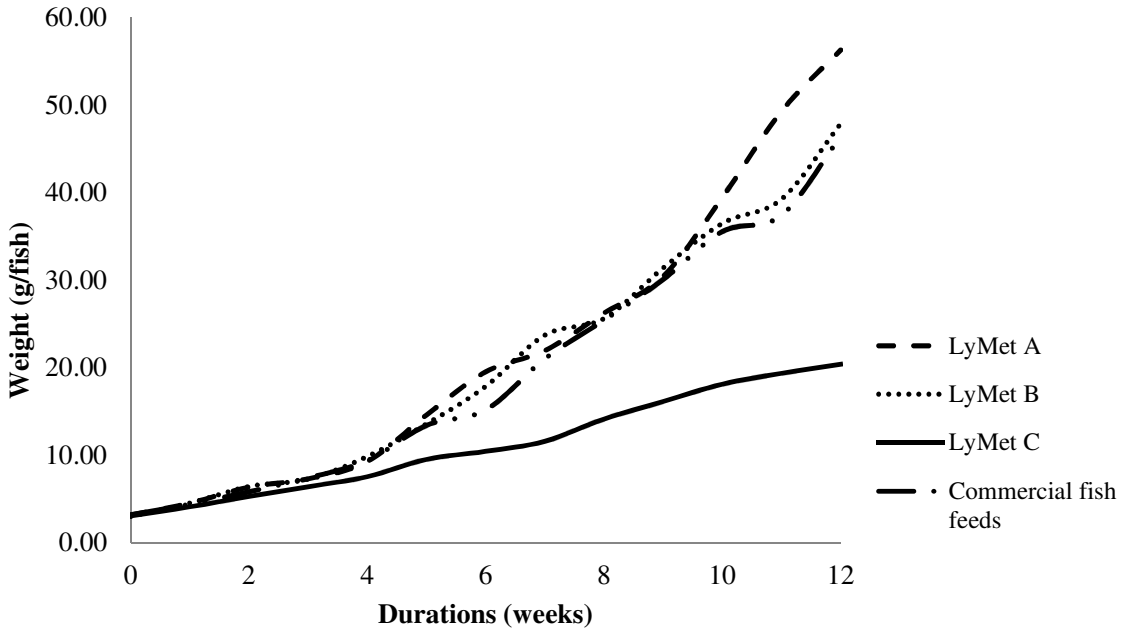

Fig. 2. Weight increment of African catfish fingerlings fed with different types of fish feeds

Figure 2 shows the weight increment of African catfish fingerlings fed with different types of fish feeds. The weight increments in the plotted graph were recorded weekly. The graph represents a linear pattern for each of the fish feeds. A linear regression analysis can be used to estimate the weight of African catfish fingerlings for a known duration. The graph pattern for LyMet A, LyMet B and commercial fish feeds were almost the same. Among all, LyMet A demonstrates the highest weight increment especially during week 10 until week 12. LyMet C fish feeds showed the slow movement from the first week until week 12. This is anticipated due to high composition of methionine and lysine in LyMet A compared to low composition of methionine and lysine in LyMet C fish feeds. The differences of the weight increment among given fish feeds were resulted from the initiation of protein synthesis that occur in fish. The limiting amount of methionine and lysine will limit the protein synthesis in fish thus, the muscle buildup in fish became slower and only small increase occurred. The weight increment of African catfish fingerlings fed with LyMet A for the 12 weeks is 94.37 % while for LyMet B, LyMet C and commercial fish feeds were 93.73 %, 85.18 % and 93.08 %, respectively.

3.4. Feed utilization

FCR is an indicator used to measure the acceptability of formulated feeds by fish. Table 6 shows the FCR of African catfish fingerlings fed with different diets. In this study, the FCR for the prepared diets were in range 1.30 until 1.88 and comparable by other study [6, 32-34] and even have lower FCR than certain study [2, 35]. Low FCR indicates that the feed were converted efficiently and lead to increase production efficiency [36]. LyMet A and LyMet B have the lower FCR compared to commercial fish feeds. This may reduce production cost of adult African catfish because the fish feeds need by the fish is lower than fish feeds with high FCR.

Table 6
FCR of African catfish fingerlings fed with different fish feeds

Fish feeds	FCR
LyMet A	1.35±0.01
LyMet B	1.30±0.01
LyMet C	1.88±0.14
Commercial	1.39±0.04

3.5. Methionine and Lysine Digestibility

The results for the methionine and lysine digestibility of African catfish fingerlings fed with different diets were shown in Table 7. By considering the leaching of protein-bound amino acid is ±2.00 %, the highest $ADC_{methionine}$ and ADC_{lysine} was obtained by LyMet A diet which is 90.48 and 96.38% of methionine and lysine, respectively. The high digestibility of lysine obtained in present study is relevant due to high requirements of lysine in catfish fingerlings diet [3]. The $ADC_{methionine}$ and ADC_{lysine} were significantly increasing with increase of methionine and lysine composition in fish diets. Digestibility more than 88% indicates that the methionine and lysine were highly digestible in fish diet [37]. However, in this study, only LyMet A has more than 88% digestibility of methionine which represent that methionine in LyMet B, LyMet C and Control diet are poorly digestible by African catfish fingerlings. For lysine, all diets are highly digestible except for LyMet C diet which only 72.36% are digestible. LyMet C consist no fish meal and low composition of earthworm powder hence, making it poorly digestible for both methionine and lysine.

Present results achieved by LyMet A is slightly higher than 92.20 and 94.30 % digestibility of methionine and lysine, respectively on juvenile catfish as reported by Nyina-Wamwiza [3]. The high methionine and lysine digestibility achieved by African catfish fed with LyMet A diet was the highest among all diets, suggesting that African catfish fingerlings are able to utilize protein-bound methionine and lysine. It is common for all amino acids to have high digestibility especially more than 90% whether dispensable or indispensable amino acids [3]. Based on the results obtained in present study, it is suggested that the African catfish fingerlings diet can be formulated without supplementation of amino acid with adequate mixture of feedstuff ingredients.

4. Conclusion

In conclusion, results obtained from this study indicate that LyMet A diet which contained 2.06 and 8.11 % of protein-bound methionine and lysine in dietary protein, respectively is enough to fulfill African catfish fingerling diet without addition of any synthetic amino acids to achieve ideal weight gain, better feed efficiency and highly digestible methionine and lysine. In comparison to commercial fish feeds, the WG and SGR of LyMet A diet has improved by 24.54 % and 7.74 % respectively, while FCR decreased 2.88 % rendered it as the potential low-cost replacement for commercial fish feeds. LyMet A also has achieved better methionine and lysine digestibility in comparable with commercial fish feeds which ensure proper nutrient uptake by African catfish fingerlings.

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