Original Research Article

Effects of Growth Promoter-Enriched Farm-Made Feeds on the Growth, Flesh Quality and Economics of *Labeorohita* Culture

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ABSTRACT

Aims: The study compared the effects of three growth promotersenriched farm-made feeds with a commercial carp grower feed on growth, fleshcompositions and palatability, and economics of*Labeorohita*culture.

Study design: The study was carried out through four treatments with three replications in each treatment. A commercial carp feed was used as control (T_1, without growth promoter) and three experimental feeds were formulated by using locally available ingredients with addition of three growth promoters viz., charger gel (CG), eon fish grower (EG) and aquazyme plus (AP) treated as T_2, T_3 and T_4, respectively. Fifteen juveniles *of L. rohita* with initial weights ranging from 131.17±9.60g to 133.33±8.84g were stocked in each cage (96ft^3) and served a daily ration of 5% body weight.

Place and Duration of Study: The study was conducted in 12 cages set in an experiment pond at Department of Fisheries, University of Rajshahi, Bangladesh for 80 days (June-August, 2022).

Methodology: Growth and feed utilization parameters by means of weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and chemical composition viz., crude protein, lipid, carbohydrate, fiber, ash and moisture content of feed and fish flesh along with palatability indicators viz., odour, taste and texture were evaluated according to standard formulae, AOAC methods and organoleptic sensory technique, respectively.

Results:CG, EG, and AP enriched feed exhibited significantly better growth performance, feed efficiency, flesh composition and palatability indicators compared to the control feed. Additionally, CG enriched feed was the most economically efficient followed by EG and AP enriched feed in terms of feed cost/kg production.

Conclusion: The findings conclude that charger gel enriched farm-made feed is more economical and yields better growth and flesh quality of *L. rohita* than the selected commercial feed.

Keywords: *Growth Promoter, Feed, Growth, Flesh Quality, Palatability, Economics, Labeorohita*

1. INTRODUCTION

Aquaculture is one of the most prolific and emerging enterprises in Bangladesh. This sector has continuously provided safer and high-quality animal protein, significantly contributing to the country's economy, food and nutrition security. Recently, aquaculture in Bangladesh has been advancing towards intensification, low-density to high-density aquaculture, due to the rising demand for food fish and the declining yield of capture fisheries. This change from low-density to high-density aquaculture subsequently leads to an unprecedented demand for fish feeds (Khatun et al. 2018). There have been growing concerns about the health effects, environmental impact, and overall cost-effectiveness of producing aquafeeds and their subsequent use. Unfortunately, most feed manufacturers fail to provide high-quality feed owing to a lack of quality feed components, the use of contaminated component, and other factors (Khatun et al. 2018). Moreover, the labeled nutritional value of commercial fish feeds is often misleading (Rahman et al. 2014).

As aqua feed production heavily relies on fish meal and the fish oil industry as sources of protein and lipids, it will most likely face significant sustainability issues in the future. As a result, emphasis is now placed on using locally available plant-based feed ingredients to produce more sustainable fish feed. Under such conditions, the on-farm formulation of fish feed using locally accessible feed ingredients has the potential to reduce the ever-increasing cost of feeding and increase feeding efficiency. In addition to that, several types of feed additives known as growth promoters are being used by farmers at present to obtain better growth performance for fish. The growth promoters enhance fish growth by increasing digestibility, immune stimulation, nutrient assimilation, and supplying essential micro-nutrients in the diet or combining these functions.

Recent studies (Chowdhury et al. 2015; Rahman et al. 2017; Anwar et al. 2018) show that β-glucan, dietary multi enzymes, vitamins, and mineral premix are among the most used growth promoters in the aquaculture sector nowadays in Bangladesh. The effect of β-glucan on growth, survival, disease resistance, immune-related gene expression, and as an adjuvant in a wide variety of fish species is well documented in numerous studies (Meena et al. 2013; Zheng et al. 2020). Exogenous enzymes supplemented as feed additives have proven to be an efficient technique to increase animal growth as it aids in the digestion of compound carbohydrates, proteins and lipids. Small but adequate quantities of vitamins and minerals are required for proper growth, improved well-being, and fish metabolism (Carmen and Geoff 2007). A sufficient supply of vitamins is mandatory for appropriate metabolic processes in enzymatic, endocrine and immunological systems (Keen et al. 2004). All these growth promoters ultimately improve the efficiency of fish feed and may reduce the operating cost of a fish farm.

Carp fattening, a form of intensive farming, is very popular in the northwestern region of Bangladesh, where *Labeorohita* is one of the most common culture species. Using locally available feed ingredients, a practical and low-cost feed formulation technique for *L. rohita* can significantly benefit the native farmers. The use of growth promoters on several species of fish like *Clarias batrachus* (Islam et al. 2017), *Oreochromis niloticus* (Islam et al. 2014), *Anabas testudineus* (Rahman et al. 2012), *Sparus aurata* (Dimitroglou et al. 2010) has been reported. However, no research has been conducted to assess and compare the impact of different commercial growth promoters on *L. rohita*while analyzing their efficacy in farm-made feed. Therefore, this experiment was conducted to evaluate the potential of growth promoter-enriched farm-made feeds as an alternative to a commercial diet while assessing its effects on growth, flesh composition and palatability, and economic feasibility in *L. rohita* culture.

2. MATERIAL AND METHODS

2.1 Experimental Design: The experiment was carried out in cages set up at an earthen pond for 80 days from August to October 2021. Twelve iron-framed cages, each measuring 96 ft^3,were constructed for this experiment and covered by synthetic nylon knotless net of 5mm mesh size with an opening at the top for feeding and sampling purposes with a suspended feeding tray hanging from the top. The investigation was conducted with four types of feeds as four treatments with three replicates. Three types of experimental feeds were formulated by incorporating three commercial growth promoters named charger gel (CG), eon fish grower (EG) and aquazyme plus (AP) in recommended dosage (**Table 1**). To compare the effects of growth promoter treated feeds to a commercial diet, a popular carp grower feed (CF) from ACI Agrovet LTD. was selected. The treatments were assigned to the cages in a completely randomized design.

Table 1. Information about the growth promoters used in this experiment

Trade Name	Manufacturer	Type	Main Ingredients	Dose/kg feed
Charger Gel	FishTech (BD) Ltd.	Immuno-modulator	1-3 D β-Glucan, Betain, and Gluco Proteins from Yeast Cell Walls and other natural sources	4g
Eon Fish Grower	Eon Group	Vitamin and Mineral Premix	Vitamin A, C, E, D, K, B complex, L-Lycine, DL-Methionine, Tryptophane, and Mineral salts.	2g
Aquazyme Plus	Eon Group	Dietary multienzyme supplement	Amylase, Xylanase, Protease, and Phytase	2g

For the formulation of the experimental feeds, locally available feed ingredients, viz. rice polish, mustard oil cake, wheat flour, fish meal, soybean oil, and molasses were used along with the selected growth promoters (**Table 2**). The proximate composition of these ingredients was evaluated, and the formulation was done using spreadsheet analysis. The protein content of the formulated feed was targeted to match that of CF (determined earlier) to obtain an isoprotein diet across all the treatments. Experimental feeds were prepared by using a pelleting machine through the extrusion process. The proximate composition of the experimental feeds was evaluated again after formulation through ensuing standard methods (AOAC 2005).

Table 2. Dietary inclusion of different ingredients in different treatments

Ingredients	CG	EG	AP
Rice polish	40%	40%	40%
Mustard oil cake	40%	40%	40%
Wheat flour	10%	10%	10%
Fish meal	8%	8%	8%
Soybean oil	0.5%	0.5%	0.5%
Molasses	1%	1%	1%
Charger Gel	4g	-	-
Aquazyme Plus	-	2g	-
Eon Grower	-	-	2g

2.2 Experimental Fish, Monitoring and Sampling: From a nearby aquaculture facility, 180 more or less similar-sized juveniles of *L. rohita* were collected. The fish were then acclimated for three days in a concrete tank. After the acclimatization period, 15 *L. rohita* juveniles were randomly released into each of the 12 cages. Weight measurements were recorded for each fish before their release, ranging from 131.17±9.60g to133.33±8.84g across the treatments (**Table 6**). Fish feeds were supplied twice daily, in the morning and the evening, at 5% of body weight. Any leftover feed in the feeding tray was collected after one hour of providing the ration and was dried and weighed. At the end of the trial, the amount of leftover feed was culled during the estimation of feed utilization parameters. Individual fish weight was measured from each cage every two weeks throughout the trial period, and the ration size was modified according to the changes in body weight. A few physicochemical parameters, such as temperature, dissolved oxygen, pH, total alkalinity, dissolved carbon dioxide and ammonium-nitrogen (**Table 3**) were recorded each week throughout the experiment. The water quality parameters didn't differ among the treatments as all the treatments were randomly placed in the same pond. The values of the water quality parameters were also found to be in the suitable range for *L. rohita* culture.

Table 3. Water quality parameters

Parameters	CF	CG	EG	AP
Temperature	26.21±1.06	26.17±0.92	26.54±1.13	26.37±0.54
DO	6.79±0.21	6.32±0.38	6.28±0.17	6.63±0.48
pH	7.63±0.33	7.52±0.18	7.58±0.23	7.55±0.27
Alkalinity	134.33±4.85	132.51±3.31	134.87±4.39	136.45±4.76
CO_2	4.09±0.09	4.24±0.12	4.38±0.07	4.17±0.14
NH_3-N	0.040±0.004	0.037±0.006	0.042±0.003	0.038±0.003

2.3 Analysis of Growth Performance and Feed Utilization: Growth performance and feed utilization parameters as mean weight gain (MWG), percent weight gain, (PWG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR) and protein efficiency ratio (PER) was calculated by using the following formulae-

$$MWG = MeanFinalWeight(MFW) - MeanInitialWeight(MIW)$$

$$PWG = \frac{MFW - MIW}{MIW} \times 100$$

$$SGR = \frac{ln(MFW) - ln\,(MIW)}{CultureDuration\,(Days)} \times 100$$

$$SR = \frac{No.ofFishHarvested}{No.ofFishStocked} \times 100$$

$$FCR = \frac{FeedFed}{LiveWeightGain}$$

$$PER = \frac{LiveWeightGain}{ProteinFed}$$

2.4 Evaluation of Flesh Quality: At the end of the trial, three fish from each replicate were sacrificed and flesh samples were collected from different portions of the body and prepared by grinding and homogenizing for subsequent analysis. Fish flesh samples were analyzed to determine protein, lipid, ash, fiber, carbohydrate and moisture content using standard methods (AOAC 2005). For the palatability test, fish fleshes in the form of loins from each treatment were cooked in a traditional boiling method. The fish loins from each treatment were marked and cooked together to avoid any cooking bias. After consuming the cooked fish, ten expert panelists gave their scores blindly on the fish's odor, taste, and texture following the specific structured scaling system (**Table 4**) described by Huss (1995).

Table 4. Organoleptic/Sensory scoring scale for palatability test

Sensory Attributes/Palatability indicators			Score
Odor	**Taste**	**Texture**	
Species-specific	Meaty flavor	Firm/elastic	10
Fresh fish	Sweet	Firm/springy	8
Slightly fishy or slightly sour	Slightly fishy	Less firm	6
Sour and stale	Slightly sour/some off flavor	Softer	4
Strong ammonia	Slightly rotten	Very soft	2
Rotten smell	Spoiled	Slippery	0

2.5 Economic Evaluation of the Experimental Feeds: The economics of the experimental feeds were determined by feed cost/kg feed and feed cost/kg fish production using the following formulae-

a) $\text{Feed cost/kg feed} = \text{summation of ingredient's price and manufacturing cost per kg}$

b) $\text{Feed cost/kg fish production}$

$$= \text{Feed cost/kg feed} \times \text{Amount of feed required for per kg fish production (FCR)}$$

***In the case of CF, the retail price was used as feed cost/kg feed.*

2.6 Statistical Analysis: Once the data's normality had been verified through Levene's test, one-way analysis of variance (ANOVA) was used for statistical analysis, followed by Duncan's multiple-range test as post hoc. Percentage data were arcsine transferred prior to the analysis. The statistically significant levels were set at P= 0.05. Statistical analysis was conducted using the computer program SPSS-25 (Statistical Package for Social Science) and Microsoft Excel 2016. Data with varying superscripts in the same row differ significantly in the presented tables.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Feeds: The proximate composition analysis showed that crude protein, lipid, ash and fiber content of the experimental feeds had no significant difference among them (**Table 5**). However, the carbohydrate content of CG, EG, and AP enriched feeds were significantly higher than CF. On the other hand, CF had significantly higher moisture content compared to the growth promoters enriched feeds.

Comment [2A1]: Underline shall be removed.

The crude protein content in CF was 20.26±0.16% against the manufacturer's declared value of 22%. Dietary protein levels in commercial feeds, particularly in carp growers, are typically lower than nutritional values advertised by certain manufacturers (Rahman et al. 2014). During feed formulation in this study, the protein content of CG, EG, and AP was kept similar to CF with no significant difference ranging from 20.21±0.17% (CG) to 20.34±0.15% (EG). The lipid content of CF was 5.20±0.10%, slightly higher than the manufacturer's labeled value of 5%. Rahman et al. (2014) and Nayeem et al. (2019) have been reported similar lipid levels in carp grower feeds. The experimental feeds were formulated with similar dietary lipid levels with no significant difference that ranged from 5.25±0.13% (CG) to 5.42±0.15% (EG).

The optimum ash content in fish feed is often between 8 and 10%. The mean ash content values in the experimental feeds were between 9.15±0.15% (EG) and 9.70±0.19% (CF), which is within the suitable range for carp. A sufficient amount of dietary fiber in feed increases binding and promotes the passage of ingested food through the digestive tract. However, fiber levels in fish diets beyond 8-12% are undesirable since elevated fiber content would degrade its nutritional quality (De Silva and Anderson 1995). In this experiment, the fiber content of the diets varied from 8.61±0.06% (CF) to 8.76±0.12% (AP), which lies within the favorable range for carp growth.

Carbohydrates are added to lower feed costs and improve the binding properties of the feed. A sufficient amount of carbohydrates in herbivorous fish diets can produce a protein-sparing effect, where a more significant portion of dietary protein is used for growth than body maintenance (Erfanullah and Jafri 1995). Saha and Ray (2001) suggest that at least 40% dietary carbohydrate is required for the optimum growth of *L. rohita*. The carbohydrate content of the feeds in this experiment ranged from 42.53±0.29% (CF) to 46.18±0.14% (EG), which satisfies the recommended carbohydrate requirement for *L. rohita*. The significantly higher carbohydrate content in farm-made feeds could also account for the improved fish growth observed in this experiment through protein-sparing.

Moisture in feed is an essential factor that determines binding capacity, nutrient quality and shelf life. The moisture content of CF was found to be 14.02±0.16% which somewhat exceeds the desirable limit. Nayeem et al. (2019) reported that several manufacturers in Bangladesh produce feeds with moisture content above 12% and exceeding 15% in some cases. However, the moisture content in the experimental feeds was kept under a reasonable limit during formulation ranging from 10.80±0.12% (AP) to 10.89±0.08% (EG).

Table 5. Proximate composition of the experimental feeds (% wet basis)

Component (%)	CF	CG	EG	AP
Protein	20.26±0.16[a]	20.21±0.17[a]	20.34±0.17[a]	20.27±0.14[a]
Lipid	5.20±0.10[a]	5.25±0.13[a]	5.42±0.15[a]	5.32±0.13[a]
Ash	9.70±0.19[a]	9.49±0.12[a]	9.15±0.15[a]	9.60±0.18[a]

Comment [2A2]: Mention about superscript meaning as a footnote in the table.

Fiber	8.61±0.06[a]	8.76±0.11[a]	8.63±0.15[a]	8.76±0.12[a]
Carbohydrate	42.53±0.29[b]	46.12±0.30[a]	46.18±0.14[a]	46.16±0.23[a]
Moisture	14.02±0.16[a]	10.88±0.12[b]	10.89±0.08[b]	10.80±0.12[b]

3.2 Growth Performance of the fish and Feed Utilization: The MWG, PWG and SGR in growth promoter treated fish groups (CG, EG, and AP) were significantly higher compared to CF treated fish group, while the best growth performance was observed in CG treated fish group (**Table 6**). Even though EG and AP treated fish groups performed better than CF treated fish group, the difference between these two treatments was not significant. The SR of the experimental fish was similar across all the treatments, and no significant difference (*P*=0.44) was noted among them. In this experiment, the outcome of the feed utilization parameters, which were expressed as FCR and PER were very similar to the results of the growth performance of the fish. The FCR and PER values obtained in CG, EG and AP treated fish groups were significantly better than in CF treated fish group (**Table 7**). Nonetheless, no significant differences were observed between EG and AP treated fish group in this regard as well (*P*=0.391 and *P*=0.378 respectively).

The findings of this experiment indicate that the inclusion of growth promoters in feed can significantly improve the growth performance of *L. rohita*. The inclusion of charger gel in feed (CG) has yielded the best MWG (237.19±8.78g), PWG (180.83±6.69%) and SGR (1.29±0.05% bwd^{-1}) compared to other treatments. The main active ingredients in charger gel are β-glucan, betaine and gluten-protein. β-glucan is a well-known immune stimulant in fin fishes (Vetvicka 2013). It also aids in the prevention of enzyme inhibition and serves as an essential methyl donor in protein and energy metabolism in tissues (Islam et al. 2017). The cumulative effect of the ingredients present in charger gel had a significant effect as a growth promoter on the experimental fish. The current finding coincides with the findings of Ray et al. (2016), in which the SGR of *L. rohita* fingerlings was significantly increased by adding β-glucan to their diet. Additionally, Cook et al. (2003) in Snapper, Jaramillo and Gatlin (2004) in striped sea bass, and Misra et al. (2006) in Rohu fingerlings reported similar results.

Eon fish grower (EG) is another growth promoter used in this experiment which also expressed significantly better growth performance in terms of MWG (206.55±6.21g), PWG (156.18±4.69%) and SGR (1.16±0.01% bwd^{-1}) compared to CF treated fish group. Eon fish grower is a premix of essential vitamins and minerals. Vitamins and minerals contribute to higher metabolic activity, better appetite, protein digestion, and lysine oxidation (Pillay and Kutty 2005; Carmen and Geoff 2007). As Yimer et al. (2015) reported, adding vitamins and minerals to fish diets can significantly boost production in *O. niloticus*. The results of the present study concur with the report of Nazeemashahul et al. (2020) who found that feeding *L. rohita* with vitamin-mineral-based nutraceuticals significantly increased its production. Barrows et al. (2008) also reported a positive effect of vitamin supplementation on the growth of rainbow trout (*Oncorhynchus mykiss*). The current findings are consistent with the findings of Rahman et al. (2018) who found that vitamin and mineral-enriched feed premix significantly increased the growth and production of the three major Indian carps (Rohu, Catla and Mrigal) in a polyculture system.

Another growth promoter used in this experiment was aquazyme plus (AP), a dietary supplement of multi-enzymes, including amylase, xylanase, protease, and phytase. Like the previous growth promoters, the use of exogenous enzyme in the feed had significantly increased MWG (212.30±6.92g), PWG (159.22±5.19%) and SGR (1.18±0.05% bwd^{-1}) of *L. rohita* compared to the CF treated fish group. Including exogenous enzymes with feed improved digestion and absorption, increased feed intake, improved metabolizable energy of diet and raised weight (Felix et al. 2018). The results of the current study agree with those of Rahman and Sarker (2019) who found that adding multi-enzyme supplements to feed significantly improved the growth of *L. rohita*. Kumar et al. (2006) also reported that supplementing *L. rohita*with an exogenous amylase-enriched diet greatly improved weight gain. Similarly, the addition of dietary enzymes to the Nile tilapia resulted in improved growth parameters (Maas et al. 2017).

The findings of this experiment also indicate that adding growth promoters has improved farm-made feed efficiency. CG inclusion in the feed exhibited the best FCR and PER values, followed by AP, EG and CF (**Table 7**). The ability of β-glucan to enhance feed efficiency in terms of FCR and PER values has been reported before by Hoang et al. (2018) in *Trachinotusovatus* and Munir et al. (2016) in *Channa striata*. Similarly, the provision of additional vitamin and mineral-enriched diet has yielded better feed efficiency in *L. rohita*and *O. mykiss,* as suggested by Nazeemashahulet al. (2020) and Barrows et al. (2008), respectively. Evidence of improved feed utilization and protein retention capabilities in fish due to dietary enzyme supplementation is well documented in previous studies as well (Felix et al., 2018; Yildirim and Turan, 2010). Increased feed efficiency can significantly reduce the environmental impact of aquaculture as reduced feed requirements will lead to less waste and less environmental pollution.

Table 6. The mean values of growth parameters

Parameters	**CF**	**CG**	**EG**	**AP**
MIW (g)	131.83±8.42^{a}	131.17±9.60^{a}	132.25±5.25^{a}	133.33±8.84^{a}
MFW (g)	317.13±15.72^{c}	368.88±15.97^{a}	338.29±14.86^{b}	345.44±18.98^{b}
MWG (g)	185.09±5.02^{c}	237.19±8.78^{a}	206.55±6.21^{b}	212.30±6.92^{b}
PWG (%)	140.40±3.81^{c}	180.83±6.69^{a}	156.18±4.69^{b}	159.22±5.19^{b}
SGR (% bwd^{-1})	1.10±0.03^{c}	1.29±0.05^{a}	1.16±0.01^{b}	1.18±0.05^{b}
SR (%)	93.33±6.66^{a}	95.56±7.69^{a}	91.11±10.18^{a}	91.11±7.69^{a}

Table 7. The mean value of feed utilization parameters

Parameters	**CF**	**CG**	**EG**	**AP**
FCR	2.56±0.05^{a}	1.99±0.06^{c}	2.32±0.04^{b}	2.28±0.04^{b}

PER	1.92±0.05[c]	2.49±0.06[a]	2.12±0.05[b]	2.16±0.04[b]

3.3 Flesh Composition of the Fish: Proximate composition of the fish flesh was analyzed to evaluate the changes in its nutritional quality due to growth promoter inclusion in feed. Fish treated with growth promoters (CG, EG, and AP) exhibited better flesh composition than CF (**Table 8**). Compared to the other experimental groups, a significantly higher quantity of crude protein, fiber and carbohydrate was recorded in the flesh of AP treated fish, whereas CG treated fish flesh exhibited significantly elevated lipid content. Although the flesh of CF treated fish contained the least amount of crude protein, lipid, fiber, and carbohydrate, it had significantly higher flesh ash content than the other treatments. The average moisture content of the fish flesh did not differ significantly from each other (P=0.54).

Fish body composition is considered significant in aquaculture as it affects fish growth, survival and efficiency of food utilization (Breck, 2014). Protein and lipid contents are frequently used to measure the fish's nutritional state and health status (Jobling, 1983). The experimental fish's proximate composition reveals a significant protein content difference, and the maximum carcass crude protein content was recorded in AP treated fish (16.31±0.13%). Yildrim and Turan (2010) and Lin et al. (2007) also found increased carcass protein due to dietary exogenous multienzyme inclusion. The flesh of fish treated with CG also exhibited significantly higher crude protein content (16.12±0.10%) than CF treated fish (15.65±0.16%). The main ingredient of charger gel is β-glucan, and its positive effect on increased protein deposition in fish has been argued in numerous studies (Mohebbi et al. 2011; Rufchaie and Hoseinifar 2014; Munir et al. 2016; Hoang et al. 2018). Significantly higher crude lipid content (2.26±0.03%) was also found in the flesh of fish supplemented with CG compared to other treatments. High lipid content in fish bodies due to the feeding of β-glucan enriched feed is previously described by Schmidt et al. (2017) in starry flounder and reasoned with enhanced blood parameters, albumin and cholesterol concentration.

Increased lipid content in red sea bream (*Pagrus major*) as a result of β-glucan inclusion in the diet has also been reported by Mohebbi et al. (2011). However, decreased level of body lipid content with an increased amount of β-glucan in the diet has been written by Hoang et al. (2018) in young pompano fish (*Trachinotusovatus*). This discrepancy might be attributed to how different fish species physiologically respond to dietary β-glucan and requires further investigation for a more accurate assumption.

Significantly higher carcass fiber (1.26±0.04%) and carbohydrate (3.39±0.04%) were also reported in fish under AP supplementation. This result agrees with the finding of Rahman and Sarker (2019), where significantly higher carcass carbohydrate content was recorded in multienzyme-fed fish. Exogenous multienzyme supplementation in fish aids in the digestive process of complex carbohydrates, allowing more nutrient assimilation from plant-based sources. According to Ding et al. (2022), enzyme supplementation promotes the absorption and digestion of carbohydrates in the feed and enhances the ability of the hepatopancreas to metabolize carbohydrates. This process may have led to the increased carbohydrate content of *L. rohita* in its body under dietary multi-enzyme supplementation. The supplementation of growth promoters in the fish diet didn't significantly affect the ash content of *L. rohita* (**Table 8**).

Similarly, the addition of vitamin premix in the extruded plant-based diet of rainbow trout (*O. mykiss*) didn't have any impact on ash content of the flesh, as Barrows et al. (2008) reported. Dietary β-glucan also didn't influence the ash content of the flesh of Pompano Fish (*Trachinotusovatus*), as argued by Rufchaie and Hoseinifar (2014). Nonetheless, flesh of CF treated fish exhibited significantly higher ash content (2.10±0.03%). This can be reasoned with the comparatively higher ash content in the commercial feed that might have contributed to the increased deposition of minerals in the fish body. In this experiment, the addition of growth promoters in the diets of *L. rohita* didn't exhibit any significant impact on its moisture content of flesh (**Table 8**). This result is backed up by the findings of Schmidt et al. (2017), where the moisture content of starry Flounder flesh was not affected by dietary β-1-3-glucan. Conversely, an increased level of moisture content in the flesh of *L. rohita* has been reported by Rahman and Sarker (2019) with increased levels of dietary enzymes in feed. Consequently, the effect of different growth promoters on body moisture content is variable and remains inconclusive.

Table 8. Proximate composition of the experimental fish (% wet basis)

Composition (%)	CF	CG	EG	AP
Crude Protein	15.65±0.16[b]	16.12±0.10[a]	16.07±0.14[a]	16.31±0.13[a]
Lipid	2.02±0.01[b]	2.26±0.03[a]	2.06±0.02[b]	2.07±0.01[b]
Ash	2.10±0.03[a]	1.26±0.04[b]	1.30±0.06[b]	1.28±0.05[b]
Fiber	0.96±0.01[c]	1.14±0.03[b]	0.97±0.03[c]	1.26±0.04[a]
Carbohydrate	2.23±0.02[d]	3.05±0.06[b]	2.88±0.05[c]	3.39±0.04[a]
Moisture	76.60±0.68[a]	76.36±0.26[a]	76.65±0.62[a]	76.47±0.33[a]

3.4 Palatability of Cooked Fish: Based on the evaluation of the expert panel, we found that the cooked flesh of CG, EG, and AP treated fish exhibited significantly better odor and flavour compared to CF treated fish. While in the case of texture, no significant difference was found among the fishes in different treatmens. The mean organoleptic score of the three sensory attributes (odour, taste and texture) reveals that growth promoter incorporated feeds induced significantly better overall palatability of *L. rohita,* whereas, CG treated fish group was the most palatable (**Table 9**). The panelists' remarks on the palatability parameters suggest that CG treated fish obtained the highest organoleptic scores followed by AP, EG, and CF treated fish groups. This might be due to the outcome of higher fish lipid content in CG treated fish. Lipids exist in foods as emulsions or as free oil/fat dispersed throughout a solid matrix and are known to affect the texture and flavor of foods. Lipids contribute to flavor by producing volatile oxidation products and imparting the taste of short-chain free fatty acids (Shahidi and Weenen, 2005). Rahman and Sarker (2019) have also documented that dietary multienzyme supplementation increases the palatability of *L. rohita*. However, studies on the impact of growth promoters on the palatability of fish are scarce, and additional research is required to draw definitive conclusions.

Table 9. Organoleptic scores of cooked fish flesh (Scale:0-10)

Parameters	CF	CG	EG	AP
Odor	7.0±0.94^{b}	8.6±0.84^{a}	8.4±0.96^{a}	8.8±0.63^{a}
Flavor	7.6±0.96^{b}	8.8±0.63^{a}	8.7±0.94^{a}	8.8±0.63^{a}
Texture	8.2±1.03^{a}	8.5±0.84^{a}	7.9±0.87^{a}	7.0±1.63^{a}
Mean Score	7.6±1.06^{b}	8.63±0.76^{a}	8.33±0.95^{a}	8.43±0.85^{a}

3.5 Economic Feasibility of the Feeds: The result showed that CG enriched feed was most-cost efficient among other experimental feeds and was 13.51±2.62% cheaper than CF. The other two growth promoter enriched feeds (EG and AP) also exhibited significant cost efficiency and were 5.98±1.82% and 5.73±1.65% more affordable, respectively (**Table 10**). The present experiment demonstrates that in terms of feed cost/kg fish production, the addition of CG (90.89±2.75 Tk.) in the diet of *L. rohita* is the most economical approach for its production using locally available feed ingredients followed by EG (98.81±1.92 Tk.) and AP (99.07±1.73 Tk.). Initially, growth promoter included feeds were more costly than the commercial carp grower feed in terms of cost per kg feed (**Table 10**) but they were significantly more cost-efficient due to higher FCR values (**Table 7**). Evidence of cost-effectiveness due to growth promoter supplementation has been reported in *O. niloticus* by Yimer et al. (2015) and Islam et al. (2014). As the prices of feed and feed ingredients have been steadily increasing, the cost of feeding cannot be directly compared to previous investigations. Nevertheless, while comparing the economics of the present experiment with earlier works of Jewel et al. (2018) and Zamal et al. (2009), it becomes clear that feeding cost in fish farming has alarmingly increased over the last decade in Bangladesh. In this context, using growth promoters in feed formulation can assist farmers and feed manufacturers achieve their objectives more efficiently.

Table 10. Economics of the experimental feeds

Economics	CF	CG	EG	AP
Feed cost/kg (Tk.)	41.00	45.75	42.65	43.45
Feed cost/kg fish production (Tk.)	105.10±2.06[c]	90.89±2.75[a]	98.81±1.92[b]	99.07±1.73[b]
Cost reduction (%) compared to CF	-	13.51±2.62[a]	5.98±1.82[b]	5.73±1.65[b]

4. CONCLUSION

Comment [2A3]: Add discussion too.

This study concludes that feed formulated using locally available ingredients with growth promoter yields better growth, body composition and palatability of flesh of *L. rohita* compared to the selected commercial feed, and the best result with the most economical outcome was obtained in charger gel inclusion. The concept of using growth promoter with feed to improve feed utilization is relatively new in the perspective of Bangladesh. Further research should be conducted to develop proper dosage and determine the long-term effect of using growth promoters in large-scale farms.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

The Rajshahi University Research Ethics Committee has exempted the need for ethics approval for this investigation in accordance with the National Wildlife Protection and Conservation Act, 2012 as the fish species used in this study are commercially produced and not a wild or endangered species. However, all procedures performed in this investigation were in accordance with the ethical guidelines provided by The International Council for Laboratory Animal Science (ICLAS) for researchers.

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IMAGE

Comment [2A4]: Add a few images of the work done on the model organism of the study