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Growth and Survival of Goldfish (*Carassius auratus*) Juveniles Fed *Tubifex*, Custard Meal and Commercial Feeds

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ABSTRACT

An experiment was conducted to investigate suitable feed for *Carassius auratus* juveniles. 30-day-old juveniles (3.31±0.14 cm and 0.84±0.3 g) were reared in glass aquaria (50×30×30 cm^3) at stocking density of 10 juveniles/aquaria in triplicates. The juveniles were fed four different feeds, *Tubifex*, egg custard, commercial feed, and aquarium feed, twice daily until satiation. After 28 days of trial, fish fed custard and *Tubifex* had significantly higher survival rates (91% and 88%) than those fed aquarium feed (81%, $P < 0.05$) and commercial fish feed (75%, $P < 0.05$). The final length, weight, absolute growth rate and metabolic growth rate were significantly varied among the treatments ($P < 0.05$). However, no significant differences were evident in weight gain and SGR ($P > 0.05$). The fish fed custard and *Tubifex* were comparable in terms of growth performance ($P > 0.05$) while a significantly lower growth rate was found in fish fed commercial feed ($P < 0.05$). Fish fed *Tubifex* and custard had significantly higher protein content (15.28% and 14.86%, respectively) than those fed commercial fish feed (13.41%) and aquarium feed (12.21%). The overall findings suggest that the formulated egg custard can promote growth and survival in goldfish.

KEYWORDS

Ornamental fish, *Tubifex*, egg custard, juveniles, growth performance

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1. Introduction

The rising interest in rearing ornamental fish has led to an increased international aquarium fish trade. Among the widely accepted ornamental fish, goldfish (*Carassius auratus*) is one of the commercially important species. It was originally popular from China to Eastern Europe and has now spread all over the world due to its wide range of morphological adaptation and diversity (Ota and Abe, 2016). Nowadays, this species is mostly reared in an intensive culture system where the growth of the fish largely depends on the nutritional quality and quantity of the food provided (Kaiser *et al.*, 2003). Food quality as well as availability affect the fish growth in all life stages and have a great influence on successful aquaculture. In a production cycle, the earlier life stage is considered the most crucial stage for most fish species (Abi-Ayad and Kestemont, 1994). The earlier life stage of goldfish requires specific environmental conditions including appropriate feeds to survive and grow (Kestemont, 1995).

The larval rearing of non-salmonid fish such as goldfish and carp largely relies on the culture of live feeds, although several efforts have been made to find suitable alternatives (Bryant and Matty, 1980). In general, living feed organisms contain higher crude protein (60–65%), moderate lipids (8–9%) and lower crude fiber (4–5%) (Sharma, 2020). Different live feeds, such as *Branchionus*, *Chirononus*, *Moina, Tubifex* and *Artemia* have long been used for rearing the larvae or juveniles of various fish species (Cruz and James, 1989; Evangelista *et al.*, 2005; Fermin and Recometa, 1988). Among the live feeds used in fish larvae culture, *Artemia* nauplii is the most widely used food item. However, the demand for *Artemia* cysts has exceeded the supply, and prices have risen exponentially, creating a bottleneck for the expansion of hatcheries (Lavens and Sorgeloos, 2000; Sorgeloos *et al.*, 2001) and increased problems for developing countries in terms of affordability (Evangelista *et al.*, 2005). Thus, research leading to the use of an alternative feed or at least optimization of cyst usage can reduce production costs.

Tubifex is one of the potential live feed candidates because of its substantial use as a nutritious food commodity for fish larvae. Although it grows in waste water with health hazard issues and the risk of spreading certain protozoan diseases in fish (Brinkhurst, 1996), the availability and appropriate size make this candidate prominent in the aquarium business as well as the commercial fish business. Moreover, *Tubifex* is rich in protein and essential fatty acids such as n-3 (C18: 3n-3 and C20: 5n-3) and n-6 (C18:2n-6 and C20:4n-6) fatty acids (Görelşahin *et al.*, 2018; Yanar *et al.*, 2003). It has already been established to increase the growth performance of different fish species such as *Chitala chitala* (Sarkar *et al.*, 2007), *Clarius macrocephalus* (Santiago *et al.*, 2003), *Notopterus chitala* (Sontakke *et al.*, 2019), and *Sander lucioperca* (Bódis *et al.*, 2007). Furthermore, several studies reported *Tubifex* as an alternative to *Artemia* for different fish species such as catfish (Arslan *et al.*, 2009; Evangelista *et al.*, 2005). Beside live feeds, studies reported that formulated egg custards are also used for larval rearing of different fish species and attained better results than commercially available formulated feeds (Malla and Banik, 2015). Formulated feeds are specially aimed to achieve optimal growth of certain fish species with species-specific, appropriate and adequate nutritional composition(Sultan Mohideen *et al.*, 2014). Moreover, these feeds are also being targeted to attain lower conversion ratio to minimize the feed cost.

Hitherto, there is no study comparing the effect of live feed and egg custard on goldfish. Therefore, the present study was undertaken to investigate the effects of live feed (*Tubifex*), homemade egg custard and two commercial feeds (fish feed and aquarium feed) on growth, survivability, and body protein content of the goldfish (*C. auratus*).

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2. Materials and Methods

2.1. Feeds:

Four experimental feeds, namely *Tubifex*, homemade egg custard, commercial fish feed (Quality Feeds Limited, Bangladesh) and aquarium feed (Sky Fish, China), were used in this study. Commercial fish feed was collected from the local feed market. Live *Tubifex* and aquarium feed commonly used for goldfish were collected from local aquarium shops. *Tubifex* was chopped into small pieces using a disinfected blade and scissors, and preserved in a refrigerator (-18°C) in the form of a small cube until use. The egg custard was prepared using several ingredients, as presented in Table 1. The ingredients were blended, and the mixture was boiled in an autoclave for 30 min. at 110–118°C. After cooling, it was cut into small pieces, individually wrapped with polyethylene film, and stored at -18°C until used. Moisture and protein contents of the experimental feeds are presented in Table 2.

Table 1: Ingredients (g/kg) used for preparing egg custard (Nik Sin and Shapawi, 2017).

Ingredients	Unit
Cornflower	50 g
Agar powder	20 g
Milk powder	500 g
Egg	325 g
Cod liver oil	5 g
Prawn meat	100 g

Table 2: Moisture (%) and protein (%, dry weight basis) of four different dietary treatments.

Dietary treatments	Moisture (%)	Protein (%)
Tubifex	82.28±0.32	55.64±3.3
Custard	70.23±0.41	43.08±2.54
Commercial fish feed	11.45±0.27	29.17±0.54
Aquarium feed	11.26±0.19	18.80±0.73

2.2. Fish Rearing:

Thirty-day-old goldfish juveniles were collected from the local fish breeders of Khulna city, Bangladesh and transported in oxygenated polythene bags to the Fish Physiology Laboratory of the Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh. Fish were randomly stocked in 12 rectangular glass tanks (50×30×30 cm^3) each containing 15 liters of water. All tanks were adorned with an air stone to ensure sufficient dissolved oxygen (DO). Key water quality parameters (temperature, pH, and DO) were checked regularly to maintain the water quality in optimum ranges (Table 3).

2.3. Experimental Procedures:

The experiment was conducted for 28 days. The four experimental feeds were randomly assigned in 12 tanks, resulting in 3 replications per treatment. In total, 10 fish with a mean initial length of 3.31±0.14 cm and weight of 0.84±0.3 g were randomly assigned to each of the 12 tanks from the common batch. About three-fourths of the water was changed twice a day before feeding (morning and afternoon). Adhered dirt inside the tank walls and small pieces of hose pipe used as fish shelters were cleaned twice a week.

The fish were hand-fed at their satiation level twice per day (8:00 am and 5:00 pm) and before every feeding, uneaten feed and feces were removed by siphoning. Before feeding, each *Tubifex* cube was thawed and given to the fish. Likewise, egg custard was thawed and crushed to make smaller pieces and sieved by a net with a 1 mm mesh size before feeding the fish.

2.4. Analytical Procedures and Calculations:

2.4.1. Fish Performance

At the end of the study, growth performances of individual fish were assayed by determining their weight and length gain, specific growth rate (SGR), and absolute and metabolic growth as described in Maas *et al.* (2021). Weight gain (g) was calculated as $W_f - W_i$, where W_f is the final weight of the fish, and W_i is the initial weight of the fish. Likewise, length gain (cm) was calculated as $L_f - L_i$, where L_f is the final length of the fish and L_i is the initial length of the fish. Absolute growth rate (GR_{abs}, g d^{-1}) was calculated as $W_f - W_i/t$, where t is the duration of the experiment. Specific growth rate (SGR, % d^{-1}) was calculated as $100 \times (\ln W_f - \ln W_i)/t$. Geometric mean body weight (W_g, g) and mean metabolic body weight (MBW_g, $kg^{0.8}$) were calculated as $\sqrt{(W_f - W_i)}$ and $(Wg/1000)^{0.8}$, respectively. Growth rate on metabolic weight (GR_{mbw}, g $kg^{-0.8}$ d^{-1}) was calculated as $(W_f - W_i)/(MBW_g \times t)$. Survival rate (%) was calculated as $N_f/N_i \times 100$, where N_f is the number of fish at the end of the experiment and N_i is the number of fish at the beginning of the experiment.

2.4.2. Determination of Moisture and Protein

At the end of the growth trial, three fish from each tank were randomly sampled to determine their protein and moisture contents. Fish samples were stored at -20°C until further analysis. Fish and feed samples were analyzed using the same methods. The pellet feed samples were analyzed as whole pellets. Frozen fish samples were ground and homogenized, and then fresh ground fish samples were taken to determine moisture and crude protein. Moisture was determined by oven drying at 105°C for 24 hours until achieving constant weight. Crude protein (N×6.25) was determined according to the Kjeldahl method (AOAC, 1995).

2.5. Statistical Analysis:

Statistical analysis was performed using the IBM Statistical Package for the Social Science (SPSS) program (version 26.0; NY, USA) (IBM, 2019). Normality was checked using the Shapiro–Wilk test. To check homogeneity of variance, Levene's test was performed. To determine group differences in growth performance, survival, and body protein, data were subjected to one-way ANOVA. The Tukey post hoc test was performed for comparison of the mean among different groups. Significance was considered at $P < 0.05$.

3. Results

3.1. Water Quality Parameters:

The ranges of temperature, pH, and DO are shown in Table 3. The results show that water quality parameters did not show any significant ($P > 0.05$) differences among the dietary treatments and were in the optimal ranges for goldfish juveniles.

Table 3: Water quality parameters (Mean±SEM) measured during the experiment over 28 days.

Parameters	*Tubifex*	Custard	Fish feed	Aquarium feed
Temperature (°C)	26.05±0.08[a]	26.35±0.22[a]	27.21±0.61[a]	26.26±0.14[a]
pH	8.29±0.23[a]	7.96±0.34[a]	8.29±0.16[a]	7.89±0.45[a]
DO (mg/l)	7.85±0.26[a]	7.59±0.21[a]	6.89±0.27[a]	7.33±0.33[a]

The same superscripts in a row indicate no significant differences ($P > 0.05$)

3.2. Growth Performance:

The influences of four dietary treatments on growth performance parameters are presented in Table 4, and Figs. 1 and 2. Initial length and weight of goldfish juveniles did not vary significantly among the dietary treatments ($P > 0.1$). All fish significantly increased in length and weight during the experiment ($P < 0.05$; Figs. 1 and 2). After the 28-day feeding trial, significant differences were observed in final total length (cm), final weight (g), length gain (cm), weight gain (g), absolute growth rate (g d^{-1}), and metabolic growth rate (g $kg^{-0.8}$ d^{-1}) ($P < 0.05$). There were no significant differences in growth performance parameters among fish fed custard and *Tubifex* except length gain. Fish fed custard had the numerically highest absolute growth rate (0.06 g d^{-1}) followed by those fed *Tubifex* (0.05 g d^{-1}) and aquarium feed (0.04 g d^{-1}). The lowest growth rate (0.03 g d^{-1}) was observed in fish fed commercial fish feed ($P < 0.05$). Similar patterns were also observed in metabolic growth rate (g $kg^{-0.8}$ d^{-1})and SGR (% d^{-1}).

Figure 1: Mean length (±SEM) of goldfish fed different dietary treatments over 28 days.

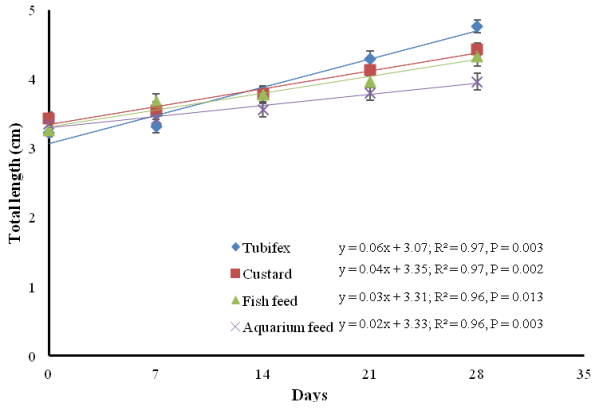


Figure 2: Mean weight (±SEM) of goldfish fed different dietary treatments over 28 days.

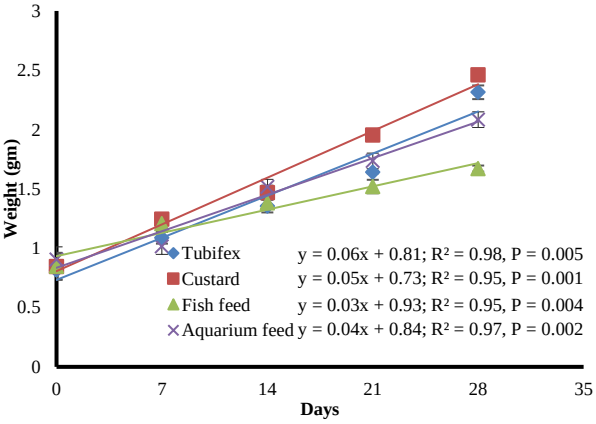


Table 4: Growth performance parameters (Mean ± SEM) of goldfish fed different dietary treatments.

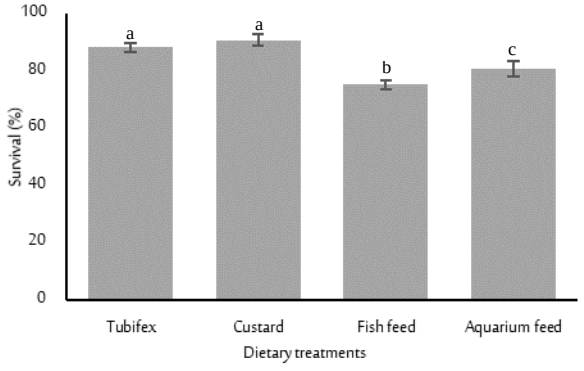
	Tubifex	Custard	Fish feed	Aquarium feed	*P* value
Length gain (cm)	1.54±0.12^{a}	1.00±0.08^{bc}	1.08±0.11^{bc}	0.62±0.04^{c}	0.001
Weight gain (g)	1.50±0.05^{ab}	1.61±0.07^{a}	0.82±0.09^{c}	1.18±0.08^{a}	<0.001
Weight gain (%)	183.59±4.03^{a}	199.56±33.53^{a}	104.84±28.55^{a}	135.35±21.58^{a}	0.087
SGR (% d^{-1})	3.69±0.05^{a}	3.85±0.40^{a}	2.48±0.47^{a}	3.03±0.34^{a}	0.081
GR_{abs} (g d^{-1})	0.05±0.00^{ab}	0.06±0.00^{b}	0.03±0.00^{c}	0.04±0.00^{a}	<0.001
GR_{mbw} (g kg$^{-0.8}$ d^{-1})	10.35±0.18^{a}	10.91±1.09^{a}	6.56±1.20^{b}	8.33±0.90^{a}	0.037

SGR = Specific growth rate; GRabs = Absolute growth rate; GRmbw = Metabolic growth rate
Different superscripts in the same row indicate significant differences (P> 0.05)

3.3. Survival:

The percentage of survival is shown in Fig. 3. The highest survival rate was recorded in fish fed custard (91%), which was higher in percentage than those fed *Tubifex* (88%), but significantly higher than those fed aquarium feed (81%) and commercial fish feed (75%). The lowest survival rate was found in fish fed commercial fish feed. Aquarium fed fish had significantly higher survival than those fed commercial fish feed (P< 0.05).

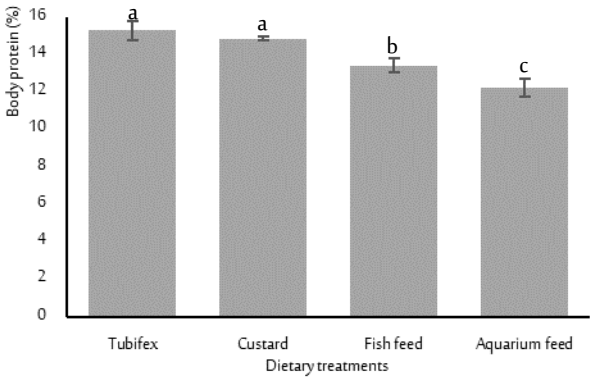
Figure 3: Survival of goldfish fed different dietary treatments. Different superscripts indicate significant differences among treatments (P< 0.05).



3.4. Protein Content in Fish Body:

Whole-body protein (%, wet weight) is presented in Fig. 4. Like survival, there was no significant difference in body protein among fish fed *Tubifex* and custard (P > 0.05). However, fish fed *Tubifex* contained numerically higher body protein (15.28%) than those fed custard (14.86%). The lowest body protein (12.71%) was found in fish fed aquarium feed (P< 0.05).

Figure 4: Body protein (%, wet weight) of goldfish fed different feeds. Different superscripts indicate significant differences among treatments (P < 0.05)



4. Discussion

Feeds and feeding are crucial elements in the culture of aquatic animals. Among the various life stages, the juvenile stage is considered one of the most sensitive phases during their life cycle. Juveniles of goldfish collected from the local market were reared for 28 days in this experiment to explore the effects of live and artificial feeds on growth performance and survival.

The results of the present study revealed that goldfish juveniles fed proteinaceous feed, *Tubifex* and custard, exhibited higher growth performance than those fed fish feed and aquarium feed. The higher growth achieved in juveniles fed *Tubifex* is similar to the findings of Mohanta and Subramanian (2002) and Mellisa *et al.* (2018) who found that goldfish fed *Tubifex* exhibited better growth performance. It has already been proved in different studies that *Tubifex* is one of the potential live feed candidates for larvae of different aquarium fish species such as zebrafish (*Brachydanio rerio*) (Bouguenec, 1992), guppy (*Poecilia reticulata*) (Görelşahin *et al.*, 2018), knifefish (*Chitala chitala*) (Sarkar *et al.*, 2006), Siamese fighting fish (*Betta splendens*) (Mandal *et al.*, 2010), and sailfin molly (*Poecilia latipinna*) (Mohideen *et al.*, 2014). Moreover, *Tubifex* also exhibited better performance in other fish species. South American catfish, surubim (*Pseudoplatystoma fasciatum*) juveniles fed *Tubifex*, for example, exhibited higher growth performance than those fed other formulated feeds (Arslan *et al.*, 2009). Likewise, higher growth performance by *Tubifex* was reported in two Asian catfish, *Pangasius bocourti* (Hung *et al.*, 2002) and *Clarias macrocephalus* (Evangelista *et al.*, 2005).

The differences in growth performance in the present study could be attributed to the nutrient composition in terms of protein content and the acceptability of the feeds, although many factors are related to making differences in growth performances. The adult goldfish can grow with vegetables matters, while juveniles of goldfish require a higher protein content in their feeds for better growth (Bandyopadhyay *et al.*, 2005). It has been reported that 40% protein is sufficient for optimal growth of goldfish (Mohanta and Subramanian, 2002). In the present study, the crude protein contents of the experimental feeds were 55.64, 43.08, 29.17, and 18.80% in *Tubifex*, custard, fish feed, and aquarium feed, respectively. Therefore, higher growth in *Tubifex*- and custard-fed

juveniles might be due to the presence of higher protein content in *Tubifex* and custard. The specific amino acids composition may play a great role in the growth performance of fish. *Tubifex* is rich in lysine (Yanar *et al.*, 2003), which acts as a growth promoter in juvenile goldfish, and it is reported that goldfish juveniles require feeds with high lysine content (Gatlin, 1987). Therefore, *Tubifex* could be a potential live feed candidate for goldfish juveniles. However, *Tubifex* is related to health hazard issues as it grows in waste water and also acts as a host of a myxozoan parasite, *Myxobolus cerebralis* (Brinkhurst, 1996). Although custard had lower protein content than *Tubifex*, fish fed custard showed numerically higher growth performance than those fed *Tubifex*. This may be due to the softness, palatability, and nutritional composition, as well as high consumption, which provided energy for fish growth. Therefore, homemade custard could be a promising alternative to *Tubifex* for ornamental fish species because the preparation of custard is easy for households. Moreover, the development of custard feed could reduce the dependency on live feeds as well as operational costs.

The poor growth rate observed in fish fed the pelleted feed suggests that the pellets used in this study were not suitable for optimal growth of goldfish juveniles. Formulated feeds can only be used for larval rearing of many finfish species if the level of acceptance is adequate and the particle intake per unit time per liter of water is high enough to prevent rapid disintegration of the feed (Pillay, 1993). In the present study, commercial fish pellet was used to compare the palatability with other feeds. The growth rate of fish with appropriate quality and adequate quantity of feed mostly depends on voluntary feed intake and assimilation of nutrients (Diana *et al.*, 1988; Sarkar *et al.*, 2007). It has been reported that feed acceptability is influenced by chemical stimuli created by the feed (Mackie and Adron, 1978). Moreover, feed intake largely depends on certain crucial factors, such as feed size, type, physical appearance, and attractiveness (Sarkar *et al.*, 2007). Although fish feed contained higher protein (29.17%) than aquarium feed (18.18%), the results showed that goldfish juveniles fed fish feed had significantly lower growth ($P < 0.05$). The crude protein present in the fish feed might have higher indigestible protein for goldfish, which, therefore, affected the feed utilization. Furthermore, it can be speculated that more energy was spent in processing the fish feed after ingestion resulted in poor growth.

Higher survival determines the success of any aquaculture operation. The survival rates were comparable between fish that consumed *Tubifex* and custard ($P > 0.05$) but significantly higher than those fed aquarium and fish feed ($P < 0.05$). In accordance with the present study, Mellisa *et al.* (2018) and Mohanta and Subramanian (2002) also recorded comparatively higher survival rates in goldfish juveniles fed *Tubifex*. The improvement in survival by *Tubifex* was also observed in *Pangasius bocourti* (Hung *et al.*, 2002), *Betta splendens*(Mandal *et al.*, 2010), *Pseudoplatystoma fasciatum* (Arslan *et al.*, 2009), *Poecilia latipinna* (Mohideen *et al.*, 2014), and *Poecilia reticulata* (Görelşahin *et al.*, 2018). Lower survival rates in goldfish juveniles fed fish feed may indicate the inappropriateness of commercial feeds for goldfish. Kaiser *et al.* (2003) also reported significantly lower survival rates in goldfish fed artificial feed than those fed live feed (*Artemia*) and combination, which is in line with the present study.

The findings of this study revealed that fish fed proteinaceous feeds had higher body protein content than those fed feed with lower protein levels. The highest body protein content was found in fish fed *Tubifex*, whereas the lowest body protein content was reported in fish fed aquarium feed. It is reported that goldfish can easily assimilate protein in their body and thus egest less nitrogen (Bandyopadhyay *et al.*, 2005); however, it depends on feed quality. Bandyopadhyay *et al.* (2005) found that goldfish fed commercial feed had higher nitrogen and lipid excretion, and therefore, protein was not assimilated in the body, which resulted in poor feed utilization. Although *Tubifex* contained comparatively higher protein content than custard, the present study revealed numerically higher growth performance in fish fed custard than those fed *Tubifex*. In the present study, nitrogen balance was not investigated; therefore, further study is needed to solve this paradox.

5. Conclusions

The present study identified suitable feed for goldfish juveniles. In goldfish juveniles, custard meal and *Tubifex* yielded better growth than those fed fish feed and aquarium feeds. Custard meal and *Tubifex* not only reduced mortality but also improved the protein content in muscle. Considering the overall growth performances, availability, price, formulation, and ecological and economic benefits, custard meal could be a suitable feed for the juveniles of goldfish and other ornamental species. However, digestion and assimilation of the tested feeds in this study deserve further investigation.

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