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Influence of Fish Meal, Yeast and Maize on the Growth and Survival of Freshwater Zooplankton *Daphnia magna*

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***Abstract*—**Culturing the zooplankton, Daphnia, in the local environment is quite challenging due to the low survival and slow growth rate. The use of locally available raw materials as their substrate may increase the survival and the growth rate of Daphnia. Therefore, this study was carried out to evaluate the effect of locally available raw materials in three different feed combinations to nourish Daphnia magna, namely; substrate 1 prepared by combining maize and yeast , substrate 2 with fish meal, and yeast, and substrate 3 with cow dung. Additionally, the dried yeast alone was used to feed D. magna as the control treatment. Each treatment and control had three replicates in which 1000 D. magna were stocked in each 10 l plastic container and fed with the same rate of substrate 1.8 mg/10 ml. The results showed that the specific growth rate of D. magna was satisfactory in both substrate 1(7.74±1.64%) and 2 (7.705±0.71 %). However, substrate 2 was recorded with significantly higher growth performance than that nourished by substrate 1. Interestingly, the propagation (survival) and growth performance of D. magna fed by both substrate 1 and 2 were better than that fed by control substrate. Therefore, the study demonstrated that the substrate 1 and 2 resulted in the best propagation and growth performance of D. magna. Furthermore, D. magna which were cultured under the substrate of fishmeal and yeast can be used to increase the survival of fish larvae.

***Keywords*—**Daphnia magna, fishmeal, yeast and maize, growth performance, mortality rate, mixed diet

I. Introduction

The increased demand for aquaculture products among the local consumers attracted the government and private institutions which are sprouting interest in various sectors of aquaculture. The quality and the quantity of the fish broods is mainly depending on the feeds which are given to the broodstocks during their reproduction (Lim et al, 2003; Kruger et al, 2001). Fish especially require protein rich live food for their better growth, efficient breeding, and survival (Lall and Tibbetts, 2009). There are three types of feed used in fish ponds namely; natural, supplementary, and commercial feeds (FAO). Feeding with natural feeds to early life stages of fish will increase the fish nutrition such as amino acids, unsaturated fatty acids, vitamins, minerals, and other components that are necessary for the health and growth of fish (Das et al, 2012). Tuchapska and Krazhan (2014) revealed that natural feeds encompass entire essential nutrients for the development and growth of fish and immunity are immensely affected by the portion of natural feeds in the fish diet by integration of artificial feed. Some of the natural food organisms for fish include phytoplankton, zooplankton, annelids, worms, insects, mollusks, etc. (FAO, 2004-2021). The cultivation of planktonic crustaceans as live feed is very important for the aquaculture and aquarium industries (Jepsen et al, 2020) as most of the fish prefer to feed on planktonic crustaceans.

Daphnia magna is small freshwater zooplankton and micro crustacean that are widely used as live food in aquaculture. *D. magna* is a highly nutritious live food containing 45-70% of protein and 11-27% of lipid (Jorge et al, 2016). The main benefit of culturing and supplying *D. magna* for fish is that they have the shortest possible generation time to reproduce, it can be actively moved and can be adapted to the size and shape of the mouth of fish immediately after fish catch them (Cheban, 2017). Nevertheless, *D. magna* can be provided sufficient content of nutrients for fish nutritional needs (Bogut et al, 2010).

Most fish rearing farms reared their post larvae in hatcheries under their best supervision by feeding on commercial feeds. In accordance to Becker (2007), 40% of the whole aquaculture productions are kept their reliance mainly upon commercial feeds. Although usage of commercial feed is popular at present, it is not enough to fulfill the nutrient requirements and survival of the early stages of the fish.

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Feeding the fish with *D. magna* may overcome the risk of high mortality during culturing post larvae in hatcheries (Khan et al, 2020). Although Artemia is used to overcome nutrient requirements in the fish industry, they are very expensive and lacks availability. Therefore, cheaper alternative diet with similar nutritional quality is needed to maintain the cost competitiveness of the fish in the global market (Khan et al, 2020). *D. magna* can be introduced without any argue for such alternative diet due to their low cost and high availability.

Feed substrate to culture *Daphnia*

Although many feed substrates are used to feed *D. magna*, it was not demonstrated rapid reproduction rate and high survival of *D. magna*. Therefore, it should be considered what feed substrates are the most applicable with their preference. Rottmann (2011) explained the use of yeast, cow manure, alfalfa, seaweed etc. as fertilizers for the cultures of *Daphnia* and *Moina*. Yeast was demonstrated as a good feed substrate for the mass culture of *Moina* (Kang et al, 2006). The Daphnia species can survive, grow, and reproduce on maize materials (Chen et al., 2021). The present study was aimed to discover the most profitable and productive feed using animal waste, cow dung to feed *D. magna*. Cow dung is easily available animal waste. Cow dung is an organic fertilizer and detritus to the growth of phytoplankton which can be consumed as feed by *Daphnia*. Furthermore, providing cereal substrate as maize to *D. magna*, is very important to enhance their growth rate by fulfilling the nutritional requirements of *D. magna*. Maize flour can serve as a nutrient enriched food source to address the problems of protein energy malnutrition and macronutrient deficiencies (Bamidele, and Fasogbon, 2020). Maize flour contained ash 3.3%, protein 12.45%, crude fiber 2.97% and total carbohydrates 60.23% and also yellow maize flour contains 16, 26.21, 7.43, 5.29, 1.40, 2.11 times higher value of Mg, K, Mn, Zn, Fe and Cu as compared to white maize flour (Qamar at el. 2017). Maize is a locally available crop and can easily be procured. Furthermore, dried fish is also a very rich source of proteins, fats, and also essential nutrients such as iodine, zinc, copper, selenium, and calcium (Bimal et al., 2020). The present study was done using cost-off dried fish waste to make fishmeal.

Due to the higher protein, fat and carbohydrate contents in the Daphnia (Cheban et al, 2018), the fish larvae reared by feeding with *D. magna* may result with rapid growth rate, high fertile and better nutritional value of fish. Furthermore, feeding the fish with high nutritious and low cost *Daphnia* will increase the profitable aquaculture by reducing the cost for commercial feed. Therefore, the present study was aimed to culture *D. magna* by using locally available feed substrates and evaluate their growth performances.

II. Materials and Methods

The 100ml of pure *D. magna* culture sample in a petri dish was isolated under a light microscope with the help of a fine dropper and kept for the reproduction of new born *D. magna* to subsequent experiments. To set up the habitat a plastic container with 10ml of chlorine free water was filled. An air pump was installed and aeration was supplied in the container to aerate water thoroughly two days prior to the introduction of *D. magna*. The pH at the level between 6.5-8.0 and the water temperature between 24-28oC were maintained. One thousand of *D. magna* were added into the container. 10-20% of the water was changed every week. *D. magna* was fed while the physical characteristics of water such as color water were not changed.

A. Experimental design

The experiment was conducted in three trials each for 7 days using 12 plastic containers with an additional backup container. While the first trial was conducted using three treatments each with four replicates, trials 2 and 3 were conducted using four treatments each with three replicates. For the first trial, each of the sixteen 50 l containers containing 10 l of chlorine free water was stocked with 1000 individuals of *D. magna*. Every container was covered with a net to protect *D. magna* from insects. In the first trial, 1.8 mg cow dung mixed in 40ml of water was used as treatment 1, while treatment 2 was prepared by mixing maize flour, soybean flour, and dried fish flour in 1:1:1 ratio, each with 0.6 mg to make 1.8 mg mixed with 40 ml of water. Treatment 3 was used as a control by mixing 1.8 mg of yeast in 40 ml water. Each substrate was given evenly for four replicates each with 10 ml. For the second and third trials, substrate 1 for treatment 1 was prepared by mixing 0.8 mg maize and 0.8 mg yeast in 30 ml of water, substrate 2 for treatment 2 was prepared by mixing 0.8 mg fishmeal and 0.8 mg yeast in 30 ml of water, substrate 3 for treatment 3 was prepared by mixing 0.8 mg cow dung in 30 ml of water, and treatment 4 was used as a control by mixing 1.6 mg of yeast in 30 ml of water. Each of the treatments was replicated and 10 ml of a substrate prepared was supplied to each replicate (Table 1).

quality parameters such as temperatures, pH, dissolved oxygen (DO) were collected daily. The density of *D. magna* was measured daily. Water quality parameters such as temperature, DO and pH were also monitored. Following formula was derived to calculate the Specific Growth Rate (r) based on the methods described by Bukovinszky et al (2012):

$$r = \frac{(Fd) - (No)}{t} \tag{1}$$

Where, Fd = Final density of D. magna ,
No = Initial density of D. magna,
t = Time interval between the initial and final density estimated,
r = Specific Growth Rate.

Calculation of Survival rate as follows,
Survival rate (%) = No. of live animals/ No.of animals initially introduced $\times 100$

B. Data analysis

The difference of the density of D.magna based on the different treatments was analyzed by One-way ANOVA by

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Table I: Constituents of the substrates used in the treatments of all three trials.

Treatments	Trial 1	Trial 2	Trial 3
T1	Cow dung (1.8 mg mixed with 40ml of water)	Maize flour + yeast (0.8 mg of each one mixed in 30ml of water)	Maize flour + yeast (0.8 mg of each one mixed in 30ml of water)
T2	Maize flour + soybean flour + fishmeal (0.6 mg of each one mixed in 40ml of water)	Fishmeal + yeast (0.8 mg of each one mixed in 30ml of water)	Fishmeal + yeast (0.8 mg of each one mixed in 30ml of water)
T3	Yeast (control) (1.8 mg mixed with 40ml of water)	Cow dung (0.8 mg mixed in 30ml of water)	Cow dung (0.8 mg mixed in 30 ml of water)
T4	-	Yeast (control) (1.6 mg mixed in 30ml of water)	Yeast (control) (1.6 mg mixed in 30ml of water)
No. of replicat	Four	Three	Three
50 l containers was filled with 10 l of water for each replicate. 10 ml of substrate was added to each replicate.			

using Minitab 16 followed by Fisher's, individual error rate test. Statistical significance was set as p 0.05.

III. Results

A. The population density of Daphnia magna

The present study demonstrated that substrate 2 prepared by mixing dried fish flour and dry yeast and supplied to *D. magna* had changed the growth performance of *D. magna* where it increased the final density and daily density as shown in (Table 2). However, there were no significant variations monitored in initial density and final density gain in T3 diet with cow dung and T4 diet with dry yeast. In trial one, the Average Daily Density gained in T3 diet with yeast (control) was significantly higher (p0.05) than other treatments and there was very low survival in T1 and T2 (Figure 1). Both trials 2 and 3, Average Daily Density gained in T1 and T2 were significantly (p0.05) higher than other treatments.

The highest density was observed in T2 diet with fishmeal plus dry yeast as compared to other treatments for all sampling days (Figure 2). The peak density of *D. magna* observed in T2 was achieved on the 7th day both trial 2 and trial 3. T2 showed the highest density of *D. magna* which was 761 ± 82.32 ind/l in trial 2 and 782 ± 85.43 ind/l in trial 3. Furthermore, the density of *D. magna* of T2 was significantly different (p<0.05) from that of T1 where *Daphnia's* density was 747 ±85.43 ind/l and 607 ± 60.06 ind/l in trials 2 and 3 respectively. T2 was also significantly different (p<0.05) from T1 which was 50 ± 11.02 ind/l in trial 2 and 64 ± 4.81 ind/l in trial 3 (Figure 3). Both T1 and T2 were significantly different (p<0.05) from treatment T3 and T4. T4 (control) was showed a density of *D. magna* which was 129 ± 43.50 ind/l in trial 2 and 322 ± 54.22 ind/l in trial 3.

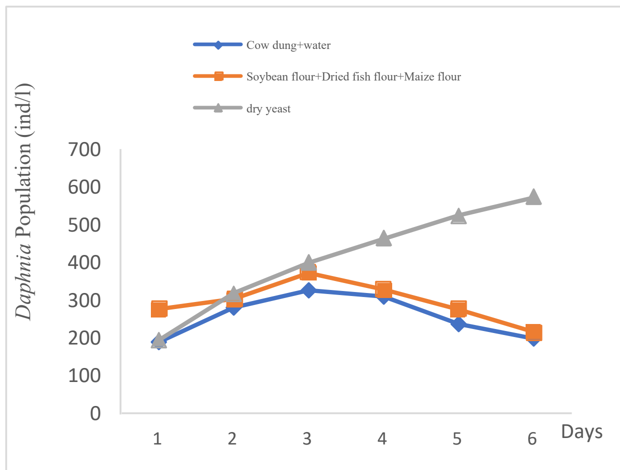

Figure 1: Mean density of *Daphnia magna* (± SD) at the trial 1 at four different feed levels.

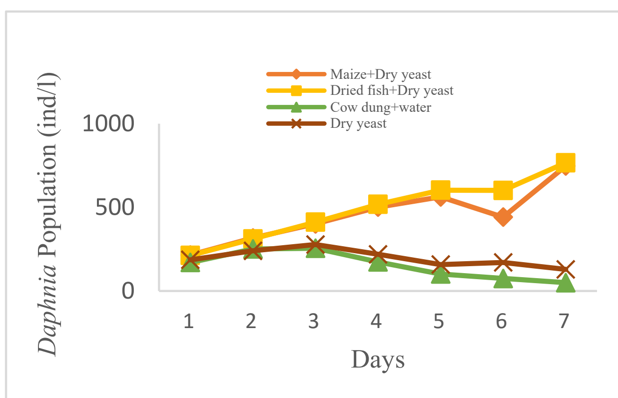

Figure 2: Mean density of *Daphnia magna* (± SD) at the trial 2 at four different feed levels.

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Table II: *Daphnia magna* mean (± SD) density at the trial 1, trial 2, and trial 3 at four different feed levels.

	Initial Density (Daphnia ind/L)			Final density gain (*Daphnia ind/L*)		
Treatments	Trial-1	Trial-2	Trial-3	Trial-1	Trial-2	Trial-3
T1	266.67 ± 86.35	191.67 ± 61.66	154.17 ± 18.71	198.96 ±14.16	747.22 ±85.43	606.94 ± 60.06
T2	254.16 ± 23.16	191.66 ± 61.16	145.83 ± 13.51	216.67 ± 55.59	761.11 ± 82.32	781.94 ± 85.43
T3	245.83 ± 54.51	195.83 ± 83.24	145.83 ± 13.51	572.91 ± 61.66	50.00 ± 11.02	63.89 ± 4.81
T4	-	191.66 ± 61.16	145.83 ± 13.51	-	129.17 ± 43.50	322.22 ± 54.22

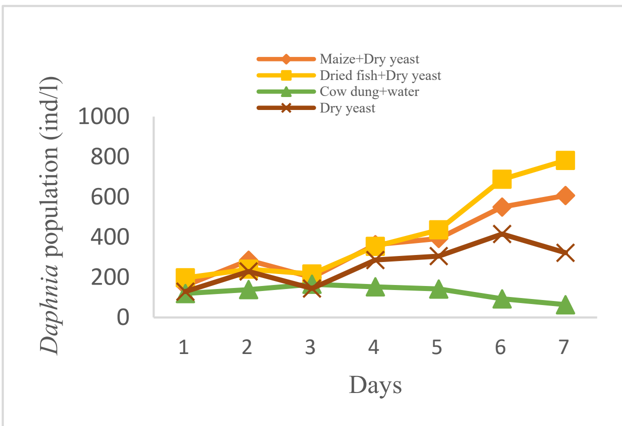

Figure 3: *Daphnia magna* mean (± SD) density at the trial 3 at four different feed levels.

B. Specific Growth rate (SGR)

The effect of the addition of three different feed combinations on SGR of *D. magna* is presented in Figures 4. The result showed that the SGR of *D. magna* was significantly ($P<0.05$) highest in T2 in both trials 2 and 3 (SGR 79±7.77 ind/l/day and 84±11.639 ind/l/day respectively). In T1 was also showed significant different ($P<0.05$) in both trail 2 and 3 which were 76±19.12 ind/l/day and 64±9.49 ind/l/day while the lowest SGR was found in T3 which was -17±5.86 ind/l/day in trial 2 and -8±3.27 ind/l/day in trial 3. According to the result, *D. magna* requires adequate nutrition content for their reproduction. T3 and T4 were fed for *D. magna* are insufficient resulting low Specific Growth Rate.

The survival rate of *D. magna* was significantly related to their types of substrate. The survival rate of *D. magna* feed on the fishmeal and yeast (T2) was 78.19% and 76.11% in both trial 2 and 3 respectively (Fig. 5).

C. Water quality management

The water quality parameters like pH, Dissolved Oxygen, Ammonia and Temperature were analyzed regularly morning (8.00 am) and evening (2.00 pm). There was not significant different between treatments (Table 3).

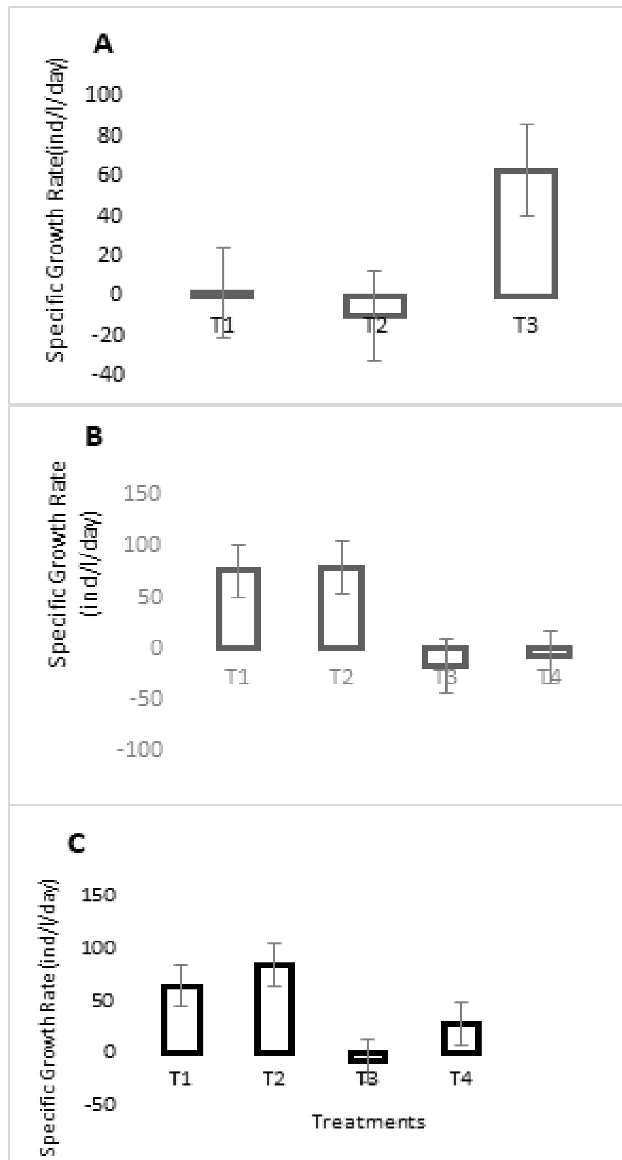

Figure 4: Changes of specific growth rate among different treatments of T1 (maize and yeast), T2 (fishmeal and yeast), T3 (cow dung) and T4 (yeast) in the Trial 1 (A), Trial 2 (B) and Trial 3 (C).

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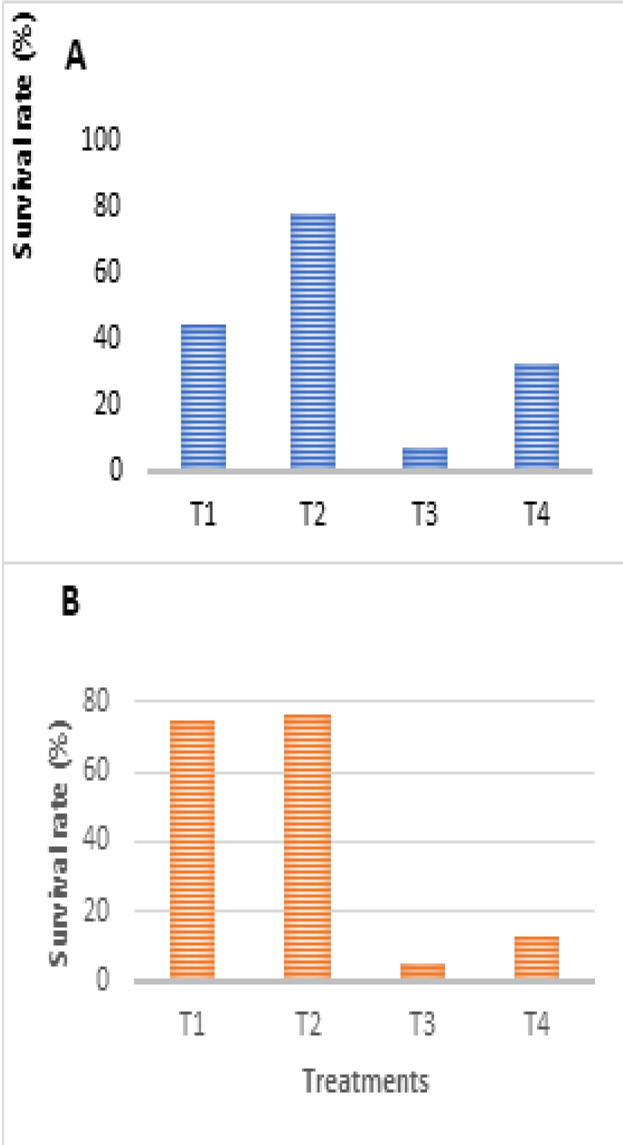

Figure 5: The survival rate of *D. magna* was raised under the substrate T1:T2: T3 and T4 in both trials 2 (A) and 3 (B).

IV. Discussion

The result of the present study proved that *D. magna* showed better performances in the mixed diet than the single diet, when the density of *D. magna* was observed using different feed trials. Similarly, Khan et al. (2020) studied the production of *D. magna* in laboratory conditions and reported that *D. magna* preferred a combined diet than a single diet. In the present study, a combined diet of T1 showed the highest population density where the T4 showed the lower population density than it. No increased population density was observed in T3 and T4 which indicated that without any supplemental diet *D. magna* showed poor growth and reproduction. Similarly, Rottman (2011) also indicated the sets of feed combinations for the growth of *Daphnia* and *Moina*. However, the recent study (Chen et al, 2021) showed that the use of different maize lines has different results in the performance of Daphnia.

Table III: Summary of the water quality parameters in the experimental tanks.

Water quality parameters	Morning	Evening
pH	7-7.5	7-7.5
Dissolved Oxygen	2.5-3 mg/l	2.5-3.5 mg/l
Ammonia	>0.1 ppm	>0.1 ppm
Temperature	25-27	26-28

In this experiment, yeast was used for making feed supplement because it has a high potential to enhance the growth of *D. magna*. Most of the experiments were found that by providing yeast they were able to increase the growth rate of *D. magna* growth performance but also fulfill their nutrient requirement. By supplying fishmeal and maize could be able to enhance their nutrient content. Maize contains a number of important B vitamins, folic acid, vitamin C, and provitamin A. It is also rich in phosphorus, magnesium, manganese, zinc, copper, iron and has small amounts of potassium and calcium (Chaudhary et al, 2014). Fishmeal is also rich in protein (amino acids), readily available throughout most of the world, a comparatively priced against other animal proteins (e.g. milk and blood) (Cho and Kim, 2010). Furthermore, Zinn et al. (2009) found that fishmeal carries large quantities of energy per unit weight and is a source of high quality protein and highly digestible essential amino and fatty acids. Moreover, the cost of feed was reduced by mixing maize flour in the fish meal and using locally available materials which are abundant everywhere in the country.

V. Conclusion

The substrate prepared out of fish meal and yeast and the substrate prepared using maize flour and yeast had increased the growth performance of *D. magna* as its daily and final density increased. Furthermore, feeding *D. magna* with the diet combined with fishmeal and yeast has demonstrated higher growth performance than that of maize flour and yeast. However, both feed combinations had significantly increased the growth performance of *D. magna* than the control diet. Interestingly, the cost of both feed combinations had reduced the cost of feed in aquaculture and caused the best propagation. The outcomes of the present experiment demonstrated that the diet combined with fishmeal and yeast could be used as the best option as an additive for *D. magna* feed for hatchery owners. Therefore, fishmeal and yeast are adaptable feed to culture *D. magna* to enhance their reproduction while enhancing the nutritive value.

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