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Evaluation Of Feed Intake, Growth, And Reproduction In The Zebrafish, Danio Rerio, Fed A Gel-Based Diet

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EVALUATION OF FEED INTAKE, GROWTH, AND REPRODUCTION IN THE ZEBRAFISH, *DANIO RERIO*, FED A GEL-BASED DIET

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A THESIS

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BIOLOGY

ABSTRACT

The zebrafish, *Danio rerio*, is a valuable pre-clinical model, aiding human health understanding and translational aquaculture. Comprehensive documentation of the culture of this model exists, with ongoing efforts to optimize husbandry requirements, particularly in the areas of diet and nutrition. Labor or mechanical costs related to feeding are high in most laboratories and alternative protocols to reduce feed handling would be beneficial and could allow the capability to monitor feed consumption. A commercially produced gel-based diet has a high moisture content, mimicking natural live prey. This diet maintains its physical integrity in the water, and one daily ration can be consumed over 24 hours. The present study goal was to evaluate *D. rerio* fed gel-based diets relative to (1) feed intake parameters, (2) long term growth, survival, reproductive success, and (3) efficacy when fed during larval development. We found that adult *D. rerio* readily accommodate to the gel diet. In addition, feed intake is highest in the morning after lights-on, although they continue to feed during the dark period. Feed intake was density dependent, with higher levels of feed intake observed at the highest densities tested (5 individuals/L). Juvenile *D. rerio* were fed one of three diets over a 12-week period, including a dry granulated reference diet, a commercial gel diet, and a dry granulated form (dehydrated and ground to the same physical form of the reference diet) of the gel diet. Survival was > 95.8% in all diets and supported growth and reproductive success. Terminal measures of weight and length in females fed the reference diet or gel-based diet did not differ significantly, whereas males fed the reference diet had the highest terminal weight and length measures. Reproductive success was similar among treatments, with all treatments exhibiting substantial egg production and embryo viability. Early replacement (at 6dpf) of a live diet for a gel-based diet negatively impacted survival and final weights. However, the gel diet supported survival and growth when fed at 12dpf or higher. Our study supports the use of a gel-based diet in daily *D. rerio* colony maintenance, but further nutritional assessments are necessary.

Keywords: Zebrafish, diet, nutrition, gel, feed intake, growth

DEDICATION

This thesis is dedicated to my family, but most importantly to my nana, Bobbie Howard. Without your love, support and encouragement, this achievement would not have been possible.

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LIST OF ABBREVIATIONS

AZ-GE	aquagel z – gel
AZ-GO	aquagel z – original
AZ-GR	aquagel z – granulated
DPF	days post fertilization
FAA	free amino acids
FCE	feed conversion efficiency
FCR	feed conversion ratio
HPF	hours post fertilization
RD	reference diet
RO	reverse osmosis
SEM	standard error of mean
TAN	total ammonia nitrogen

INTRODUCTION

Over the last few decades, the zebrafish *Danio rerio* has become an increasingly useful model organism for biomedical research (Tavares and Lopes 2013, Choi, Choi et al. 2021), aquaculture (Ribas and Piferrer 2014), environmental toxicology (Stegeman, Goldstone et al. 2010), and drug screening (MacRae and Peterson 2015) . This is in part due to their small size, high reproductive output with optically clear embryos, close genetic relatedness to humans, and relatively low maintenance cost. Although *D. rerio* has been utilized in many studies, there are concerns about formulated diets and feed management practices that have not yet been fully addressed (Watts, Lawrence et al. 2016). Among these concerns are a lack of understanding regarding best husbandry practices (Lawrence 2011), inadequate understanding of nutritional requirements, insufficient dietary reporting standards, and an absence of a standardized reference diet (Williams and Watts 2019, Watts and D'Abramo 2021). When comparing the knowledge base of *D. rerio* nutritional requirements to other biomedical models, the lack of standardization in reporting is especially concerning. Similar concerns were expressed in the early development of rodent models (Nielsen 2018). Additionally, a number of diets exist in murine models to induce specific disease states to allow for further investigation of real-world dietary maladies (Lang, Hasselwander et al. 2019).

In addition to the lack of nutritional information in zebrafish, the relatively small size of the zebrafish and the aquatic environment pose additional challenges. Small fish require either a live diet, which itself has limitations, or a granulated nutrient-dense formulated diet with a small size and high surface area: volume ratio that can promote significant leaching if not consumed rapidly. For these reasons zebrafish are often overfed to compensate for dry matter and nutrient leaching. Additionally, it is common to provide feed multiple times per day either by research personnel or through robotic assistance (Lawrence, Best et al. 2012). Daily feeding provided via research staff can require an extensive time commitment, and this can vary among personnel if protocols are not followed precisely and can add to the cost of animal maintenance. Robotic feeding requires staff to be trained in the proper loading and maintenance of the devices, and contractual assistance may be needed. These options are costly, time consuming, and can produce confounded results.

To reduce leaching, it may be possible to encase dietary ingredients within a gel matrix in the form of a wet (>20% moisture) pellet. These moist formulated diets should be stable in water to reduce nutrient losses, be physically accessible to the fish, and have the physical and chemical characteristics that would promote attractability and palatability. Gel-based diets may reduce labor associated with typical feed management practices by reducing the number of feedings per day.

Another important nutritional challenge in zebrafish husbandry is early life diets and feed management. A few laboratories have had successes with formulated diets at first feed; however, most studies that have utilized formulated diets in early life history stages have resulted in severely reduced growth (Kaushik, Georga et al. 2011). Consequently, most laboratories employ live diets of small crustaceans to support early development and digestive competence. Unfortunately, live diets can be extremely variable, costly, and may require time consume maintenance of stocks (Best, Adatto et al. 2010).The use of gel diets in early life history stages has not been considered in most facilities and requires further evaluation. We hypothesize that in addition to easing the daily burden of feeding multiple rations, gel-based diets offer a food source that more closely mimics the moisture content of natural food sources and can be available for consumption at any time when provide in sufficient quantity (Lal, Biswas et al. 2023). In this study we fed zebrafish a commercially available, gel-based diet. We evaluated 1) feed intake parameters associated with the daily introduction of a gel diet to adult zebrafish, 2) growth of juvenile fish fed a gel-based diet in comparison to a granulated dry diet of identical nutrient composition as well as a granulated reference diet, and 3) the use of gel diets in early life history stages.

METHODS

Research Approval

The research protocols involving vertebrate animal studies were approved by the UAB IACUC (Institutional Animal Care and Use Committee protocol number 20656) and followed the established *D. rerio* husbandry guidelines for housing and euthanasia.

Feed Intake Parameters

Diet Preparation and Handling

AquaGel Z 2021 gel feed formulation (AZ-GO) was provided by Clear H_2O (Westbrook, ME, USA) in a ready-to-use form in airtight packages of approximately 190g each. Packages of AZ-GO were stored at 4°C refrigeration**.** Five 1g cylindrical rounds (pellets) of AZ-GO (approximately 1cm in height and diameter) were dried to constant weight in a 50°C forced-air oven. These rounds were uncoated and homogenous in composition between surface and interior. The average weight of the dried AZ-GO feed rounds after reaching a constant weight (around 72-hr in the oven) represented the dry mass of the AZ-GO feed while the weight lost to evaporation represented the wet mass of the feed (70.8%).

In the following experiments for AZ-GO consumption, a plastic punch was used to cut out cylindrical rounds of AZ-GO that each weighed approximately 2g and were approximately 2cm in height and 1cm in diameter. Individual rounds were weighed and then placed in 50mm small Petri dish lids. Long forceps were used to place a Petri dish containing the gel round in the bottom front interior of *D. rerio* tanks, unless otherwise indicated. This method avoided direct handling of the AZ-GO feed, thus minimizing loss of material. Following experimental feeding periods, Petri dishes and remaining AZ-GO rounds were removed with forceps. AZ-GO rounds were placed on paper towels for approximately 1 min to remove excess water, then final weights were measured to 0.001g (Mettler Toledo Model AJ100 Mettler-Toledo, LLC, Columbus, OH, USA) and recorded.

In addition to the tanks containing *D. rerio*, AZ-GO rounds were also placed in 2.8L tanks without *D. rerio* to estimate weight change of the AZ-GO round when held in water for the same time period on the same recirculating aquaculture system (controls). The average weight loss or gain of the control values was used to predict the amount of AZ-GO consumed by *D. rerio*.

Husbandry

Male and female AB line *Danio rerio* provided by University of Alabama Birmingham Lab Animal Nutrition Core (Birmingham, AL) were bred together in a mass spawn and eggs produced were incubated at 28.5°C from 0 – 5 days post fertilization (dpf) in 100mm diameter Petri dishes. Hatched *D. rerio* fry were placed in a polyculture with rotifers and fortified with previously frozen *Nanno chloropsis* from 5 – 10dpf (Fowler, Williams et al. 2019). *D. rerio* fry were then moved to a recirculating Aquaneering Zebrafish Housing Rack (Aquaneering, San Diego, CA) with biological and physical filtration in 6L tanks of approximately 45 fish per tank and provided live *Artemia nauplii* through 28dpf. At 29dpf, *D. rerio* were provided a maintenance diet of the granulated dry feed GEMMA Micro 300 (Skretting, Westbrook, Maine).

Approximately 5.4L of water was exchanged hourly from each tank. Water chemistry was monitored to maintain appropriate conditions: a conductivity of 1400-1500 µS, a salinity of 0.7 ppt, pH of 7.4, and total ammonia nitrogen (TAN) below 0.25 ppt. Water temperature was maintained at 27°C.A light/dark period of 14-hr of light and 10-hr of darkness with lights turning on at 07:30 and off at 21:30 local time was maintained.

At approximately 180dpf *D. rerio* were randomly selected and stocked into 2.8 L tanks for the following experiments with 14 *D. rerio* (n = 7 males and 7 females per tank) unless otherwise noted. Tanks were provided GEMMA Micro 300 twice daily prior to and after consumption testing. *D. rerio* were weighed using an analytical balance (Mettler Toledo Model AJ100 Mettler-Toledo, LLC, Columbus, OH, USA) to 0.001 g before and after consumption testing.

Accommodation

For this experiment, the tanks (n = 5) were administered a round of AZ-GO in place of dry feed, with continued feeding of the AZ-GO diet for 7 consecutive days. Each day, the tanks were provided with a single 2g cylindrical round, placed in a 50mm Petri dish, at 08:00 local time using the handling methods previously described. *D. rerio* were allowed to consume the AZ-GO round for 7 hr. The adult *D. rerio* utilized in this experiment had an average weight of 254 ± 15 mg before the start of the study.

Diurnal Patterns of Feed Intake

During this experiment, each tank (n = 2 per feeding period each day) was proffered a 2g cylindrical round of AZ-GO diet placed in a 50mm Petri dish. After 4, 8, or

24-hours rounds were removed from the tanks and weighed. This was repeated over three days with feed period assignments rotating among tanks so that each feed period had n = 6. After each feed period ended, the remainder of the AZ-GO round was returned to the respective tank for the remainder of the 24 hours so that all *D. rerio* were provided the same amount of food each day over the three days of experimentation. The adult *D. rerio* utilized in this experiment had an average weight of 282 ± 12 mg before the start of the study.

Diel Patterns of Feed Intake

Tanks (n = 6) were provided 2g cylindrical rounds, placed in a 50mm Petri dish, of the AZ-GO diet during daytime hours – 07:30 to 21:30 local time – after which the remaining AZ-GO rounds were removed and weighed. A new 2g AZ-GO round was placed in each tank immediately after removing the initial round and it remained in the tank during nighttime hours – 21:30 to 07:30 local time. The following day at 07:30 local time, the remaining AZ-GO rounds were removed and weighed. This feeding regimen was then repeated once more for a total of 24 hour time periods. The adult *D. rerio* utilized in this experiment had an average weight of 322 ± 18 mg before the start of the study.

Feed Position

D. rerio tanks (n = 2 per placement) were provided a 2g cylindrical round , placed in a 50mm Petri dish, of AZ-GO at one of three randomly assigned placements: A) one 2g round in the front of the tank, B) one 2g round in the back of the tank, or C) one 1g round in the front of the tank and one 1g round in the back of the tank. AZ-GO rounds remained in the tanks for 8 hours. This protocol was conducted over three consecutive days with feed placement assignments rotating among tanks so that each feed placement had n = 6. The adult *D. rerio* utilized in this experiment had an initial average weight of 281 ± 22 mg.

Animal Density

Adult *D. rerio* were fed AZ-GO diet *ad libitum*. Tanks (n = 2) containing either 2, 8, or 14 The adult *D. rerio* utilized in this experiment had an initial average weight of 573 ± 54 mg. Each tank was proffered a 2g cylindrical round, placed in a 50mm Petri dish, of AZ-GO for 8 hours during the light period. The remainder of the AZ-GO rounds were removed and weighed. This was conducted three times over 3 consecutive days. Equal numbers of each sex were weighed after experimentation to determine average individual weights.

Feed Intake Analysis and Statistics

In the previously described experiments, the total weight of *D. rerio* in each tank was divided by the number of *D. rerio* to determine average weights of individuals. Net wet mass consumed in each experimental tank was calculated by subtracting the average weight lost by AZ-GO rounds in control tanks of the same treatment from the weight lost by the AZ-GO round in the experimental tank. The net wet mass consumed per *D. rerio* was calculated by dividing this value by the number of *D. rerio* in the experimental tank.

$$\frac{Wet\ mass\ proffered - wet\ mass\ recovered}{Number\ of\ D.rerio}$$

The percent body weight consumed per *D. rerio* in each experimental tank was determined by dividing net wet mass consumed per *D. rerio* by the average weight of individuals in each tank and multiplying by 100.

$$\frac{Wet\ mass\ consumed}{Average\ weight\ of\ individual}\ x\ 100$$

The average percent body weight consumed per *D. rerio* in wet mass was then calculated for each treatment. For the light cycle experiment, percent body weight consumed per *D. rerio* was converted to percent body weight consumed per *D. rerio* each hour to account for the 14:10 hour light/dark cycle. For the feed intake and placement in tanks experiment, corrections were made for the loss of three *D. rerio*.

The data were analyzed for normality and outliers prior to testing using Shapiro-Wilk test function and the identify outliers function in R Studio. To determine if there were any significant differences in feed intake during the acclimation study a one-way repeated measures ANOVA was used with outliers removed. To determine if there were significant differences in feed intake for the feed placement study and animal density study a two-way ANOVA was conducted followed by Tukey’s post hoc test. All figures were generated, and all statistical calculations were completed using RStudio (The R Foundation for Statistical Computing, 2021, v4.1.0).

Growth

Diet Preparation

During this trial, three different diets were evaluated. These diets included Aquagel Z 2022 formulation (AZ-GE), a gel-based diet produced by Clear H2O (Westbrook, MA, USA) (Table 1), a dry granulated form of the Aquagel Z 2022 formulation (AZ-GR) (Table 1), and a reference diet (RD) comprised of 35% casein, 20% fish protein hydrolysate, and 7.2% supplemented oil (Table 2). The RD formulation also included cholesterol, menhaden oil, a custom vitamin mixture from MP Biomedicals, mineral premixes (MP Biomedicals, Cat. no 290284, Irvine, CA, USA), and alginate binders. The protein content was obtained from fish protein hydrolysate (Sopropeche, Cat. no CPSP90, Wimille, France) and casein (MP Biomedicals, Cat. no 904798, Irvine, CA, USA). All ingredients were precisely measured using an analytical balance (Mettler Toledo New Classic MF Model MS8001S or Model PG503-S Mettler-Toledo, LLC, Columbus, OH, USA) and combined using a Kitchen Aid Professional 600 Orbital Mixer (Kitchen Aid, Benton Harbor, MI, USA). The diet was then cold extruded into strands to preserve its nutritional properties, employing a commercial food processor (Cuisinart, Stamford, CT, USA). The strands were subsequently air-dried for 24 hours on wire trays and ground to ca. 500 um.. To create the dried form of the AZ-GE diet, the diet was frozen (-80C) and then transferred to a freeze dryer (Lyph-Lock 12 Liter, Model 77540/77545, Labconco, Kansas City, MO, USA) and processed for an additional 24-hours as per the manufacturer's instructions. After completion of the process, the dried gel was pulverized into small fragments using a mortar and pestle. These fragments were further ground in a feed grinder (Kitchen Aid, Model KPCG100OB1, Benton Harbor, MI, USA) to ensure a consistent size suitable for consumption by *D. rerio*. The third diet to be employed in the study was a formulated standard reference diet (RD)

Husbandry and Dietary Trial

Male and female AB line *D. rerio* provided by University of Alabama Birmingham Lab Animal Nutrition Core (Birmingham, AL), were bred together in a spawning event of 20 breeding tanks, each containing 2 adult males and 2 adult females. The breeding occurred over a red surface (P. Oden, Pers.comm). Water for all breeding and embryo housing was prepared by using 50% water from the recirculating system, under parameters listed below, and 50% RO filtered water of the same conductivity and pH. The evening prior to a breeding event, breeding pairs were transferred to 1-L breeding tanks (Aquaneering) with approximately 500mLs of water between 17:30 – 18:00 hours local time with a divider separating the pair in each tank. Full water exchanges were performed for each breeding tank, dividers were removed, and tanks were tilted at an angle to encourage shoaling behavior associated with reproduction at 07:00 hours local time (when the lights turned on). The *D. rerio* breeding pairs were allotted a 3-h period to spawn and embryos were collected. The embryos were then placed into 100mm Petri dishes (n = 30 embryos per dish) and incubated at 28.5°C until 5 days postfertilization (dpf). At 2dpf, a 50% water exchange was performed on all Petri dishes. At 4dpf, 0.5mLs of rotifers were added to each Petri dish. At 5dpf, the hatched larvae were place in polycultured in 6-L static tanks (n = 60 larvae per tank) along with the rotifer *Branchionus plicatilis* L-type (obtained from Reed Mariculture, Campbell, CA, USA) at a salinity of 5 ppt. The rotifers were enriched using a combination of 6 microalgae (RotiGrow Plus, Reed Mariculture, Campbell, CA, USA). When the larvae reached 11dpf, all the tanks were transferred to a recirculating aquaculture system (ZS560 Standalone System, Aquaneering, San Diego, CA, USA), and their diets were switched to stage-1 *Artemia nauplii* until 36dpf. At this time, all *D. rerio* from the 6-L tanks were consolidated and randomly distributed into 2.8-L tanks (n = 14 *D. rerio* per tank). Each tank was then randomized to one of three dietary treatments (n = 12 tanks per treatment) and maintained on that treatment for a 12-week period. Prior to the start of the experiment, the initial weights of a subset of *D. rerio* (n = 52) were individually measured to obtain their baseline weights. For the first two weeks of the feeding trial, *D. rerio* receiving AZ-GR or RD were provided with a daily ration equivalent to 10% of their initial body weight. Adjustments to the rations were made every two weeks based on weight gain and food conversion ratios. For the AZ-GE, cylinders of the gel diet were produced by using a test-tube with an internal diameter of approximately 11mm as a hole punch to produce approximately 1g cylindrical gel rounds (Figure 1A-1B). *D. rerio* receiving AZ-GE diet had the gel rounds skewered on to a 304 stainless steel solid round rod that were approximately 1.5mm by 200mm. This rod had a 90-degree angle bend at the bottom appox.10 mm to serve as a foot to keep the rod upright inside the tank (Figure 2A-2C). To keep the AZ-GE rounds in the approximately middle of the rod, a small piece of airline tubing was placed to serve as a stopper. *D. rerio* receiving the AZ-GR diet were fed twice daily, at 09:00 hours and 15:00 hours local time and those receiving the AZ-GE diet had the AZ-GE rounds changed daily at 09:00 hours local time.

The tanks in the commercial recirculating system were held at a temperature of 28.5°C and system water conductivity was maintained at 1450-1500µS/cm. Approximately 5.4L of water was exchanged hourly from each tank. Municipal tap water was filtered using a 1µm sediment filter, an activated carbon filter, reverse osmosis filter, and a cation/anion exchange resin. Synthetic sea salts (Instant Ocean, Blacksburg, VA, USA) were added to adjust the conductivity of the system water. Sodium bicarbonate was added as necessary to maintain a pH of 7.4 in the system water. Colorimetric methods were employed weekly to measure total ammonia nitrogen, nitrite, and nitrate levels in the water. (Test Kit API, Campbell, CA, USA) A daily water exchange of 10% was carried out to maintain optimal water quality in the sump of the recirculating system. Prior to re-entering the tanks, the water passed through activated charcoal and UV sterilization to decrease the presence of persistent compounds that may originate from feed or microbial organisms. Tanks were maintained on the same recirculating system throughout the experiment, they were cleaned and relocated to a new randomized position on the system every two weeks to minimize potential confounding environmental factors such as noise, light, vibration, or other unidentified sources due to tank location from interfering with the results.

The *D. rerio* were subjected to a light/dark period of 14-hr of light and 10-hr of darkness, with the lights being turned on at 07:00 hours local time. At the end of the feeding trial, *D. rerio* were humanely euthanized by swiftly submerging them in ice-cold water for a minimum of 10 minutes and ensuring cessation of opercular motion. *D. rerio* were then individually identified by their sex, weighed with a precision of 0.001g (Mettler Toledo Model AJ100 Mettler-Toledo, LLC, Columbus, OH, USA) and length measured by placing individuals on dorsal side on top of a ruler, measured from caudal peduncle to snout, to the closest 1mm. Subsequently, secondary euthanasia was performed through decapitation.

Reproductive Performance

At the end of the 12-week feeding trial, breeding pairs were maintained under the same husbandry conditions as above in sex segregated 2.8-L tanks for reproduction assays (n = 16 male and female pairs for AZ-GE and AZ-GR diets, n = 8 male and female pairs for RD). Male and females were randomized into breeding pairs while avoiding being paired with tankmates that they had been housed with during the 12-week feeding trial. Each breeding pair (one male and one female) represented one breeding event. After the first breeding event, males and females were given a 1-week resting period before being randomized to new breeding pairs for a second breeding event. This resulted in 32 breeding events for AZ-GE and AZ-GR diet fed *D. rerio*, and 16 for RD fed *D. rerio*.

Breeding and water preparation for embryos were accomplished as described previously. At 10:00 hours local time (0hpf for all embryos) male and female *D. rerio* were returned to their respective tanks. At 4hpf (14:00) the total number of eggs produced from each breeding pair (clutch size) was counted and recorded under a dissecting microscope. The eggs were sorted for viability and placed in Petri dishes (n = 30 eggs per dish) with water prepared as stated as above. The Petri dishes were then incubated until the following day at 28.5°C. At 10:00 hours local time, 24hpf viability counts were performed. Embryos exhibiting a stage of development at 4hpf consistent with the blastula period and embryos exhibiting a stage of development at 24hpf consistent with the pharyngula period were considered viable (Kimmel, Ballard et al. 1995).

Statistical Modeling and Analysis

Data from this study was analyzed, and graphs were generated, using RStudio Statistical Software (R Core Team, 2023, v06.1+524, Vienna, Austria). All terminal analyses for continuous outcomes were stratified by sex. Terminal wet body weight, and total body length were compared separately by linear random effects model with tank as a random variable. All data were analyzed for normality and equal variances. Any datasets with a non-normal distribution were log-transformed. For total embryos produced, previous examination of similar datasets revealed over-dispersion with excessive truncated zeroes (non-successful breeding events), indicating that these data were well-suited for a hurdle negative binomial model (Hothorn, Bretz et al. 2008). Data for total embryo production were fitted to a hurdle negative binomial model with help of the pscl package of the R language (Zeileis, Kleiber et al. 2008). Diet and week of breeding were included as predictors in the model and analyzed for main effects on total embryo production. The outcome for embryo viability is a proportion between 0 and 1, with two types of zeroes present: truncated (non-successful breeding events) and sampling (zero viable embryos produced). For this reason, a zero-inflated beta regression model (BEZI) is selected as the most appropriate model. The first component of the BEZI model uses logistic regression and the parameter nu (controls the probability that a 0 occurs) to analyze the zero counts and determine the probability of 0 viable embryos produced. The second component analyzes the positive counts by fitting a beta regression to compare the expected proportion of viable embryos and includes the parameters mu (mean) and sigma (variance) (John Dawson, Dept. of Biostatistics, personal communication). The best fit model usually includes all three parameters and is selected with help of the gamlss package of the R language (Rigby and Stasinopoulos 2005)

Early Larval Feeding

Diet Preparation

During this trial, two different diets were fed to post-hatch larvae. The initial diet employed was Aquagel Z 2022 formulation (AZ-GE), a gel-based diet produced by Clear H2O (Westbrook, MA, USA) (Table 2). The second diet employed was a dried, powdered variation of Aquagel Z 2022 formulation (AZ-GR) (Table 2). To create this form of the diet, the diet was frozen and then transferred to a freeze dryer (Lyph-Lock 12 Liter, Model 77540/77545, Labconco, Kansas City, MO, USA) and processed for an additional 24-hours as per the manufacturer's instructions. After completion of the process, the dried gel was pulverized into small fragments using a mortar and pestle. These fragments were further ground in a feed grinder (Kitchen Aid, Model KPCG100OB1, Benton Harbor, MI, USA) to ensure a consistent size suitable for consumption by *D. rerio*.

Husbandry

A mass spawning of AB strain *D. rerio* males and females from the University of Alabama Birmingham Lab Animal Nutrition Core (Birmingham, AL) were used to produce embryos. Embryos were incubated from 0 to 5dpf in 100mm Petri dishes (n = 50 embryos per dish). At 6dpf, *D. rerio* fry were transferred to 2.8-liter tanks (n = 14 fish per tank) and randomized to 1 of 5 treatment groups (n = 6 tanks per treatment) based on the day the larvae were started on AZ-GE or AZ-GR (6DPF, 12DPF, 18DPF, 24DPF, and

30DPF). Fish in the 6DPF treatments received AZ-GE or AZ-GR from 6dpf until 36dpf on a recirculating system (Aquaneering, Sen Diego, CA) with a flow rate of at least 2 exchanges water in the tank per hour. All other treatments were maintained in static tanks at 1500µS water (Instant Ocean, Blacksburg, VA) in polyculture with rotifers and fortified previously frozen *Nanno chloropsis* (Reed Mariculture). At 10dpf all remaining treatment tanks were also placed on water recirculation and provided a live diet of Stage I *Artemia nauplii.* Treatments 12DPF, 18DPF, 24DPF, and 30DPF received AZ-GE or AZ-GR at 12dpf, 18dpf, 24dpf, and 30dpf, respectively, and were fed these diets until 36dpf. Diets were provided *ad libitum* at 07:00 and 15:00-hr local time.

The tanks in the commercial recirculating system (Aquaneering, San Diego, CA) were held at a temperature of 28.5°C and had a conductivity of 1500µS/cm. Approximately 5.4L of water was exchanged hourly from each tank. Municipal tap water was filtered using a 1µm sediment filter, an activated carbon filter, reverse osmosis filter, and a cation/anion exchange resin. Synthetic sea salts (Instant Ocean, Blacksburg, VA, USA) were added to adjust the conductivity of the system water. Sodium bicarbonate was added as necessary to maintain a pH of 7.4 in the system water. Colorimetric methods were employed weekly to measure total ammonia nitrogen, nitrite, and nitrate levels in the water. (Test Kit API, Campbell, CA, USA) A daily water exchange of 10% was carried out to maintain optimal water quality in the sump of the recirculating system. Prior to re-entering the tanks, the water passed through activated charcoal and UV sterilization to decrease the presence of persistent compounds that may originate from feed or microbial organisms. Tanks were maintained on the same recirculating system throughout the experiment. The *D. rerio* were subjected to a light/dark period of 14-hr of light and 10-hr of darkness, with the lights being turned on at 07:00 hours local time. At the end of the feeding trial, *D. rerio* were humanely euthanized by swiftly submerging them in ice-cold water for a minimum of 10 minutes, ensuring cessation of opercular motion. Subsequently, secondary euthanasia was performed through decapitation. At the end of the study, survival and weight gain were recorded. Fish were measured to the nearest 0.001g (Mettler Toledo Model AJ100 Mettler-Toledo, LLC, Columbus, OH, USA).

Statistical Modeling and Analysis

Data from this study was analyzed and graphs were generated using RStudio Statistical Software (R Core Team, 2023, v06.1+524, Vienna, Austria). To determine if there were significant differences in survival and weight gain a two-way ANOVA was conducted followed by Tukey's post hoc test.

TABLE 1. Nutritional information for Aquagel Z per 100 grams, as per manufacture website. AZ-GE is a commercially available gel diet (Clear H2O), AZ-GR is the dried, granulated form of AZ-GE, and RD is the reference diet.

	AZ-GE[a]	**AZ-GR[a]**	**RD[b]**
Calories (kcal)	62.2	239.470	423.1
Protein (g)	14.4	55.440	47.28
Carbohydrates (g)	1.8	6.930	10-15
Sugars(g)	0.5	1.925	
Dietary Fiber (g)	1.1	4.235	
Fat (g)	3.0	11.550	11.04
Saturated Fat (g)	0.3	1.155	
Cholesterol (g)	2.9	11.165	
Moisture (%)	72-76	0	~10
Calcium (mg)	481	1851.850	
Phosphorus (mg)	433.9	1670.515	
Potassium (mg)	161.7	622.545	
Sodium (mg)	55.1	212.135	

[a] Proximate composition of AZ-GE and AZ-GR were provided by Clear H2O
[b] Composition of RD was provided by Midwest Labs.

TABLE 2. Ingredient composition of formulated reference diet.

Ingredients	**RD[a]**
Casein - low trace metals	350
Fish protein hydrolysate	200
Wheat starch	22.6
Maltodextrin	50
Alpha cellulose	10
Diatomaceous earth	125.7
Menhaden fish oil (ARBP) Virginia Prime Gold	26
Safflower oil	45.5
Alginate	20
Lecithin, sunflower	40
Vitamin premix (Dyets Std. Fort. Mix)	40
Mineral premix (Dyets AIN 93G)	30
Canthaxanthin (10%)	23.1
Potassium phosphate monobasic	11.5
Glucosamine	2.5
Betaine	1.5
Cholesterol	1.2
Ascorbylpalmitate	0.4
Total	1000
Calculated protein level (%) as fed	47.28
Calculated protein level (%) as dry	52.53
Calculated lipid level (%) as fed	11.04
Calculated lipid level (%) as dry	12.26
Calculated energy level (cal/g) as fed	4231
Calculated energy level (cal/g) as dry	4701

[a] RD, amounts listed in g/kg.

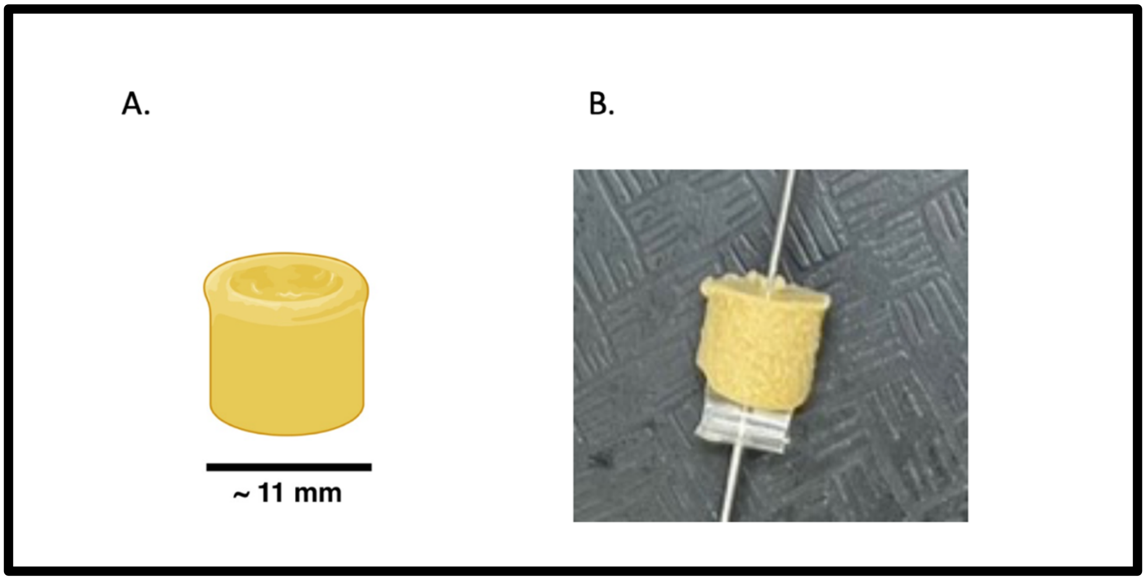

FIGURE 1. Representative size and dimensions of the gel-based diet proffered to *D. rerio*. (A) Dimensions of the gel-based visualized via Biorender. (B) Gel-based (AZ-GE) diet pellet impaled on a metal rod skewer prior to suspending into a tank.

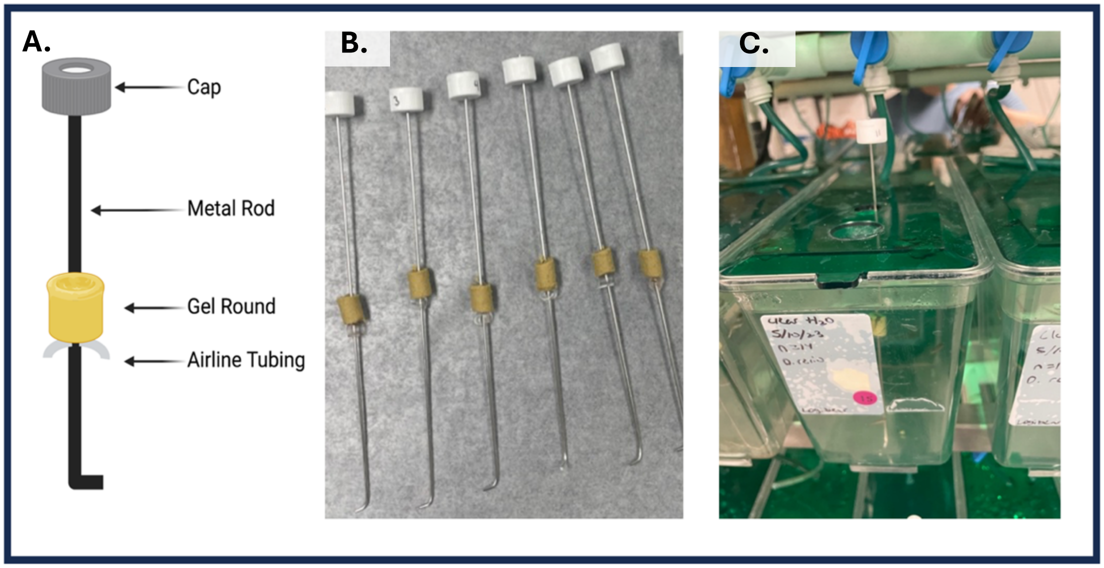

FIGURE 2. (A) Schematic of gel-based feed applied to a metal rod skewer visualized via Biorender and (B) photo of metal rod skewer supporting gel-based feed. (C) Photo of insertion of gel rods in a 2.8 L tank.

RESULTS

Zebrafish Feed Intake Parameters

Accommodation to the Diet

Based on daily feed intake, *D. rerio* fed previously a dry reference diet did not require time to accommodate to the AZ-GO gel diet (Figure 3). Feed intake did not vary significantly over seven days of feeding.

Diurnal Patterns of Feed Intake

After the initial feeding response to daily introduction of the gel round at lights-on in the morning, feed intake of AZ-GO by *D. rerio* decreased over the course of the day. *D. rerio* consumed an average of 11.16% of body weight over 24-hours (as fed). Total feed intake per fish was 0.74% of wet body weight consumed per hour in the initial 4-hours of feeding (Figure 4 and 5). The apparent rate of feed intake was lower during the 8-hour and 24-hour intervals, resulting in 0.52% and 0.47% wet body consumed per hour, respectively.

Diel Patterns of Feed Intake

The majority of the daily feed ration was consumed in the 14-hour period of light, with 82.6 % of the daily feed ration consumed during light and 17.4 % of the daily feed ration consumed during dark. The average individual fish consumed 0.65 % of their bodyweight per hour during the light, and the rate decreased to 0.24 % per hour during the dark period. (Figures 6 and 7).

Feed Position

D. rerio consumed the same amount of AZ-GO diet regardless of whether the feed round was positioned at the front or the back of the tank, or whether the ration was positioned at both locations, with no significant difference in the amount consumed per fish among the different feed placement sites ($p = 0.279$) (Figure 8).

Animal Density

D. rerio consumed more AZ-GO per fish when were stocked at 8 fish per tank compared to 2 fish per tank ($p = 0.002$) (Figure 9). There was no significant difference between AZ-GO consumption per fish stocked at 14 fish per tank compared to 2 or 8 fish per tank ($p = 0.064$).

Zebrafish Growth Experiment

Body Composition Metrics

All three diets sustained *D. rerio* growth and development over the 12-wk feeding trial (Figure 10). Initially, RD-fed fish showed the highest rate of growth in terms of weight gain; however, by week 12, AZ-GE fed fish exhibited similar weight gain to those fed RD. Dry granulated diets were readily consumed from the water surface. Fish on all diets appeared healthy. No aberrant behavioral or morphological features were observed, suggesting that there were no apparent nutritional deficits in any of the diets proffered.

The survival rate exceeded 95% within all diet treatments. Upon the termination of the experiment, the sex distribution in all diets exhibited a moderate bias favoring males (Table 3).

At the end of the study, female *D. rerio* fed the RD and AZ-GE diet exhibited a higher terminal wet body weight compared to AZ-GR fed females (P < 0.01) (Figure 11A). Male *D. rerio* fed the RD diet exhibited a higher terminal wet body weight compared to AZ-GE and AZ-GR fed males (P < 0.01) (Figure 11B). Likewise, female *D. rerio* fed the RD and AZ-GE diet exhibited longer average length compared to AZ-GR fed females (P < 0.04) (Figure 12A). Male *D. rerio* fed the RD diet exhibited a longer average length compared to AZ-GE and AZ-GR fed males (P < 0.01) (Figure 12B).

Reproductive Performance

Diet impacted several parameters of reproductive performance. The percent of females expressing gametes was high (Table 4) and the average number of eggs produced in individual *D. rerio* did not differ among fish fed the different diet treatments ($p > 0.996$) (Figure 13A). However, embryo viability of fish fed RD was significantly higher compared to fish fed AZ-GE (at 4hpf, $p = 0.001$ and 24hpf, $p = 0.001$) and AZ-GR (at 4hpf, p = 0.032 and 24hpf, $p = 0.028$) (Figure 13B and 13C).

Feed Intake

Feed intake was not measured in fish fed the RD or AZ-GR diets and was measured weekly in fish fed the AZ-GE. For those fed the AZ-GE diet, feed intake increased concomitant with the observed increase in fish mass. By the end of the study

D. rerio consumed approximately 252.91 mg per fish per day, or 963.47 mg/g wet weight of fish/day. Based on estimated feed intake, FCR was 43.142 ± 0.503. These feed intake and FCR estimates are unusually high and are discussed below.

Zebrafish Early Gel Consumption Experiment

Survival

D. rerio proffered AZ-GE or AZ-GR at 6dpf exhibited significantly reduced survival versus those fed these diets starting at 12dpf ($p < 0.027$) (Figure 14). Survival was not significantly different ($p > 0.877$) among treatments started on AZ-GE or AZ-GR at 12dpf or later, with an average survival among these groups ranging between 74% and 87%.

Growth

Final wet weight at 36dpf increased with a longer delay in the introduction of AZ-GE and AZ-GR (representing a longer period in the provision of live diets). 30dpf *D. rerio* proffered AZ-GE and or AZ-GR exhibited the highest final weight ($p < 0.001$) (Figure 15). For *D. rerio* proffered AZ-GE and AZ-GR at 18dpf and 24dpf, *D. rerio* proffered AZ-GR diet exhibited higher final weight ($p = 0.017$, $p = 0.023$, respectively).

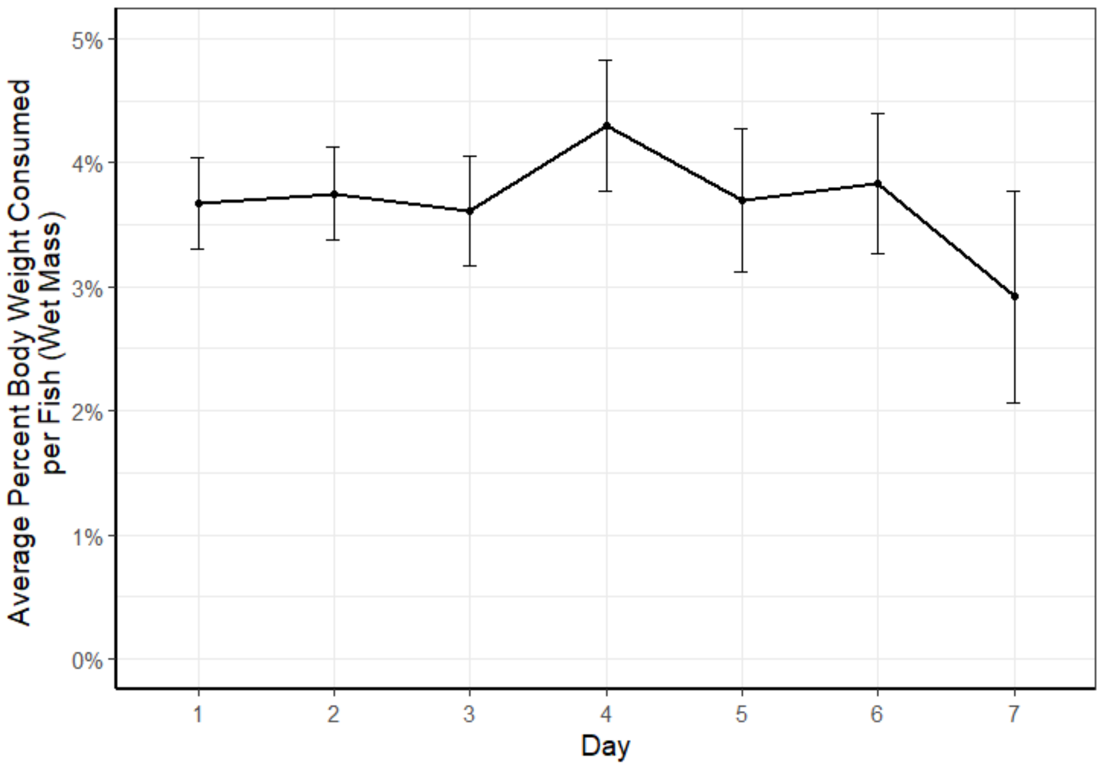

FIGURE 3: The average total amounts of AZ-GO feed consumed as a percentage of body weight per fish each day during a 7-day feeding period (n = 5 tanks, 7 adult males and 7 adult females per tank). Dots represent mean amount of feed consumed and error bars represent SEM.

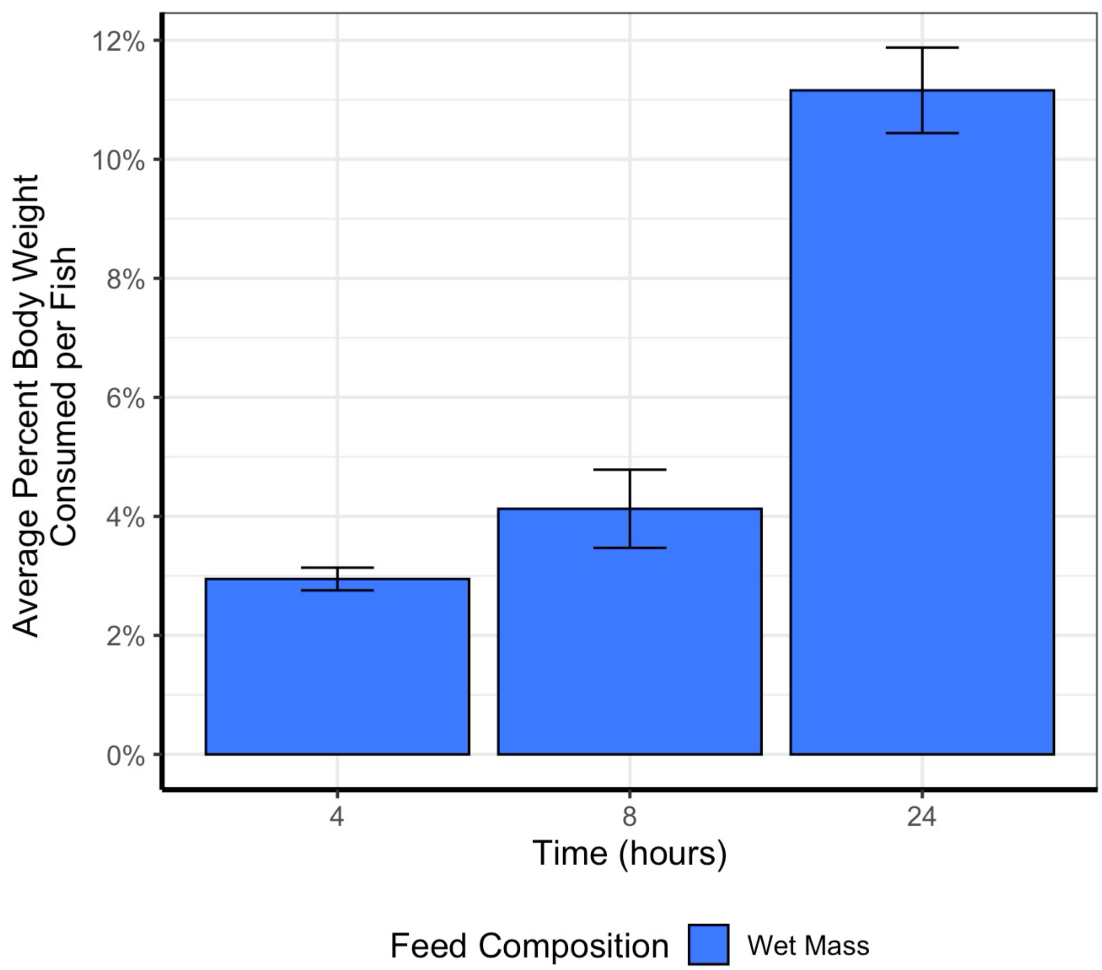

FIGURE 4. The average amounts of AZ-GO feed consumed as wet mass (as fed) as a percentage of body weight per fish at different exposure periods (n = 6 tanks, 7 adult males and 7 adult females per tank) initiated following the onset of the morning light phase. Bars represent mean amount of feed consumed and error bars represent SEM.

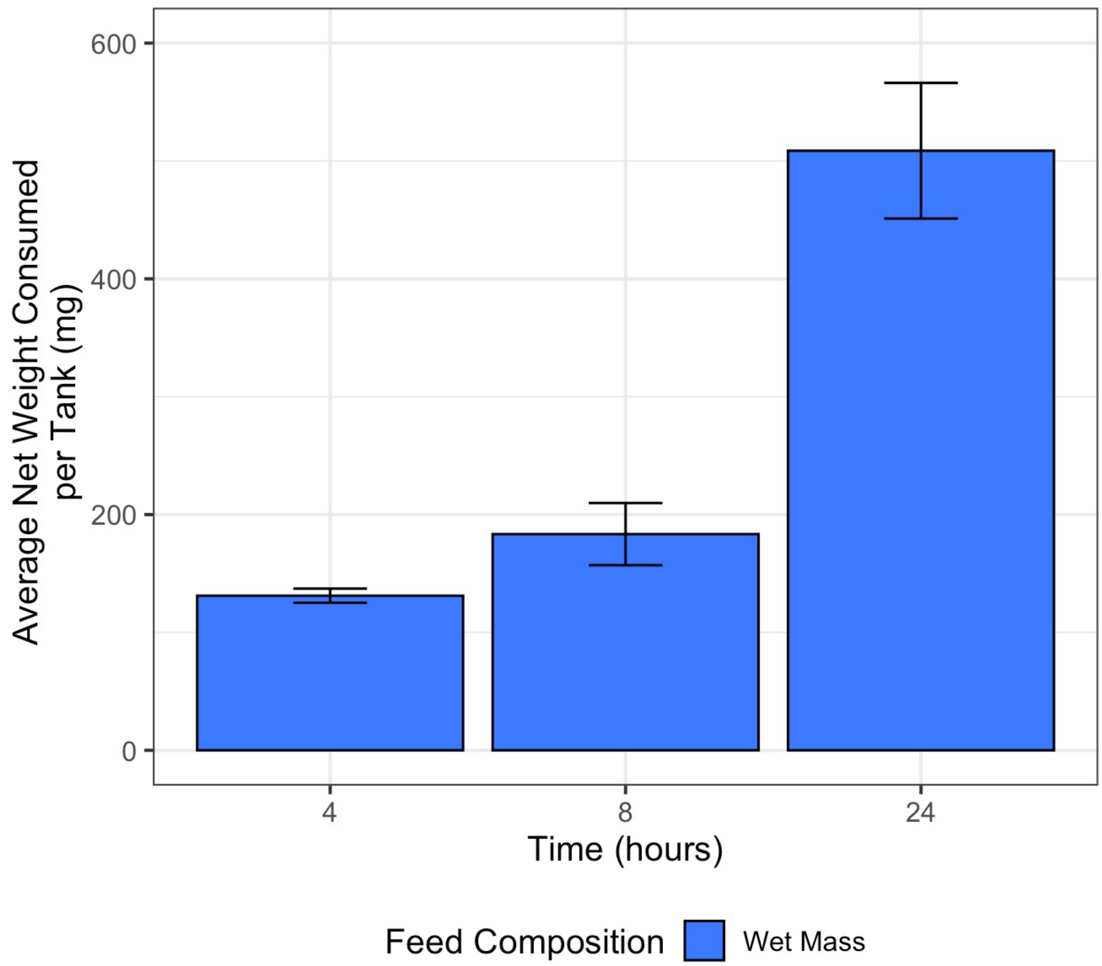

FIGURE 5. The average amount of AZ-GO consumed per tank (n = 6 tanks, 7 adult males and 7 adult females per tank) as wet mass (as fed). Bars represent mean amount of feed consumed and error bars represent SEM.

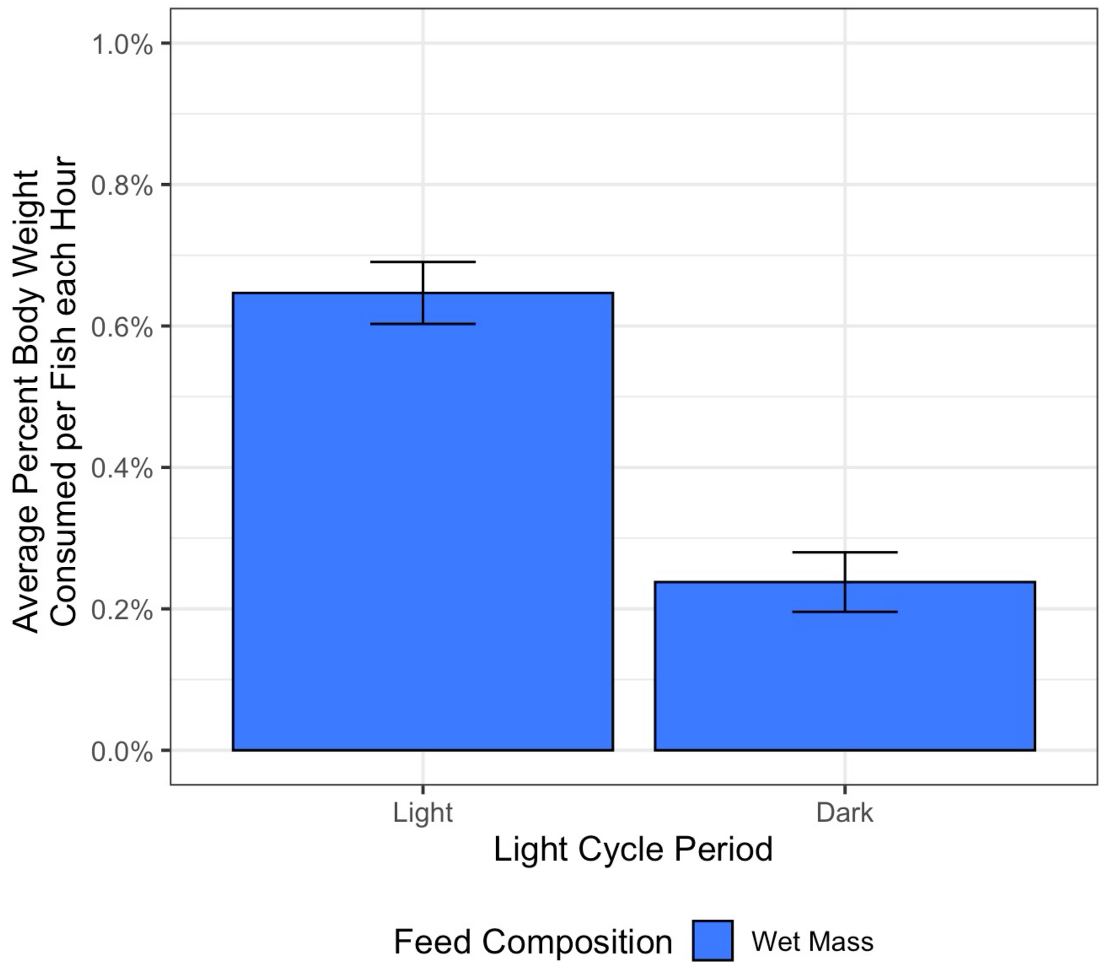

FIGURE 6. The average amounts of AZ-GO feed consumed as a percentage of body weight per fish per hour during both 14-hour light and 10-hour dark periods (n = 6 tanks, 7 adult males and 7 adult females per tank). Bars represent mean amount of feed consumed and error bars represent SEM.

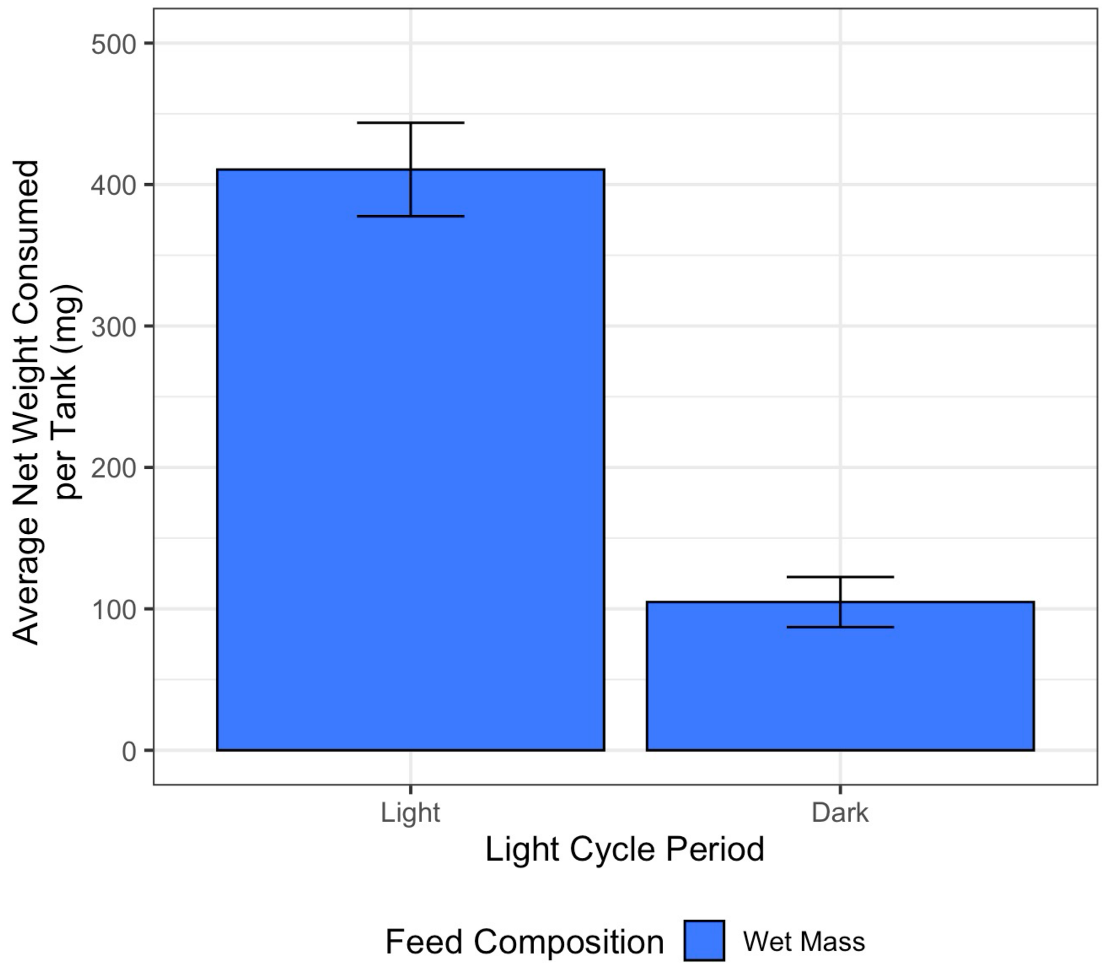

FIGURE 7. The average amount of AZ-GO (wet mass, as fed) consumed per tank (n = 6 tanks, 7 adult males and 7 adult females per tank) when fish were held in the 14-hour light or 10-hour dark periods. Bars represent mean amount of feed consumed and error bars represent SEM.

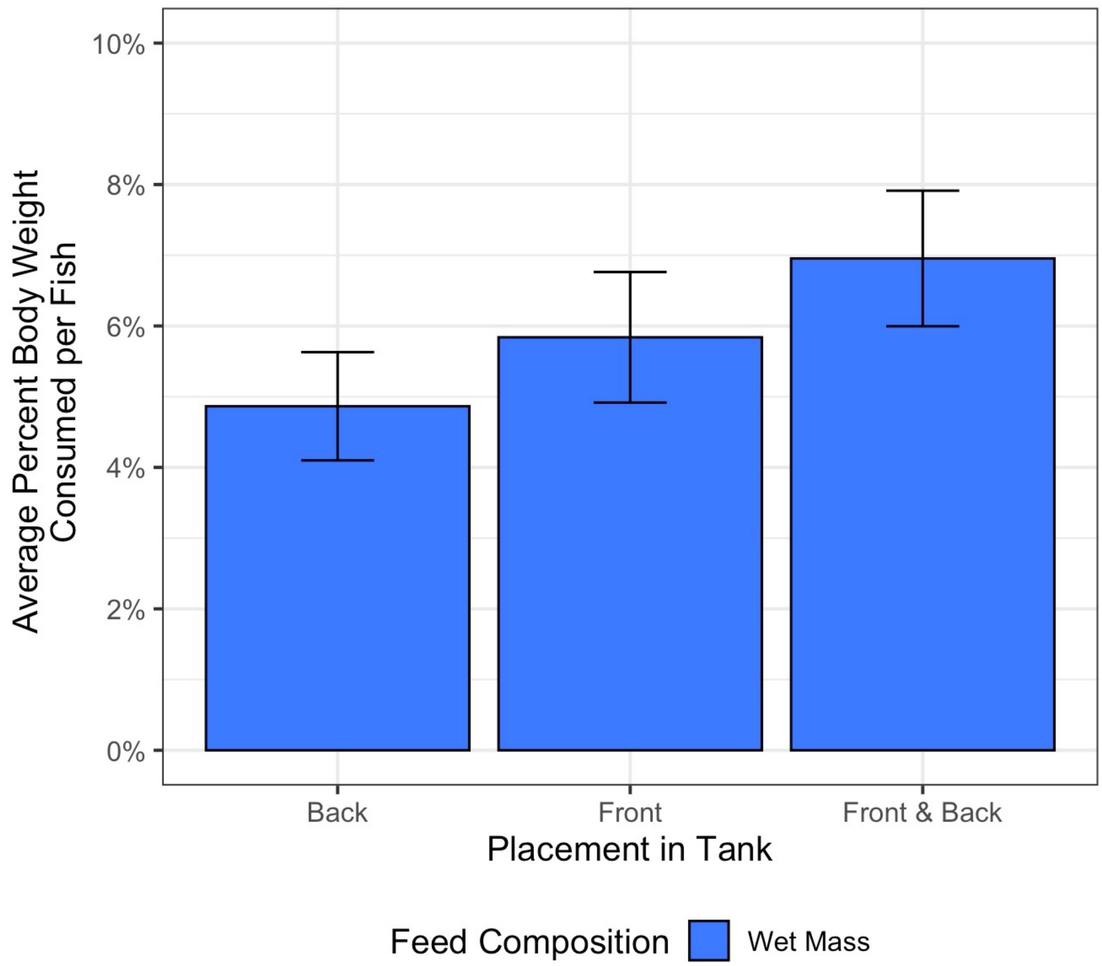

FIGURE 8. The average amounts of AZ-GO feed consumed as a percentage of body weight per fish in tanks where the food source was placed in only the rear of the tank, in the front of the tank, or evenly divided between the front and the rear. Each tank contained 7 adult females and 7 adult males. Bars represent mean amount of feed consumed and error bars represent SEM.

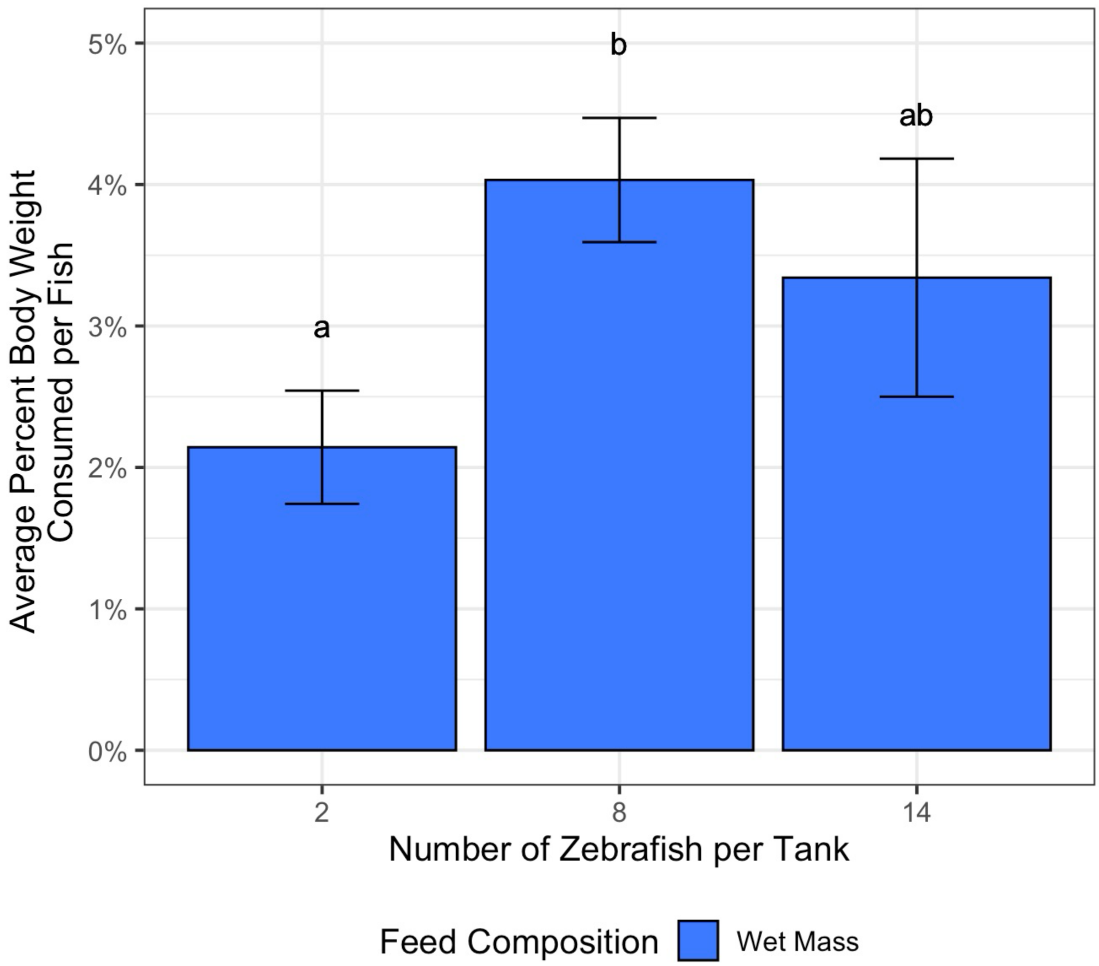

FIGURE 9. The average amounts of AZ-GO feed consumed as a percentage of body weight per fish in tanks stocked with 2 adult fish, 8 adult fish, or 14 adult fish ($n = 6$ tanks per stocking density). Bars represent mean amount of feed consumed and error bars represent SEM. Letters indicate where significant differences were observed among treatments ($p \leq 0.05$).

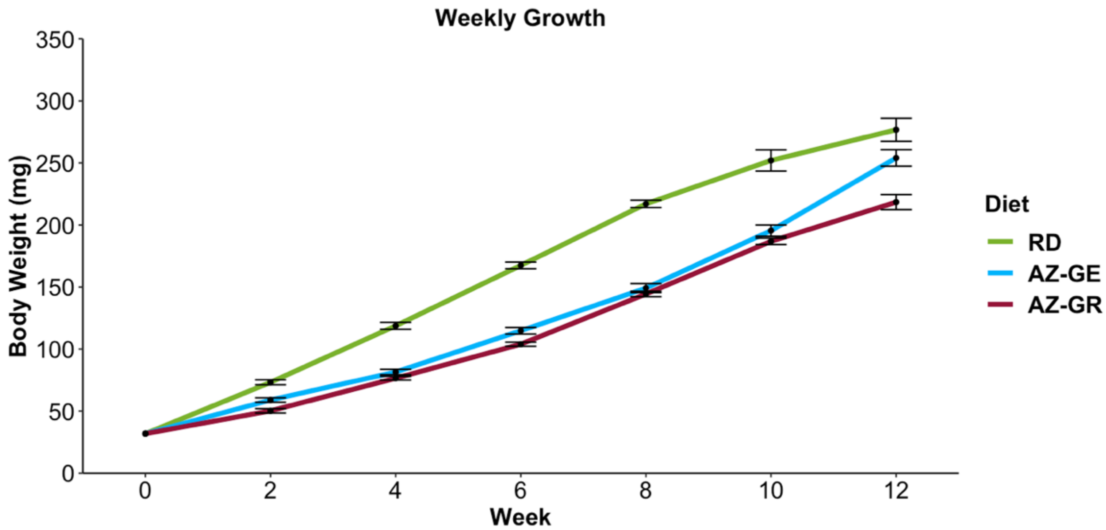

FIGURE 10: Average fish body mass of juvenile *D. rerio* fed the reference diet (RD), the gel diet (AZ-GE), or the dry granulated form of the gel diet (AZ-GR) over a 12-wk feeding period (n = 12 tanks per treatment, 14 fish per tank). Dot represents mean body weight and error bars represent SEM at each respective time point.

TABLE 3. Weight and length by sex among diet treatments.

Diet	n	*Weight, mg*	*Length, mm*
RD Diet			
Males	93	278±7	25.08±0.2
Females	73	227±7	23.42±0.2
AZ-GE Diet			
Males	99	227±5	23.49±0.2
Females	65	252±8	23.25±0.3
AZ-GR Diet			
Males	113	214±3	23.67±0.1
Females	48	194±8	22.06±0.2

Data are given as mean ± standard error of the mean.

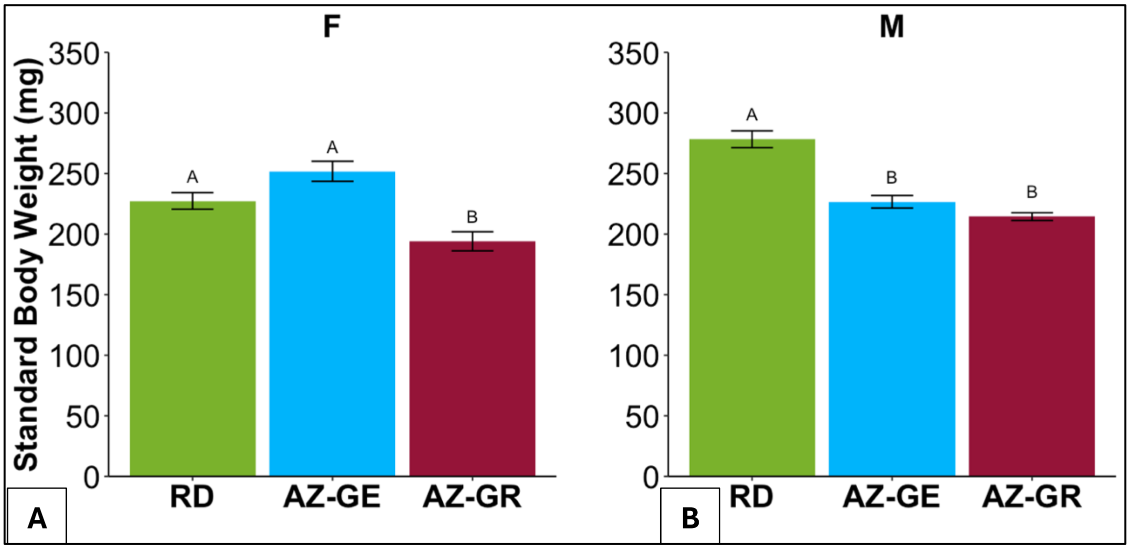

FIGURE 11: Terminal fish mass by sex. Female *D. rerio* fed the RD and AZ-GE diet exhibited higher wet body weights compared to AZ-GR fed females ($P < 0.04$). Male *D. rerio* fed the RD diet exhibited higher wet body weights compared to AZ-GE and AZ-GR fed males ($P < 0.01$). Bars represent mean body weight and error bars represent SEM. Letters indicate where significant differences were observed among treatments ($p \leq 0.05$).

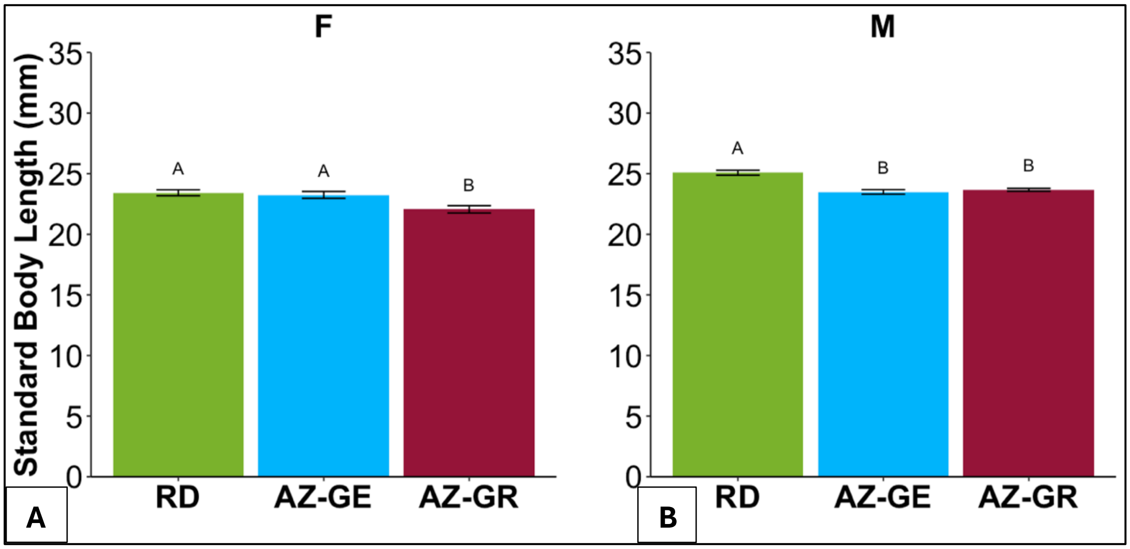

FIGURE 12: Terminal fish mass by sex. Female *D. rerio* fed the RD and AZ-GE diet exhibited higher wet body mass compared to AZ-GR fed females ($P < 0.04$). Male *D. rerio* fed the RD diet exhibited higher wet body mass compared to AZ-GE and AZ-GR fed males ($P < 0.01$). Bars represent mean body length and error bars represent SEM. Letters indicate where significant differences were observed between treatments ($p \leq 0.05$).

TABLE 4. Spawning success by diet. AZ-GE is a commercially-available gel diet (Clear H2O), AZ-GR is the dried, granulated form of AZ-GE, and RD is the reference diet.

Diet	*No. of successful[a]/total*	*Proportion successful*
RD	14/16	0.875
AZ-GE	32/32	1.00
AZ-GR	28/32	0.875

[a] Number of successful breeding events in which eggs were released by the female relative to the number to total breeding attempts.

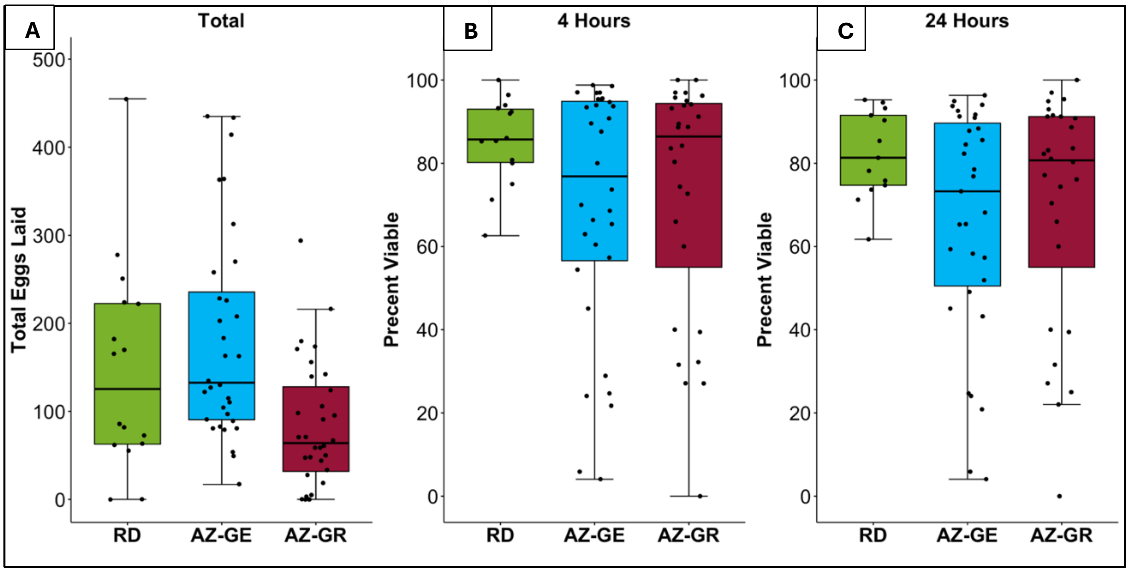

FIGURE 13: Fish fecundity as (A) number of total eggs released, (B) viable eggs per treatment at 4 hours, and (C) viable eggs per treatment at 24 hours. RD had significantly higher percent of viable eggs at 4 hours (P = 0.04) and 24 hours (P = 0.03). Dots represent eggs released from a single breeding pair. Boxes represents upper and lower interquartiles and whiskers represent upper and lower outerquartiles.

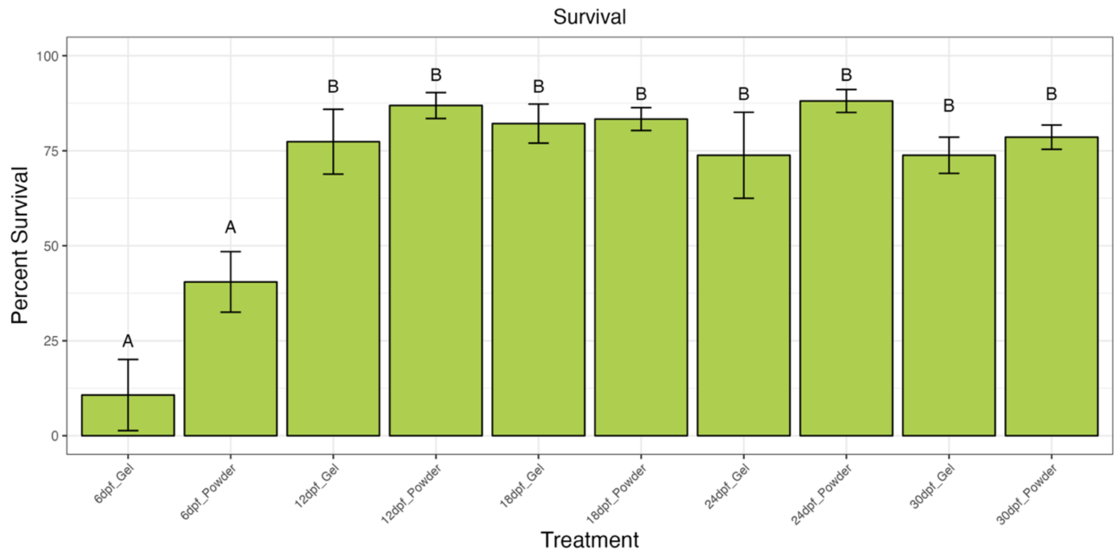

FIGURE 14: Survival rate across all treatments showing significant differences in AZ-GE (gel) and AZ-GR (dry). Bars represent mean value of percent survival and error bars represent SEM. Letters indicate where significant body weight differences were observed among treatments ($p \leq 0.05$).

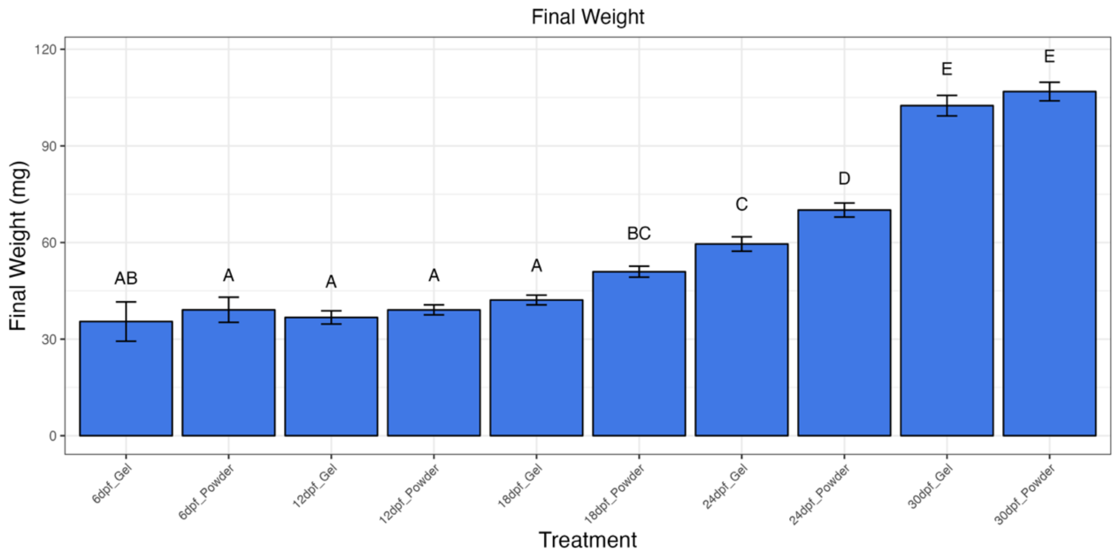

FIGURE 15: Terminal weight distribution summarized across all treatments. Bars represent mean final weights and error bars represent SEM. Letters indicate significant body weight differences observed among treatments ($p \leq 0.05$).

DISCUSSION

Rapid accommodation of *D. rerio* to a gel-based diet was observed. This accommodation is likely related to their natural feeding behavior in the wild. *D. rerio*, like many species, are opportunistic and omnivorous feeders (Spence et al. 2008) and will consume a variety of foodstuffs. They are predicted to more readily accommodate to novel diets compared to strictly carnivorous or herbivorous species (Wu, McAlpine et al. 2012, Karandikar, Serota et al. 2022). Although *D. rerio* are generally considered sight-feeders, they most likely can also detect a variety of compounds associated with a food source, regardless of the physical form of that food source (Spence, Gerlach et al. 2008). Many of these compounds may serve as attractants or feed stimulants.

Danio rerio consumed feed more readily during the initial daily introduction of the gel-based diet to the system. Feeding rate decreased over time during the day, although consumption continued throughout the 24-hour light and dark period. We hypothesize that upon introduction of the gel-based feed to the tank, leaching rate is highest and therefore the diet may be more attractable to the fish at that time. Yúfera et al. (2002) showed that microencapsulated diets for use in aquaculture leached out free amino acids (FAA), which can act as feed attractants, upon initial introduction into water and that leaching was reduced over time. Alternatively, the increased feed intake following the initial daily introduction of the gel-based feed may be linked to the start of the period of prolonged light. Chemical leaching should be evaluated in gel-based diets for the *D.*

rerio model, as well as other parameters that may impact attractability such as time of feeding, feed texture, shape, color, and size (Biswas and Takeuchi 2003).

Although *D. rerio* consumed the gel-based diet more readily during the 14-hour light period, they continued to consume food during the 10-hour dark period, although at a reduced rate. Tucker et al., (2006) noted that changing the photoperiod can affect overall weight gain in Australian snapper, *Pagrus auratus,* a related teleost species. In their paper, they highlighted a positive correlation in weight gain with an extended photoperiod, an 18-hour light and 6-hour dark period, rather than a 12-hour light and 12-hour dark period. Not all teleosts respond positively to an increased photoperiod. Biswas and Takeuchi (2003) altered photoperiods for Nile tilapia, *Oreochromis niloticus,* by increasing the light period in 3-hour increments up to a 24-hour light period. Extended photoperiod did not impact fish length or weight but did increase feed intake and reduce feed conversion efficiency (FCE), which would increase rearing cost in a production facility. Researchers did not measure activity levels of the fish, but it can be hypothesized that those with a longer photoperiod had a higher caloric demand necessitating an increased feed intake. Additional studies evaluating both quantity and quality of light are warranted (Villamizar et al. 2014).

D. rerio were able to locate the food source immediately upon its introduction to the tanks. The location of the food source in the tank, defined by placing the source at the front or at the back of the tank, did not affect food intake. In addition, placing the food source at both positions simultaneously did not increase population feed intake, suggesting that the amount of feed provided was adequate to support normal feed intake in these culture tanks regardless of the location of the food source or the number of food sources available.

Population density for *D. rerio* has been shown to impact a number of variables including growth, reproduction, and the stress response (Andersson and Kettunen 2021). Leibold and Hammerschmidt (2015) found that densities between 2 and 20 fish/L did not impact feed intake but did result in growth differences, suggesting metabolic changes within populations is influenced by density. They found that the animals in the higher densities grew more than the lower density though food intake did not change. To a point, higher densities are better for *D. rerio* as highlighted in Sarma et al. (2023), who showed that low stocking densities increased aggressive behavior and cortisol secretion. In our study we observed decreased feed intake at the lowest stocking density, and we did not observe aggressive behavior. We hypothesize feed intake is increased at higher densities due to competition triggering more sustained or rapid feeding. Future studies should examine feeding behavior and feed intake patterns at different population densities.

All diets supported positive growth rates over the 12-week study period. The terminal size of the *D. rerio* consuming the gel form of the AZ diet was not significantly different from the granulated form of the AZ diet until week 12. The nutritional content was identical in these diets, with diets varying only in physical form. Highest growth rates were observed in *D. rerio* fed the RD diets, although the terminal size of the *D. rerio* fed the RD was not different from those fed the AZ-GE at 12 weeks. The nutrient content of the RD may be different from the AZ diets, whose contents are proprietary. Honorato et al. (2010) found that pacu, *Piaractus mesopotamicus,* provided various diets had higher protein efficacy ratios (PER) using a pelleted rather than extruded feed. In contrast, a study of channel catfish, *Ictalurus punctatus,* consuming pelleted or extruded diets of the same chemical composition did not show significant differences in growth performance, whole-body composition, and nutrient retention (Xu, Li et al. 2017). Impacts of the feed form may be highly dependent on the species.

It has been suggested that nutrients are allocated differentially to specific tissues over the early growth period to ensure somatic and gonadal growth (Fowler, Williams et al. 2019). It is also typical to see dietary responses that differ by sex for these fish (Williams, Palmer et al. 2021, Williams, Lawrence et al. 2023). In our study egg production was not impacted by diet, regardless of the nutritional content or the physical form of the diet; however, viability of the embryos was impacted. RD fed fish had significantly higher embryo viability at 4-hours and 24-hours. Many studies in *D. rerio* have shown that diet strongly impacts fecundity especially when comparing dietary lipid quality. *D. rerio* provided a diet of *Artemia* supplemented with olive oil had increased male sperm density and motility and a significant difference was observed in follicle abundance at different stages of gametogenesis (Samaee, Manteghi et al. 2019). *D. rerio* diets supplemented with phosphatidylethanolamine had higher sperm counts and motility in males while also showing larger diameter eggs in females (Diogo, Martins et al. 2015). Refinement of the nutritional content of gel-based diets may be needed to optimize reproductive output in *D. rerio.*

Despite the positive growth rates observed in response to all diet treatments, it was not possible to determine the total feed intake of any of the diets in this study. Some level of leaching and feed waste is normally observed when *D. rerio* are fed a dry granulated diet. Attempts were made to quantify the amount of gel feed consumed by weighing the gel diet both before and after introduction to the tank. Despite adding corrections for leaching, the amount of food consumed could not be accurately assessed. Visual observations indicated small particles of the gel feed were displaced during normal feeding behavior and these particles accumulated on the tank bottom or were removed from the tank during active water flow typically associated with a recirculating aquaculture system. A calculation of feed conversion ratio indicated an unusually high FCR ratio, which suggested the majority of the gel feed was lost and not consumed. The loss of the gel feed during normal feeding behavior may be comparable to the amount of feed lost during the feeding of a dry granulated diet; however, there are many factors that could influence feed loss in dry granulated diets, including the size of the pellet, the binder, pellet buoyancy, the number and size of feed rations, and the solubility of ingredients and associated nutrients. Additional work is needed to enhance both dry granulated and gel diets, leading to the improvement of feed intake and limiting waste.

In our study we observed that replacement at the earliest larval stages of live diets with a gel-based diet, or with a granular form of the gel-based diet, resulted in a decreased survival and final body weight. We suggest that fish of this age do not have a competent digestive system to consume and/or assimilate these diets. In most labs, *D. rerio* of this age and size are usually fed live diets, although it is possible to successfully feed these young larval fish with a dry granulated diet (Barton, Johnson et al. 2016). At 12dpf or later, survival was high in individuals fed the gel diets; however, dry granulated diets of equal nutrition improved weight gain in those fed at 18 and 24dpf as compared to the gel diet. Eid et al. (2018) attempted replacement of live diets with dry diets for Seabream, *Sparus aurata,* larvae and showed a negative impact on both survival and growth with earlier replacements of live diets. Johnston et al., 2008 found that full replacements of live feeds (*Artemia* and Greenshell mussel gonads) in spiny lobster, *Panulirus ornatu,* starting at 9 days of age resulted in 100% mortality within 8 days of feed replacement. However, when researchers provided a partial replacement at 75% formulated feed and 25% *Artemia*, the partial replacement performed as well as the 100% live diet and lobster showed no differences in final length or carapace measurements. Based on this information, the gel diets used in this study may need to be supplemented with live diets during early life stages.

Our study indicates that the gel diet can be successfully fed to juvenile and adult *D. rerio*. *D. rerio* readily consumed the gel diet. Our study further suggests that this gel diet can support *D. rerio* growth of juveniles, leading to successful reproduction. In practical applications, we can infer that a large advantage of gel diets could be in the reduction of the daily burden of feeding multiple rations of a live or granulated diet, as we were able to apply the gel diet as one daily feeding ration. Based on growth and reproductive results in our study, the nutritional characteristics of the gel diet could be refined to maximize its nutritional competency. The current value of this gel diet as a larval feed form cannot be established at this time and requires further evaluation.

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APPENDIX A

IACUC APPROVAL FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

MEMORANDUM

DATE: 26-Sep-2022

TO: Watts, Stephen A

FROM: Shannon M. Bailey

Shannon M. Bailey, Ph.D., Chair

Institutional Animal Care and Use Committee (IACUC)

SUBJECT: **NOTICE OF APPROVAL**

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on 26-Sep-2022.

Protocol PI:	Watts, Stephen A
Title:	Zebrafish Nutrition
Sponsor:	UAB DEPARTMENT
Animal Project Number (APN):	IACUC-20656

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

This protocol is due for full review by 25-Sep-2025.

Institutional Animal Care and Use Committee (IACUC)

403 Community Health on 19th | 933 19th Street South

Mailing Address:

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