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STUDY OF IMPACT OF DIFFERENT NUTRITION ON ZEBRAFISH IN REFERENCE OF GROWTH

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ABSTRACT

*From the beginning of the 20th century, the pharmaceutical and aquaculture industries, among others, have come to recognise the value of using model animals in their study due to the widespread implications for human health.The main aim of the study is Study of impact of different Nutrition on Zebrafish in reference of growth***.** *When proper statistical analysis was completed, the data was normalised using arcsen.Common procedures in many zebrafish facilities include feeding zebrafish commercial foods and live feed without a balanced and regulated diet, which may introduce bias into the findings and make replication of the studies more difficult.*

Keywords: *Pharmaceutical, aquaculture, zebrafish, growth, nutirition*

1. INTRODUCTION

The beginning of the 20th century, the pharmaceutical and aquaculture industries, among others, have come to recognise the value of using model animals in their study due to the widespread implications for human health. Manipulability, physiological tractability, and, most importantly, high extrapolation to human applications are essential requirements for model animals. Animal models include the fruit fly (Drosophila melanogaster), nematode worm (Caenorhabditiselegans), Medaka (Oryziaslatipes), zebrafish (Daniorerio), amphibian African clawed toad (Xenopuslaevis), and the mouse (Musmusculus). For a variety of reasons, including its short life cycle, high quantity of eggs, large embryos, and the possibility of external fertilisation, as well as its quick growth and great resilience to experimental study, zebrafish have been more popular in recent years. In addition, zebrafish are simple to keep and breed in captivity, which allows for its manufacturing to take place in a laboratory setting. Successful research into human genetic illnesses has been conducted using zebrafish because of the strong similarity between several important zebrafish and human genes. The drawbacks of the zebrafish include its little size and its great evolutionary separation from humans. Knockout technology has been slow in coming, but recent advances in high-throughput targeted mutagenesis methods using gene inactivation through zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases have begun to fill this gap (TALENs).

1.2 Zebrafish Nutrition

Insufficient data exists to fully understand the function of nutrition in zebrafish development and reproduction, and there is no general agreement on the commercial diet used to sustain these fish. Some research has measured how different meals affect larval development, juvenile development, and reproductive success. Paramecia, rotifers (Brachionus sp.), and artemia are often used to feed zebrafish of all ages since their nutritional needs are not well understood (Artemia sp.). Since live feeds do not provide all of the nutrients that zebrafish need, this feeding method cannot provide a complete diet for this species. Fish stress may be triggered or made worse by a number of factors, including a deficiency in, excess of, or inability to absorb certain bioavailable minerals. Yet, live feeds are crucial for zebrafish upbringing because they encourage the fish to engage in their natural predatory behaviour, reducing the stress associated with confinement and enhancing the fish's well-being. Since artemianauplii have the right size and digestibility for zebrafish larvae, they are the most practical life feed, and artemia also seems to boost gamete production, favourable fertilisation rates, and spawning performance in adults..

1.3Zebrafish bone mineralization

Aquaculture (skeletal abnormalities) and biomedical research both benefit greatly from an improved knowledge of the bone remodelling mechanisms in teleost fish (skeletal development). Most teleosts have acellular bone, meaning they don't have osteocytes, which allows them to grow and mature at any age. Acellular bone is characterised by a lack of osteocytes, haematopoietic bone marrow, many tiny mononuclear osteoclasts, phosphorus mineral balance, and skeletal resorption at specific places. The zebrafish possesses cellular bone, which is more akin to the shape of human bones. Osteoclasts (either mononucleate or multinucleate) resorb bone and are followed by osteoblasts (bone-forming cells) forming new bone throughout the zebrafish bone remodelling process.

2. LITERATURE REVIEW

Karga, J. &Mandal, Sagar (2016) The purpose of the current study was to examine the impact of diets containing variable quantities of protein on the development, survival, and reproductive success of zebrafish, Daniorerio. Test diets containing 350 g kg1 protein (T2), 400 g kg1 protein (T3), and 450 g kg1 protein (T4) were prepared and given to fish for 210 days; the control diet (T1) was wild-collected zooplankton from nearby fish ponds. Mean weight increase and specific growth rate were both considerably ($P < 0.05$) higher in T1, with T3 and T4 showing comparable results. T1 had the greatest mean egg production and relative fecundity, followed by T4 and T3, with T2 having the lowest mean egg production and relative fecundity ($P < 0.05$). There were no statistically significant changes ($P > 0.05$) in brood survival, fertilisation, or hatching rates between the diets. Fry survival was greatest in generation one ($P < 0.05$), then generations three and four. Hence, it seems that the growth, reproductive performance, and fry survival rate were all higher on the control diet (i.e., mixed zooplankton). Nevertheless, the growth, survival, and reproductive performance of zebrafish were similarly unaffected by a diet containing 400 g kg-1 crude protein.

Watts, Stephen & Lawrence, Christian & Powell (2016) The zebrafish (Daniorerio) has become increasingly significant as a model organism in biomedicine and other scientific fields in the course of just around four decades. Standards for the production and upkeep of healthy animals for tests are necessary as zebrafish research grows in scope and complexity. Fish nutrition is an often-overlooked factor in fish health. Traditional methods of feeding zebrafish in the lab have been developed to maximise development and reproductive success. Nutritional priorities for these animals, hitherto focused only on basic production, are shifting towards the upkeep of clinically healthy study subjects as the discipline develops. In this article, we highlight the drawbacks of present methods and argue for the need of creating specified, standardised diets to support and promote the expansion of the zebrafish model system.

O'Brine, Timothy &Vrtělová (2015) Concern for the well-being of fish kept only as decorations has grown in recent years. A fish's diet may have a major effect on its well-being, resulting in behavioural and physical changes. Daniorerio, also known as the zebrafish, is kept as an aquarium decoration despite the fact that very little is known about the species' dietary needs, which might have serious consequences for its health. Here, we looked at how zebrafish behaved, how much oxygen they used, and how much development they experienced when fed diets that varied in crude protein content but were otherwise isocaloric and isonitrogenous. In our study, we observed that feeding fish 5% of their body mass (BM) per day had no significant influence on the specific growth rate or oxygen consumption, however the greatest crude protein and fat diet did raise condition factor. Zebrafish, when given a 2% BM feed once daily, showed no behavioural changes when fed any of the crude protein diets. Our zebrafish were able to reach their growth targets on a diet consisting of 32% crude protein and 8% crude fat when provided at a 5% BM ration, as revealed in this research. The fish's physical health was maintained by these diets, which was good for their overall well-being.

Smith, Daniel & Barry, R & Powell(2013) Many model species have shown that certain dietary components are crucial to achieving desired growth and body composition results. Although zebrafish (Daniorerio) have long been used in labs to better understand developmental biology and genetics, the impact of food on fundamental growth outcomes has been less well documented. The present investigation compared the effectiveness of four different protein sources when fed either alone (through isonitrogenous diets) or together (via a specified laboratory diet). During 12 weeks, groups of fish (n=60) were kept in tanks with a maximum capacity of 1.8 litres each and given a three-times-daily ad libitum diet. Length, weight, and overall fish health were measured and analysed (lean and fat mass). The length, weight, and lean mass of male and female fish given wheat gluten protein were considerably lower compared to those fed alternative diets, but the percentage of body fat was higher. Male and female fish on a casein diet were much shorter and lighter than fish fed other diets, and they had lower percentages of lean and fat mass (leanest). The fish given fish protein hydrolysate had much reduced lean mass and a high % body fat, whereas the fish fed a soy protein isolate diet fared similarly to fish fed a mixed-protein control diet. Our findings imply that in the context of a semipurified, specified diet, the protein source, together with associated amino acid ratios or other protein source changes, significantly affects growth and body composition outcomes in zebrafish.

Mehrad, Bahar&Jafaryan, Hojatollah&Taati, Majid (2011) The effects of varying vitamin C intakes on zebrafish (Daniorerio) growth, survival, fertility, and egg size were investigated. During 20 weeks, zebrafish were randomly assigned to one of five treatments, with each treatment being reproduced three times. Each of the four experimental diets (250, 500, 1000, and 2000 mg ascorbic acid kg-1) and one control group consumed the same total amount of vitamin C. (0 mg kg-1). There was a strong correlation between zebrafish body weight gain (BWI), specific growth rate (SGR), and reproductive success in vitamin C treatments ($P < 0.05$). The diameter of the eggs did not vary significantly across the treatments. Compared to other groups, zebrafish given diets containing 1000 and 2000 mg kg-1 AA had a greater survival rate. The importance of food in reproduction was highlighted by these findings, lending credence to the theory that feed additives might increase reproductive success. These findings provide credence to the idea that feed additions like vitamins may boost reproduction in all vertebrates, including humans, which is significant given the zebrafish's well-established status as a vertebrate model for biomedical research.

3. METHODOLOGY

3.1 Rearing conditions

The department of zoology A N College Patna provided the lab space and zebrafish breeding tanks for the experiments. Adult zebrafish were kept in a 14:10 light-dark photoperiod cycle in a ZEBTECH (Tecniplast, Italy) recirculating system with 3L glass fish tanks and a water turnover rate of 7,3 L h -1. In the past, a reverse osmosis system (Aquatic biosystems) has been used to keep the water at a consistent temperature of 28 ± 1^{0}C, a conductivity of 680 S, and a pH of 7.5. In this experiment, the levels of nitrite, nitrate, and ammonia were tracked weekly and remained well below the safe range the whole time (< 0.1mg L-1).

3.2 Nutrition

A total of 9 treatments were used: a control diet (CD), a purified diet (PURE), and 7 supplemented purified diets (consisting of a total of 18 diets). To evaluate the effects of these diets, 3-month-old fish from wild type stock were split into groups of 8 (5 females and 3 males) and fed with each diet for 20 days before the reproduction trials began. As a comparison, we employed a commercially available, high-quality marine fish larval diet (CD, 60% Crude protein; 14% Crude Lipid) and a sterile diet. Phosphatidylcholine (5 g/Kg), phosphatidylethanolamine (5 g/Kg), selenium (1 mg/Kg), zinc (750 mg/Kg), manganese (1 mg/Kg), iodine (20 mg/Kg), vitamin D3 (4000 IU/Kg), and iodine (20 mcg/Kg) were added to the purified diet for the remaining diets. Fish larvae were given pellets 200 m in size whereas adult fish were fed pellets ranging from 600 to 800 m in size. The experimental diets were provided twice daily on an ad libitum basis, and the tanks were emptied and cleaned about 30 minutes after each feeding to remove any leftover food.

3.3 Statistical analysis

When proper statistical analysis was completed, the data was normalised using arcsen. Hatch rate, number of abnormalities, charge of deformities, and presence of deformities by area were analysed using One-way ANOVA ($p \leq 0.05$). Repetitive measures analysis of variance ($p \leq 0.05$) was used to examine data from SL and TL measurements of breeders, weight measurements of breeders, TM, PM, LIN, VCL, and VSL measurements of sperm, and SL and TL measurements of larvae. Tukey's multiple range test ($p \leq 0.05$) was used to compare means across all data. All the numbers were crunched in (SPSS 21.0).

4. RESULTS

4.1 Growth in length and weight

Length (TL and SL) and weight were used as biological performance measures to compare the development of adult zebrafish fed various supplemented meals at three separate time periods. Standard length (SL) was significantly different between the D3 diet (D3; 28.9 mm) and the control commercial diet (CD; 35.9 mm) at 25 days of trial (M; $p = 0.002$). (Fig. 1a). Zebrafish given CD had more SL development than those fed PE, ZN, MN, I, or D3 at the conclusion of the trial (E; 68 days) ($p = 0.002$). The D3 diet had the worst performance across the board (Fig. 1a).

At 28 days (M) into the trial, the CD and PC diets produced considerably longer fish than the D3 diet did, as measured by total length (TL; $p = 0.002$). (Fig 4b). At the conclusion of the trial (E), the CD and PC groups had higher TL values than the PE, MN, I, and D3 groups ($p < 0.0001$). Significant differences were also seen between zebrafish given the D3 diet and those fed the purified diet (PURE) and the SE diet (Fig. 2b). Similar findings to what was shown for length were also seen for the adult zebrafish's weight (Fig. 2). Significant weight differences ($p < 0.001$) between the ZN, I, and D3 diets and the CD diet were seen at 25 days (M). There were also notable variations between the PC diet and the D3 diet. Compared to the D3 diet, the CD diet (0.60 mg) produced a greater weight gain after 68 days (E) (0. 28 mg). No statistically significant differences were seen between the CD and D3 groups and the other diet groups (Fig. 2).

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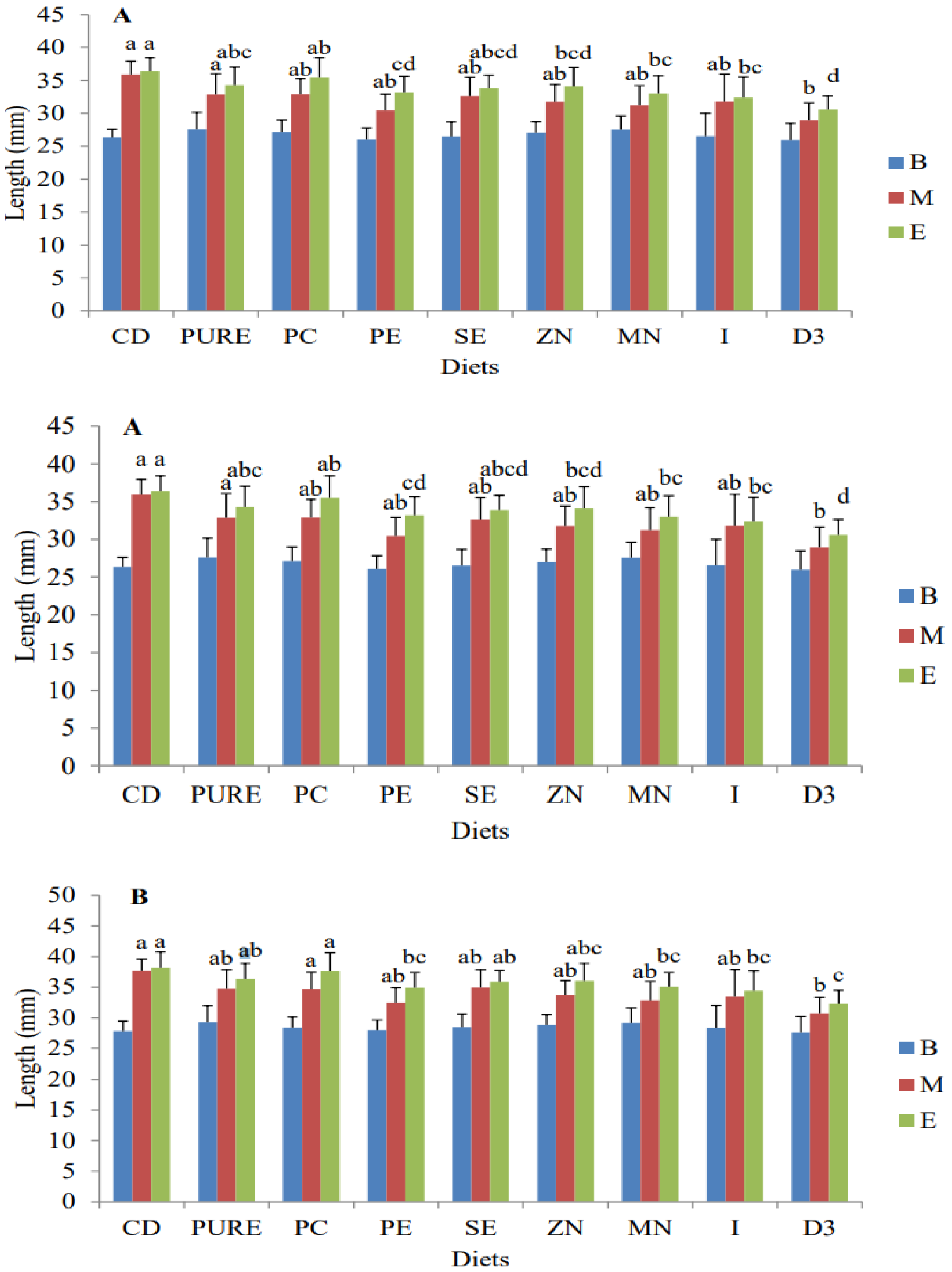


Fig. 1Zebrafish (*Daniorerio*) length is affected by commercial diet (CD), purified diet (PURE), and supplemented diets (PC, PE, SE, Zn, MN, I, D3) (mm) A series of readings taken at the start of the experiment (0 days, B), in the middle (25 days, M), and at the conclusion (68 days, E). Length (A) is the standardised length (SL), and Length (B) is the overall length (TL). Letters stand for statistically significant differences (p <0.05).

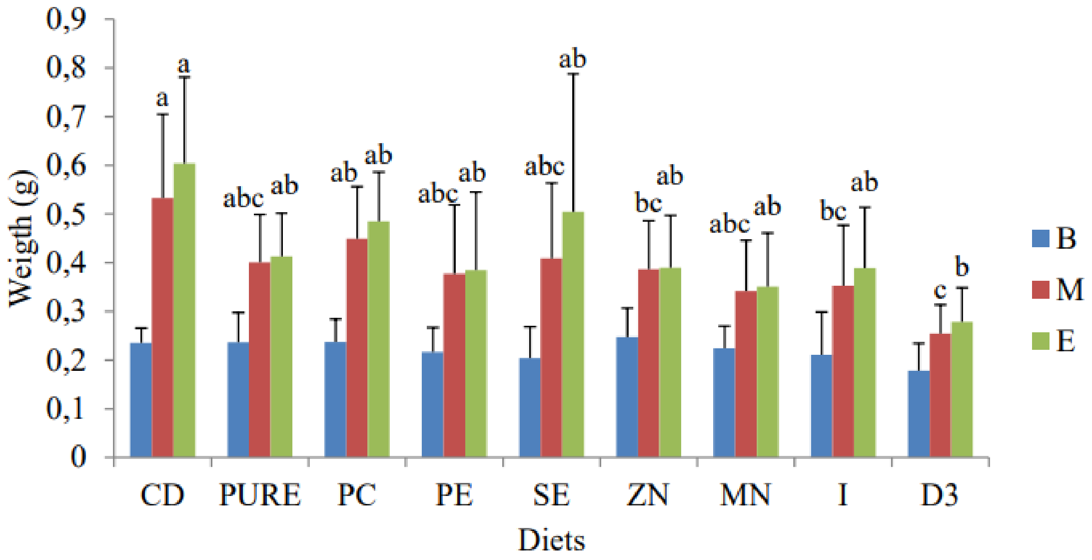
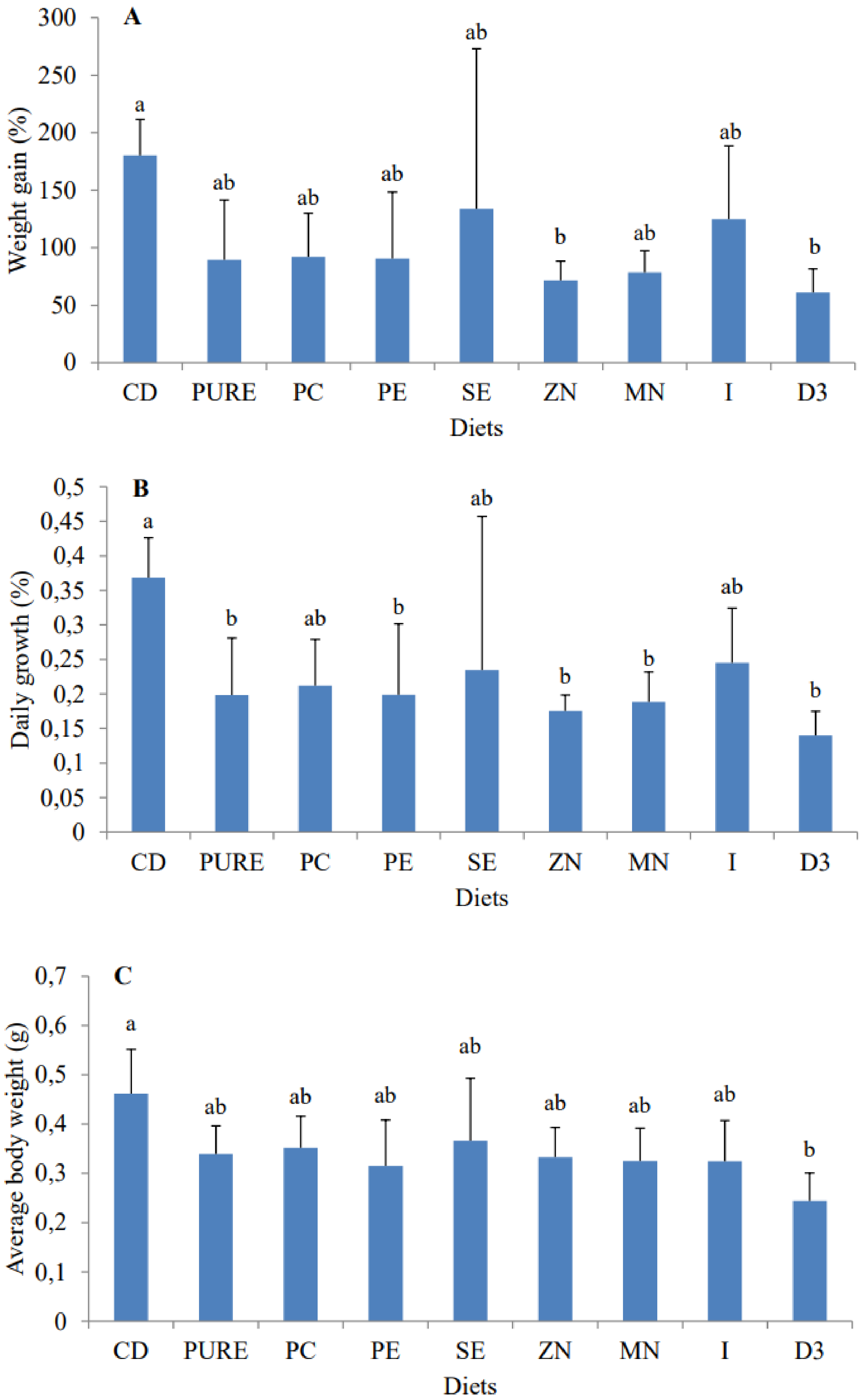
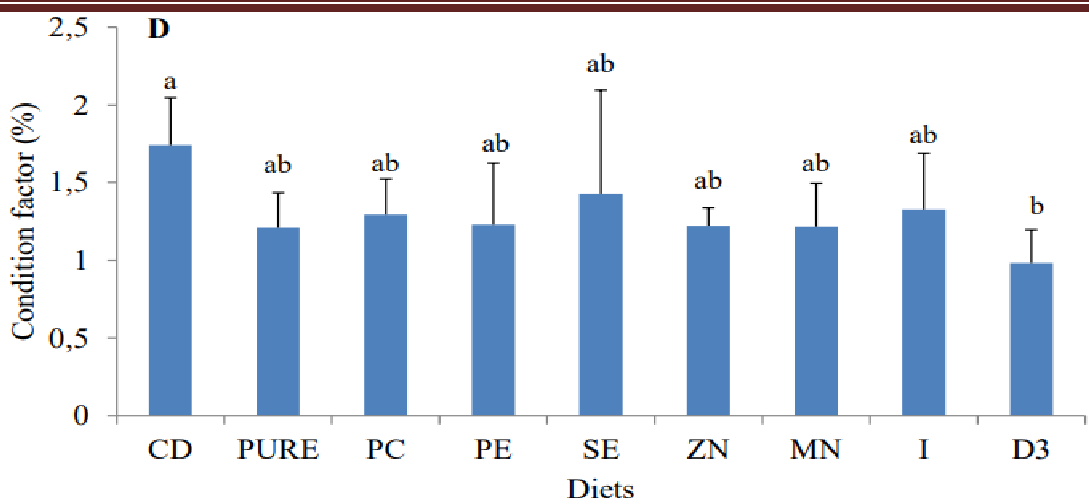


Fig. 2Zebrafish (*Daniorerio*) breeders' body weight changed as a result of exposure to several dietary conditions: the standard diet (CD), a purified diet (PURE), and various supplemented diets (PC, PE, SE, ZN, MN, I, D3) (g). Beginning (B), intermediate (M; 25 days), and ending (E; 68 days) values were recorded. Variations indicating statistical significance (p <0.05) are shown with letters.

4.2 Growth analysis

In order to better understand the effects of the diet, numerous indices were employed in addition to size and weight measurement (Fig. 3). Control diet (CD) specimens (180, 3%) showed a greater increase in body weight (IBW) than those given either ZN (71, 6%) or D3 (61, 3%) diets ($p = 0.026$). (Fig. 3a). In contrast, zebrafish given the other diets did not vary statistically ($p = 0.010$). (Fig. 3a). Among the dietary interventions tested (PUR, PE, ZN, MN, and D3), the CD diet resulted in the greatest daily growth (DGI) ($p = 0.011$). (Fig 3b). Compared to the other diets, the PC, SE, and I diets showed no distinguishing features (Fig 3b). Fish given CD had significantly higher ABW ($p = 0.010$) and CD ($p = 0.050$) indices compared to fish fed a D3 diet, with the greatest values being recorded in the former (Fig. 3c, d). We found no statistically significant differences between any of the diets (Fig 3c, d). Growth values for the CD diet were the greatest ($p = 0.044$), while values for the ZN and D3 diets were the lowest (Fig. 3e). Contrasted to the other treatments, no changes were seen (Fig. 3e).


A
Weight gain (%)
300
250
200
150
100
50
0
a
ab
ab
ab
ab
b
ab
ab
b
CD
PURE
PC
PE
SE
ZN
MN
I
D3
Diets
B
Daily growth (%)
0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
a
b
ab
b
ab
b
b
ab
b
CD
PURE
PC
PE
SE
ZN
MN
I
D3
Diets
C
Average body weight (g)
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
a
ab
ab
ab
ab
ab
ab
ab
b
CD
PURE
PC
PE
SE
ZN
MN
I
D3
Diets



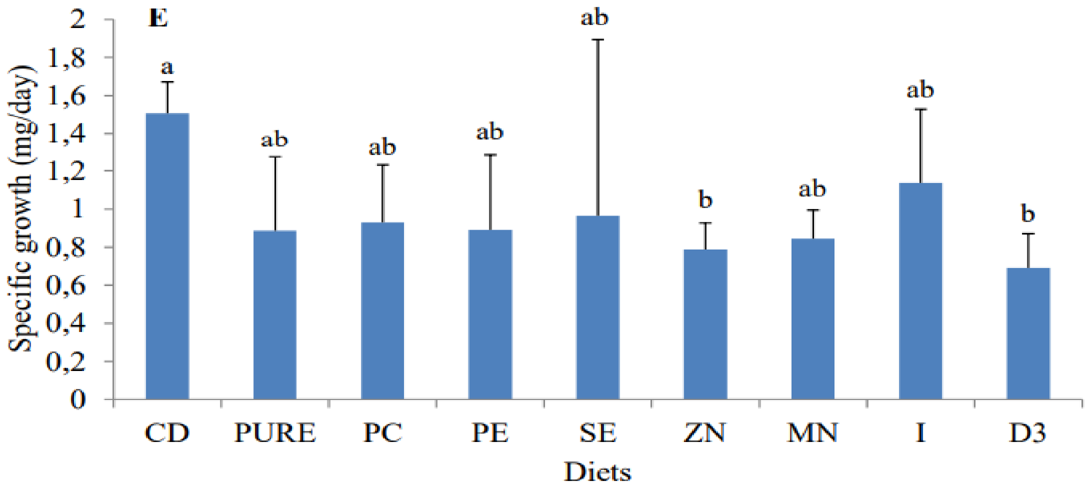
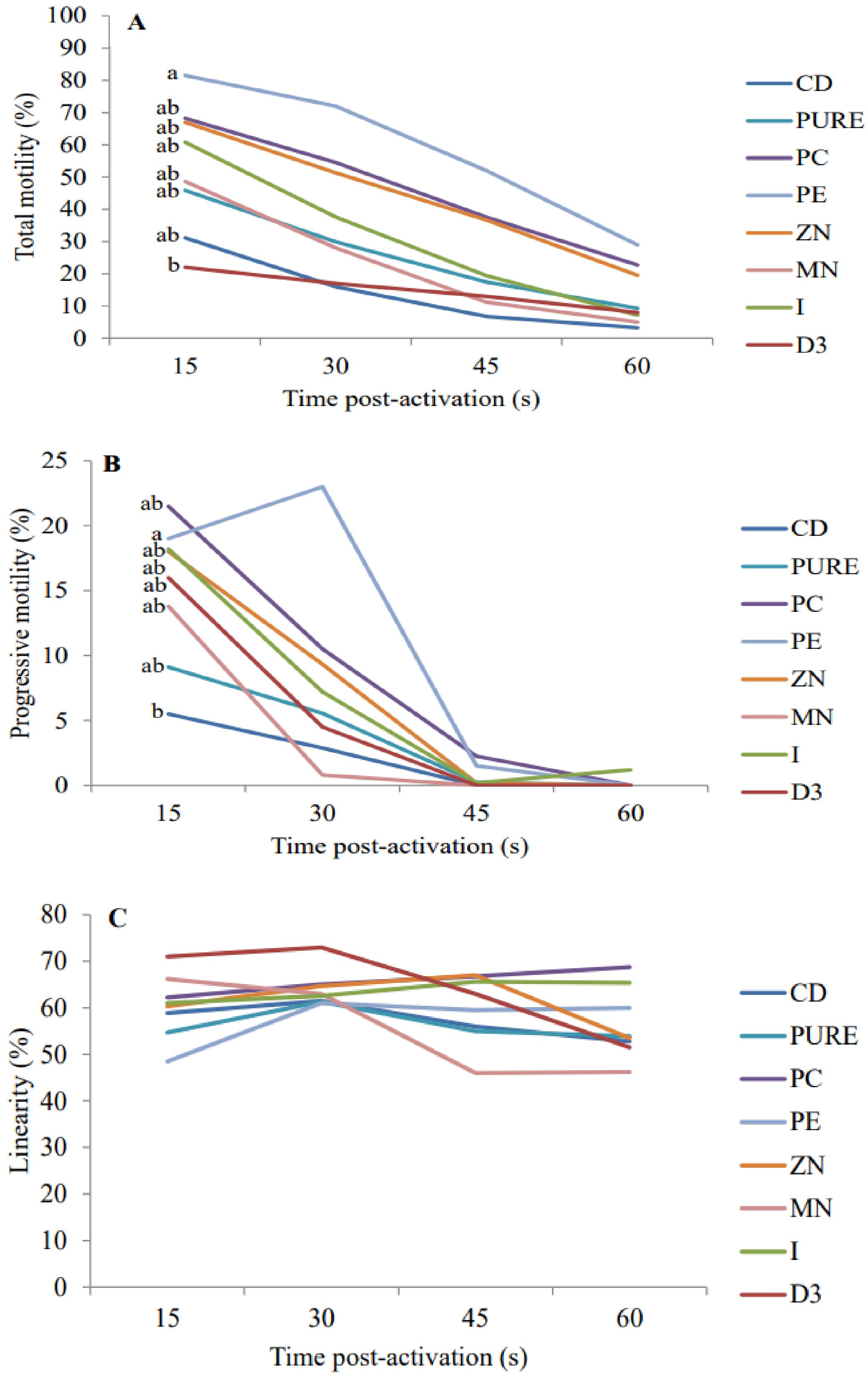
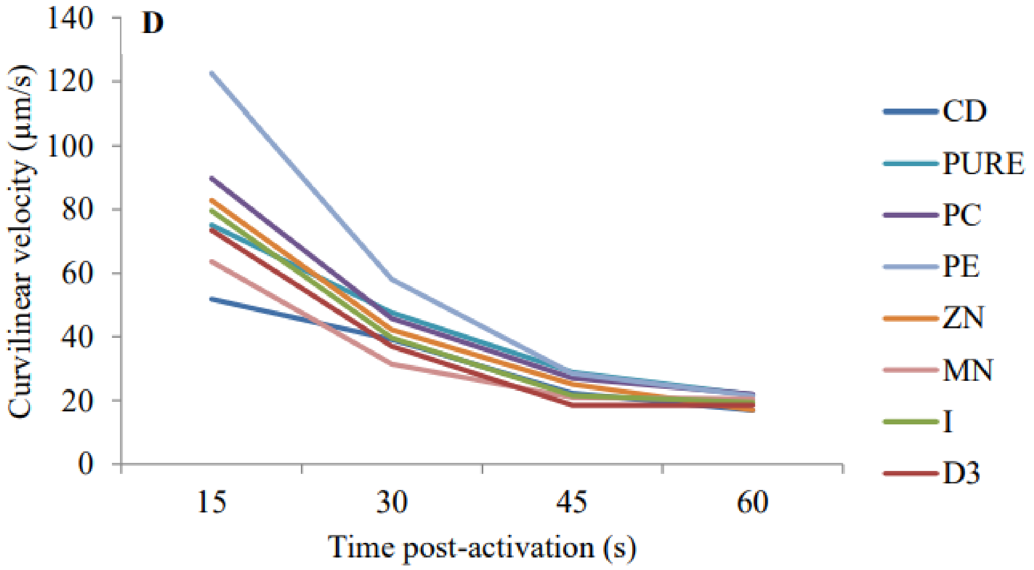


Fig. 3Impact on Growth Performance Indices of Control (CD), Purified (PURE), and Supplemented (PC, PE, SE, Zn, MN, I, and D3) Diets. Weight gain (IBW), DGI, ABW, CF, and SGR are all measures of physical development (SGR). Variations indicating statistical significance ($p < 0.05$) are shown with letters.

4.3 Sperm quality analysis

Several sperm circumstances, including total motility (TM), linearity (LIN), progressive motility (PM), curvilinear velocity (VCL), and straight line velocity (VSL), were analysed (Fig. 4). There were changes in TM and PM motility, but no further differences could be established. Significant differences ($p = 0.005$) were seen between the TM of the PE diet and the decreased motility values seen with the CD and D3 diets (Fig. 4a). As compared to groups given the other diets, the PE diet group performed similarly well in terms of PM ($p = 0.009$). (Fig. 4b). There were no statistically significant changes ($p < 0.05$) between the control group and the diet-treated group on the remaining sperm quality measures (linearity, curvilinear velocity, and straight line velocity) (Fig 4 c, d, e).


A
Total motility (%)
100
90
80
70
60
50
40
30
20
10
0
a
ab
ab
ab
ab
ab
ab
b
15
30
45
60
Time post-activation (s)
CD
PURE
PC
PE
ZN
MN
I
D3
B
Progressive motility (%)
25
20
15
10
5
0
ab
a
ab
ab
ab
ab
ab
b
15
30
45
60
Time post-activation (s)
CD
PURE
PC
PE
ZN
MN
I
D3
C
Linearity (%)
80
70
60
50
40
30
20
10
0
15
30
45
60
Time post-activation (s)
CD
PURE
PC
PE
ZN
MN
I
D3



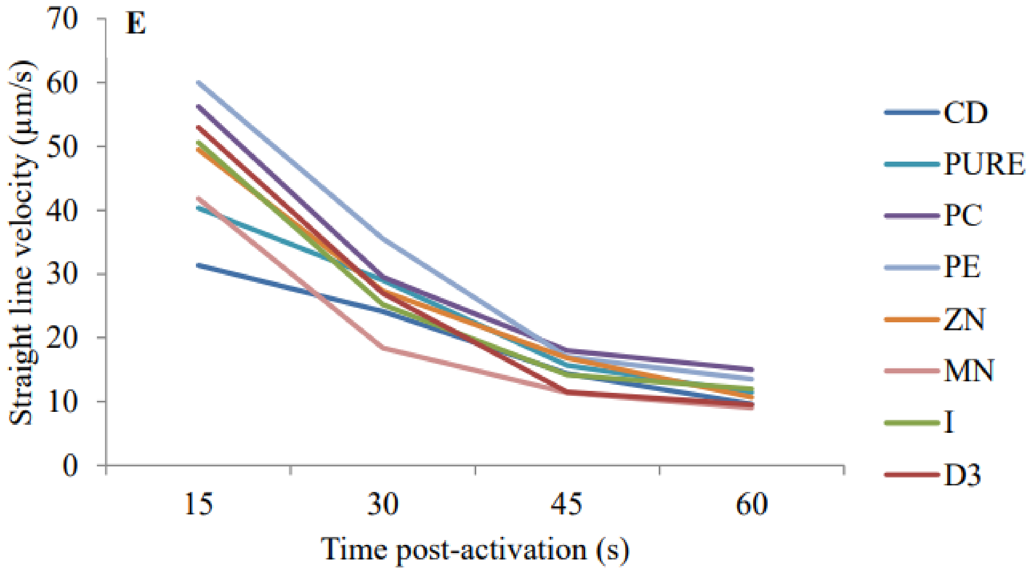


Fig. 4Zebrafish (Daniorerio) sperm motility after being exposed to several diets and supplements (control diet [CD], purified diet [PURE], and supplemented diets [PC], PE], SE, Zn, MN, I, and D3] (68 days). Total motility (A), progressive motility (B), linearity (C), curvilinear velocity (D), and straight line motility (E) are all characteristics of sperm motility. Variations indicating statistical significance ($p < 0.05$) are shown with letters.

5. CONCLUSION

Common procedures in many zebrafish facilities include feeding zebrafish commercial foods and live feed without a balanced and regulated diet, which may introduce bias into the findings and make replication of the studies more difficult. As zebrafish are such an important study species, a number of dietary studies are needed to determine what they need. Results showed that PURE food, with its reduced lipid content, had the same outcomes as other diets employed with greater lipid content and seemed to be acceptable for growing zebrafish, bringing this diet very near to the optimal values required for zebrafish. The various supplements had varying effects on zebrafish of both sexes and at various stages of development. Phospholipid PC resulted in longer adults, larger eggs, and more perivitelline space, but it also caused abnormalities in the larvae. Higher values for perivitelline space showed a positive effect from the PE on total motility (TM) and progressive motility (PM) of sperm, whereas longer larvae were the consequence of the poorest outcomes. To improve sperm motility and, by extension, fertility, these findings lend credence to the adoption of a diet of a comparable concentration.

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