Non-Caffeinated Soda Concentration and the Effects on Zebrafish Embryos Development

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Abstract:

The *Danio rerio* or more commonly known as Zebrafish were the model organism used in research. To be more exact the effects on the development of the Zebrafish in the different environment of the non-caffeinated soda was the topic of interest. The purpose of this experiment was to determine the effects of Zebrafish embryos when they were placed in an environment that contained non-caffeinated soda (Fanta). More specifically, to determine if we added non-caffeinated soda to the embryo’s environment would this cause the embryos to grow larger. Non-caffeinated soda was chosen to test the effects of sugars on the embryos. The bigger picture was to look at how humans could be effected. Soda is not always viewed as a harmful substance. Sugar is a part of everyday life, however this could be the very reason it may be doing harm to the body and maybe embryos.

Introduction:

Zebrafish were excellent models for learning about development vertebrate development. The embryos exhibited several characteristics that made them great models. They developed outside of the mother and were produced in large quantities. This allowed for multiple trials for the experiment. Zebrafish exhibited the same development in time (synchronous development) in the clutch. The optically clear and quick development helped to observe the embryos throughout the experiment. The synchronous nature of the embryos made them easy to take on substances in their environment (University of Wisconsin-Milwaukee). The experiment was to concentrate on the sugar effect of soda so caffeine was eliminated. Sugar is what leads to obesity, tooth decay, and weak bones for humans. Several animal studies have linked aspartame to a higher cancer risk (10 Ways Sugar Harms Your Health). The high fructose corn syrup is associated with poor development of collagen and dehydration. The phosphoric acid tends to draw calcium away from the bone as the body tries to recreate a balance in the internal pH of the body. This lowers bone density (10 Ways Sugar Harms Your Health).

The question for the experiment was if the embryos were in the environment of non-caffeinated soda (Fanta), would that then cause the embryos to grow larger
because of the association with obesity. The hypothesis was if the non-caffeinated soda (Fanta) was added into the embryo’s environment, then the embryos would grow to be larger than when placed in a normal environment because of the sugar in the soda. The embryos could also have developed irregular heartbeats. Both of those factors could have been due to the high sugar content and harmful acids in the soda that was linked to lower bone density and a slower growth.

**Materials and Methods:**

List of Materials:
- 1 Bottle of Fanta (Non-Caffeinated Soda)
- Zebrafish Embryos (About 5 to 7 embryos per well)
- 1 Bottle of Instant Ocean
- 2 Multi-well Plates (1 control and 1 experimental)
- 1 Dissecting Microscope
- 1 Compound Microscope
- 2 Beakers (100ml or more)
- Stir Stick
- Pipettes (As many as needed)
- Deionized water (Enough for solution)
- Tape (To label)
- Marker
- Incubator (Set at 28°C)

To start the experiment all of the materials were gathered. The multi-wells were rinsed to make sure the solution would not be mixed with other contents from the wells. Then one multi-well was labeled with tape that said control (just instant ocean) and the other well was labeled with tape as well but said experiment (non-caffeinated soda). When the wells were labeled and cleaned the instant ocean then could be placed into the control wells. Some of the Zebrafish embryos were then placed into the 6 wells filled with the instant ocean. To find the amount of soda to mix with the instant ocean was done with a calculation. A normal soda serving for humans (12oz) was converted to mL
which came out to be about 354mL. Then 354mL was divided by how many mL of water is in the human body (56,000mL). The soda to water ratio was about 0.006mL. Then this was used to determine the ratio of soda to Instant Ocean. For every 250mL of Instant Ocean 1.5mL of soda was added to the solution. For every 10mL of Instant Ocean, .06mL of soda was needed, and for every 100mL of Instant Ocean, .6mL of soda was needed. The instant ocean was then mixed with the proper amount of soda. When the solution was mixed it was added to the other wells that were labeled experiment. The embryos were then placed into the solution. All of the embryos were counted and the number alive embryos were recorded into the chart. The wells containing the embryos and solutions were placed into the incubator for 24 hours. After the 24 hours the wells were placed under the dissecting microscope to look at the presence of life and if the embryos were hatched. The number counted was then recorded in chart. Then one of the embryos was sucked up using a pipette and placed under the compound microscope to observe a closer look at the embryo. After recording the observations the embryo was placed back into well. Finally, the solutions were switched by sucking out the old solution and replaced with new. After the solutions were switch the plates were placed back into incubator. The same process was done after 48 hrs. 72 hrs., and 96 hrs.

The outcomes of the experiment were recorded by counting the embryos showing the presence of life (heartbeat present) and if they were hatched or not (fish were swimming). The data was collected in a data table to show the presence of life and if hatched. The data was analyzed by comparing the control group and experimental group numbers of hatched and alive.

Safety:
For this experiment precaution was taken. Protective eyewear was worn to keep solutions out of the eyes. The materials were kept out of our mouths so none of them were consumed.
Results:

The experimental design was to have two multi-welled plates containing Zebrafish embryos living in the environments of Instant Ocean and Instant Ocean mixed with Fanta (non-caffeinated soda) to watch the stages of the development of the embryos. There were two solutions to compare the different development stages and effects. One constant of this experiment was to keep the same amount of each of the solutions in each of the wells. To keep the temperature the same in the incubator over the time the plates were kept in there was constant or controlled variable also. The embryos were kept in the same wells throughout the entire experiment so no new contents were added. The independent variable was the embryos in the instant ocean mixed with non-caffeinated soda (Fanta). The dependent variable was the development of the embryos and their size. If they survived then the heartbeat would have been observed closely. The relationship between them was linked with the sugar in the Fanta. The aim of this experiment was to see if the embryos would develop.

<table>
<thead>
<tr>
<th>H= Hatched</th>
<th># Starting of Embryos</th>
<th>24 Hrs</th>
<th>48 Hrs</th>
<th>72 Hrs</th>
<th>96 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>L= Living</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment: Instant Ocean and Fanta</td>
<td>43</td>
<td>H: 0</td>
<td>L: 40</td>
<td>H: 0</td>
<td>L: 40</td>
</tr>
<tr>
<td>Percents</td>
<td>6 hrs</td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>72 hrs</td>
<td>96 hrs</td>
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</tr>
<tr>
<td>Alive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Instant Ocean)</td>
<td>100%</td>
<td>87%</td>
<td>87%</td>
<td>83%</td>
<td>78%</td>
</tr>
<tr>
<td>Experiment (Fanta and Instant Ocean)</td>
<td>100%</td>
<td>93%</td>
<td>93%</td>
<td>93%</td>
<td>81%</td>
</tr>
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</table>

<table>
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<tr>
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<th>96 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatched</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Instant Ocean)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>19%</td>
<td>60%</td>
</tr>
<tr>
<td>Experiment (Fanta and Instant Ocean)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>5%</td>
<td>77%</td>
</tr>
</tbody>
</table>

108 embryos was the starting number for the control. Starting day (6 Hrs) 100% were alive and 0% were hatched. 24 Hrs 87% were alive and 0% were hatched. 48 Hrs 87% were alive and 0% were hatched. 72 Hrs 83% were alive and 19% were hatched. 96 Hrs 78% were alive and 60% were hatched. These percentages were based off of the 108 embryos from the start.

43 embryos was the starting number for the experiment. 6 Hrs 100% were alive 0% were hatched. 24 Hrs 93% were alive and 0% were hatched. 48 Hrs 93% were alive and 0% were hatched. 72 Hrs 93% were hatched and 5% were hatched. 96 Hrs 81% were alive and 77% were hatched. These percentages were based off of the 43 embryos from the start.
6 Hpf Observations:
- Most of the embryos were fertilized and have a clear color
- We removed all of the white (dead) embryos from all of the trays

24 Hpf Observations:
- A lot of the embryos were moving
- Almost all of the embryos survived
- Both solutions were switched to fresh solutions
- Both solutions looked as if they were in stage 16 hpf
- The plates were not in the incubator overnight.

48 Hpf Observations:
- One of the control embryo died
- All of the Fanta embryos were alive
- Soda- looked like 48 Hpf stage they had pigment and longer tails
- Control- looked like 19 Hpf stage they had no pigment but were growing in shell
  - After more observation the embryo moved position and presented pigment
    so the stage of Hpf was changed to 48 Hpf
- The solutions were switched to keep the embryos in fresh solutions

72 Hpf Observations
- All of the embryos seemed to be alive and developing
- Soda- seemed to be behind in development, looked like they were only at 48 Hpf
- Control- They were doing well
  - 12 Hatched
  - They looked like they were at 60 Hpf
- Heartbeats were more visible and closely observed
- The solutions were switched to keep the embryos in fresh solutions
96 Hpf Observations

- **Soda**: The fish were swimming differently from the control
  - They did not move much but when they did move they were slow and shook
  - Rapid heartbeat
  - More were dead and mostly died in the shells
- **Control**: Mostly all of the embryos were hatched
  - They swam quick and moved around more than the experimental group
  - Slower heartbeat
- The solutions were switched to keep the embryos in fresh solutions

**Results:**

**6 Hpf Pictures**
(All control)
24 Hpf Pictures

Control

Fanta

48 Hpf Pictures

Control

72 Hpf Pictures

Fanta
Data Presentation and Analysis:

- Both solutions decreased in number of alive embryos over Hrs
- Fanta seemed to have a consistent rate of number alive
- Control seemed to decrease in number of alive more than the Fanta

- Control hatched at a faster rate
- Fanta had a very slow rate for hatching
As the hours increased the percentages of both solutions decreased in the number of embryos alive.

As the hours increased the percentages of both solutions increased as well in the number of hatched embryos.

**Discussion:**

In the pre-lab it was hypothesized that if non-caffeinated soda or Fanta was added to the embryos environment, then the embryos would grow larger than their normal environment (Instant Ocean) because of the sugar added to the solution. They also could develop irregular heartbeats due to the high sugar content and harmful acids present in the Fanta. This was hypothesized based on the knowledge that non-caffeinated soda can slow metabolism and contribute to an onset of obesity in humans, and can be related back to the Zebrafish embryos. If they survived the environment change, it was then hypothesized that their heartbeat would be irregular, because of the exposure to a large amount of sugar. Sugar is converted to glucose more quickly than
the foods typically found in the diet of Zebrfish. The data collected showed that the experimental group developed at a much slower pace than the control group. The experimental fish were able to develop but some not to their full potential. Some of them were never able to hatch and unfortunately die inside of their shells. When the fish were able to hatch and move around inside of the well, they barely moved. If they did swim around they were much slower than the control group. The experimental fish would also had a distinct shake to their movement. It was a fast back and forth movement of their tails. These movements could be due to the effects the sugar in the Fanta can have on the development of the vertebrae of the embryos. The phosphoric acid in the Fanta could be another probable cause of the movements of the experimental Zebrafish embryos. When taken in in concentrated or large amounts phosphoric acid can cause dermatitis, pain, tearing, and blurred vision, difficulty swallowing or breathing and gastrointestinal problems. The embryos exposed to phosphoric acid could have been experiencing some of these effects such as blurred vision or difficulty breathing, which would explain their sudden, jerky movements.

The control group was able to hatch at a faster rate than the experimental. The control group fish were much more active. They swam around with speed. There was no shake to the control group’s tail as well. There was no sugar effect to the control to cause the embryos to develop differently. For both groups the embryos developed more overtime (hours), but the experimental group lagging behind about 24 hours. The control group was more successful at hatching than the experimental group. The data collected supports that there were effects on the Zebrafish embryos when non-caffeinated soda was added to the instant ocean. It was determined that the heartbeats were irregular based on the rapid heartbeat in the experimental embryos, which was due to the high sugar content in the Fanta. Also because many of the experimental embryos did not hatch. We concluded that the embryos did not hatch because of dehydration from the sugar. Fortunately, we were able to see the more predominant effects of the sugar such as the slow movement and the shake of the vertebrae.

Even though some of the effects of the sugar were seen, errors were still present as well. The very first night the trays were not placed into the incubator. The room
temperature may not have been warm enough for the embryos to develop correctly. This could have affected both of the groups with their development process. Room temperature was not recorded. Too many embryos in one well could have affected the control group. In some of the wells of the control group, 10 or more embryos were placed in each. There might not have been enough space for the fish to develop correctly. The embryos may have not been receiving the correct amount of nutrients due to overpopulation. When the fish were observed, both groups were under intense light. This could have also caused the embryos to develop differently.

This experiment was done to evaluate the effects of non-caffeinated soda (sugar) on Zebrafish embryos development. The results might correlate to human development in humans who consume greater amounts of sugar, and can be linked to the development of the vertebrae in the embryos of this experiment as well. The degradation of bone density in both humans, and the Zebrafish embryos can be linked to the presence of phosphoric acid in Fanta and other non-caffeinated sodas. This experiment could be followed up by more extensive research on the effects of phosphoric acid on Zebrafish embryos. Since the sugar content was intended to be the isolated variable and it is not known what its specific effects were, it would be helpful to carry out an experiment where phosphoric acid was the only variable tested. This would allow for more definitive evidence on the effects of sugar only, phosphoric acid only, and a combination of both on the Zebrafish embryos and in turn, humans.

References

