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Amino Acid Preferences in Bumble Bees

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in Biology and the Honors Program

by:

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Abstract

Bumble bees (*Bombus impatiens*) forage for nectar and pollen alike, however, little is known as to whether bees sense the nutritive value of different pollens. Pollen is a major source of protein for bumble bees, as they feed it to larvae and use it to make eggs (Nicolson, 2011). In comparison to honey bees (*Apis meliifera*), bumble bees collect pollen from more diverse plant species and return to the colony with pollen loads higher in essential amino acids (Leonhardt et al., 2012). Whether or not a bumble bee can detect these amino acids through taste and/or smell and choose pollen on this basis is unknown (Brito Sanchez et al., 2007). I asked whether bumble bees have the ability to taste/smell the difference between different amino acid solutions, specifically Glycine, Threonine, Proline, and Phenylalanine. In honey bees, research has shown that these amino acids may play an important role in nutrition, but little is known as to the importance in bumble bees. In a series of feeding choice assays, I tested these amino acids at different concentrations to determine if there was an effect on bumble bee preference at high or low concentrations. This study lays the groundwork for understanding what role gustation plays in the recent North American bumble bee declines. These declines affect pollination of agricultural crops and native plant species (Cameron et al., 2010). Thus, understanding bumble bee nutrition is important to keep their species thriving and consequently our species as well. This research will shed light on a basic aspect of bumble bee nutrition and assess whether their preferences for different quality pollens potentially contribute to their current decline.

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Introduction

Amino acids play an important role in all organisms by providing the building blocks for proteins, which can then be used in numerous biological functions. Essential and non-essential amino acids differ for different species. Essential amino acids are those that the body is unable to produce on its own, but that are necessary for biological processes and therefore must be obtained through foods (Nutriology, 2008). Nonessential amino acids, in contrast, can be produced through the breakdown of other amino acids, and thus can be produced by the body (Nutriology, 2008). Essential amino acids, although important to all species, differ slightly across species in the ratio necessary for survival. Many species can survive for long periods of time without amino acids, while others need them on a regular basis (DeGroot, 1953). For example, research using mammalian cell cultures found that a total of 13 essential amino acids had to be present in order for cell survival (Eagle, 1959). In rats, it was found that an increase in phenylalanine levels led to a decrease in amino acids in the brain (McKean et al., 1968). In flies, it was found that serine was not essential (DeGroot, 1953). Thus, essential and nonessential amino acids differ across species. Many experiments on animals such as rats, butterflies, and honey bees have explored whether or not these animals can detect the presence of amino acids in their food source and furthermore, if they can differentiate between essential versus non-essential amino acids. However, whether bumble bees can detect amino acids is unknown, despite previous research. Leonhardt et al. (2012) found that bumble bees may be able to differentiate between pollen qualities but, because in this study, the content of returning foragers' pollen loads was measured, it is not clear if this is due to amino acid presence, lipid presence, or some other factor.

It is important to understand the dietary nutrition of bumble bees as they act as major pollinators of various plants. For example, bumble bees are used specifically for tomato plant pollination, sweet peppers, and strawberries (Hood, 2015). Thus, as their population declines, crops and plants will be negatively impacted (Cameron et al., 2010). In order to analyze their dietary needs it may help to identify what amino acids are necessary for their survival so that efforts can be made to supplement the bees with their necessary amino acids in order to help the population survive. Additionally, it can aid conservationists in selecting species of plants that offer pollen or nectar rich in these amino acids, such as *Heteromeles arbutifolia*, that would be readily pollinated by bumble bees (Jha, 2013).

An analysis of previous research will aid in the understanding of bee nutrition, specifically, their ability to detect (e.g. by taste and/or smell) amino acids and differentiate between non-essential and essential amino acids. In rats, for example, it was found that when fed a low protein diet for several days, and then switched to a diet lacking essential amino acids, the rats had a shortened eating period and ultimately rejected the essential amino acid-lacking meals (Koehnle et al., 2003). This suggests that rats may be able to detect the presence of amino acids in their diets. Similarly, in butterflies it was found that when given nectar rich in amino acids, the butterflies laid more eggs (Mevi-Schutz et al., 2005). Thus, the presence of amino acids seems to have an effect on their reproductive success. Many studies have been done in honey bees as well. In an experiment testing pollen preferences, which is a primary source of amino acids, honey bees were found to prefer oilseed rape pollen which contained more of the essential amino acids (such as leucine) in comparison to the field bean pollen (Cook et al., 2003). However, apparently no research has been conducted on bumble bees to determine what amino acids are essential and nonessential for this group and whether they can even detect the presence of amino acids in nectars and pollens. The large amount of research done in honey bees and the minimal work done in bumble bees provides a starting point to better understand bumble bee nutrition.

Pollen and nectar are important sources of amino acids in honey bees (Nicolson, 2011). Nectar is a primary energy source for bees while pollen contains various proteins, fats, and vitamins necessary for development. Different plants produce pollens that vary greatly in protein content (Auclair et al., 1948). An obvious question is whether bees prefer to collect pollens with particular amino acid contents. Leonhardt et al. (2012) found evidence in support of this hypothesis, as bumble bees collected pollen that was higher in essential amino acid content but honey bees did not. In addition, it was discovered that plants that were visited often by pollinating bumble bees produced higher quality pollen (Hanley et al., 2008). Thus, it seems possible the bees seek protein-rich pollens and nectars in order to meet biological requirements. In honey bees, further experiments have been conducted testing their ability to differentiate essential versus non-essential amino acids. It was found that they were unable to do so and furthermore, showed a preference for amino acids present at lower concentrations in solution (Inouye et al., 1984). This suggests that honey bees are unaware of the amino acids that are important in their diet and possibly that they are unable to sense amino acids present in solutions. Despite this possibility in honey bees, the research has not yet been conducted in bumble bees.

Out of the many amino acids present, some have been noted to play especially important roles in bee attraction and function. In honey bees, it was found that tryptophan and lysine play an important role in growth, while cystine, aspartic acid, and glutamic acid are irrelevant for growth (DeGroot, 1953). Additional experiments suggest that proline, glycine, phenylalanine, and threonine also play a role in important bee physiological functions (DeGroot, 1953).

In an experiment testing the preferences of honey bees for amino acids in nectars, it was found that proline, glycine, and phenylalanine all produced a positive response in bees at a 2mM concentration (Nicolson, 2011). Similarly, an earlier experiment highlighted proline and glycine as dispensable, or non-essential amino acids, and phenylalanine and threonine as essential amino acids for honey bees (De Groot, 1953). Phenylalanine was found to be an attractant while Glycine seemed to deter the honey bees (Hendriksma et al., 2014). Phenylalanine was also characterized as an important amino acid in honey bees as it is produced in the royal jelly that worker honey bees make (Webster et al., 1987).

Despite proline being a nonessential amino acid, it was also found to have an important role in honey bee flight physiology. Proline levels were found to be lower immediately following flight, suggesting that it is important in supplying the necessary energy for flight (Barker et al., 1972). Threonine also plays a vital role as it has been stated that it must be present in pollens at a certain level in order to fulfill the requirement needed by the bee (De Groot, 1953; McCaughey et al., 1980).

This research looks at the putative essential amino acids phenylalanine and threonine as well as the putative nonessential amino acids glycine and proline in an attempt to better understand bumble bee nutrition and foraging behavior. Specifically, I assessed bumble bees' (*Bombus impatiens*) preference for amino acids to see if the results were similar to those found in other organisms. I hope that this work will be able to provide a better understanding of the importance of amino acids in a bee's diet and the necessity to provide bees with the important amino acids necessary for survival for their conservation and pollination of plants.

Materials and Methods

Phase 1: Preparation

I collected bumble bee foragers (*Bombus impatiens*) from the large foraging arena with dimensions 38 x 38 x 35.5 inches the night before I ran the experiment. The foraging arena had four bumble bee colonies attached to it obtained from Koppert Biological Systems (Michigan, USA), a cotton wick feeder with 15% sucrose that was always available for the bees, and artificial flowers for environmental enrichment. These bees were also provided about 1 tablespoon of pollen every day. I froze the bees for approximately 3-4 minutes to immobilize them and then placed them in individual preference chambers, large test tubes with a feeder at the end measuring 5.25 x 1 x 5.25 inches (See Figure A below). The feeder consisted of a cotton wick placed in the end of an open-ended 1.5 mL microcentrifuge tube. I filled the feeder with approximately 750 microliters of 15% (w/w) sucrose. The bees had access to this feeder overnight, for approximately 12 hours. In the morning, I removed the sucrose feeder and starved the bees for two hours.

Phase 2: The Experiment

I then presented each bee with two 500 microliter capillary tubes measuring 2.5 x 0.125 x 2.5 inches, one containing an amino acid solution and the other containing deionized water. The amino acids included Phenylalanine, Proline, Glycine, and Threonine. I tracked bees' consumption of each solution by measuring the level of solution to the closest 0.5mm at the time intervals of 15, 30, 45, 60, 90, and 120 minutes. It was previously calculated that 1mm of solution measured was equal to approximately 12 microliters consumed. I also set up an empty preference chamber that contained the same solutions as the current experiment, but without the bees. I later subtracted these values from the data to account for evaporation.

I tested approximately 15-20 bees on the four amino acids (200 bees). In the second part of the experiment, I tested the amino acid in 15% sucrose against 15% sucrose. In this case, a stock solution of 16.7% sucrose was made. I took 1mL of the 0.1M amino acid solution and added it to 9mL of 16.7% sucrose solution in order to make a 0.01M amino acid in 15% sucrose solution. I then ran the experiment according to the same protocol outlined for the deionized water. In each case I compared the absolute volume of amino acid solution consumed at the different concentrations for both water and 15% sucrose solution. In parts 1 and 2 of the experiment, I tested the amino acids against water/sucrose at two different concentrations, a low concentration of 0.01M and a high concentration of 0.1M. (Table A.)

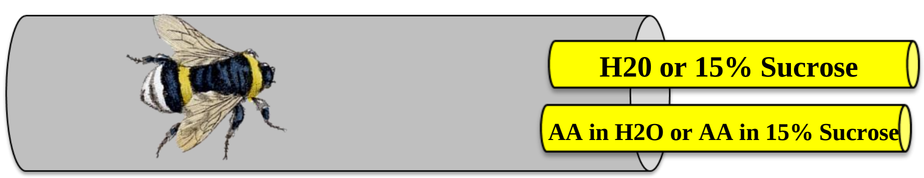

Figure A.

Experiment	Amino Acid Solution	Non-AA Solution	Concentration
1a	Phenylalanine, Glycine, Threonine, or Proline	Water	0.01M
1b	Phenylalanine, Glycine, Threonine, or Proline	Water	0.1M
2a	Phenylalanine, Glycine, Threonine, or Proline	Sucrose	0.01M
2b	Phenylalanine, Glycine, Threonine, or Proline	Sucrose	0.1M

Table A.

Results

Overall, concentration was important in determining preferences, and amino acid responses differed in sucrose as compared to water. Finally, bumble bees did not show a preference for essential amino acids. It is useful to first examine each amino acid in water and sucrose to determine its individual effects on preference in a general overview format. Then, I will discuss important comparisons between amino acids and their statistical values.

Proline: In sucrose, the lower concentration of 0.01M amino acid solution was preferred (mean: 9.6 mm consumed at 120 minutes) over the higher concentration of 0.1M (mean: 2.8 mm consumed at 120 minutes). However, in general, the bees consumed more sucrose at both concentrations. Bees consumed 9.6 mm of amino acid in sucrose at 120 minutes versus 9.6 mm of sucrose at 120 minutes at the lower concentration. Similarly, the bees preferred the sucrose solution (mean: 6.5 mm consumed at 120 minutes) over the amino acid in sucrose solution (mean: 2.8 mm consumed at 120 minutes) at the higher concentration of 0.1M (*Figure 1*). In water, the bees showed a slightly higher preference for proline at the lower concentration (mean: 0.89 mm consumed at 120 minutes) than at the higher concentration (mean: 0.58 mm consumed at 120 minutes). However, overall, it seems that the bees consumed more water than amino acid in water at both high and low concentrations (*Figure 2*). Bees consumed an average of 0.89 mm of amino acid in water solution at 120 minutes compared to 1.1 mm of water at 120 minutes at the low concentration. At the high concentration, bees consumed an average of 0.58 mm of amino acid in water solution at 120 minutes compared to 0.95 mm of water at the high concentration.

Glycine: In sucrose, at the lower concentration of 0.01M, the bees consumed more amino acid solution (mean: 8.3 mm consumed at 120 minutes) than at 0.1M (mean: 4.2 mm consumed at 120 minutes). At both concentrations, the bees seemed to prefer the sucrose solutions to the amino acid in sucrose solutions (*Figure 3*). Bees consumed an average of 8.5 mm of sucrose at 120 minutes compared to 8.3 mm of amino acid in sucrose at 120 minutes at the low concentration. At the high concentration, bees consumed an average of 6.0 mm of sucrose compared to 4.2 mm of amino acid in sucrose at 120 minutes. In water, the bees consumed more of the lower concentration amino acid solution (mean: 1.6 mm consumed at 120 minutes) than higher concentration (mean: 0.9 mm consumed at 120 minutes). Interestingly, the bees did not show a strong preference between water and amino acid in water at either concentration. At the low concentration, bees consumed an average of 1.6 mm of amino acid in water versus 1.7 mm of water at 120 minutes. At the high concentration, bees consumed an average of 0.9 mm of amino acid in water versus 0.8 mm of water at 120 minutes. Preference between the two groups was very similar (*Figure 4*).

Threonine: In sucrose, at the lower concentration of 0.01M, the bees did not consume more amino acid solution (mean: 3.8 mm at 120 minutes) than at 0.1M (mean: 3.8 mm at 120 minutes). However, overall, they preferred the sucrose solutions at both concentrations over the amino acid in sucrose solutions (*Figure 5*). At the lower concentration, bees consumed an average of 7.4 mm of sucrose compared to 3.8 mm of amino acid in sucrose at 120 minutes. At the higher concentration, the bees consumed an average of 9.3 mm of sucrose compared to 3.8 mm of amino acid in sucrose at 120 minutes. In water, the preferences were not as clear-cut. At the lower concentration, the bees drank more of the amino acid solution (mean: 1.2 mm at 120 minutes) than at the higher concentration (mean: 0.92 mm at 120 minutes). It seems that they preferred the water solution to the amino acid in water solution at both concentrations. However, there was only a slight preference for water over amino acid in water (*Figure 6*). At the low concentration, bees consumed an average of 1.4 mm of water compared to 1.2 mm of amino acid in water at 120 minutes. At the high concentration, bees consumed an average of 1.3 mm of water compared to 0.92 mm of amino acid in water at 120 minutes.

Phenylalanine: In sucrose, the bees preferred the amino acid solution at the lower concentration of 0.01M (mean: 3.2 mm consumed at 120 minutes) in comparison to the higher concentration of 0.1M (mean: 1.5 mm consumed at 120 minutes). However, at both concentrations, the bees preferred the sucrose solution to the amino acid in sucrose solution (*Figure 7*). At the low concentration, bees consumed an average of 8.6 mm of sucrose compared to 3.2 mm of amino acid in sucrose at 120 minutes. At the high concentration, bees consumed an average of 8.0 mm of sucrose compared to 1.5 mm of amino acid in sucrose at 120 minutes. In water, the bees consumed more of the amino acid solution at the lower concentration (mean: 0.69 mm at 120 minutes) than at the higher concentration (mean: 0.5 mm at 120 minutes). They did not seem to show a strong preference between water (mean: 0.75 mm consumed at 120 minutes) and amino acid in water (mean: 0.69 mm consumed at 120 minutes) at the lower concentration of 0.01M. However, at the higher concentration, the bees preferred the water (mean: 0.9 mm consumed at 120 minutes) to the amino acid in water solution (mean: 0.5 mm consumed at 120 minutes) (*Figure 8*).

Analysis 1: Did bees show a preference for all amino acids vs. water or sucrose?

After pooling the data from all of the amino acids, I compared the preference for water and sucrose in general. In water, at the lower concentration of 0.01M, the bees consumed more of the amino acid in water solution (mean: 1.11 mm at 120 minutes) in comparison to the higher concentration of 0.1M (mean: 0.67 mm at 120 minutes). At both concentrations, more water was consumed than amino acid in water. At the low concentration, bees consumed an average of 1.28 mm of water in comparison to 1.11 mm of amino acid in water at 120 minutes. At the high concentration, bees consumed an average of 0.92 mm of water in comparison to 0.67mm of amino acid in water at 120 minutes. However, at 120 minutes, a Wilcoxon Signed Ranks Test showed that there was no statistically significant preference for the 0.01M amino acid in water solution vs the water solution (*Figure 9*; P=0.398). In contrast, at the higher concentration, with a P-value=0.039 (same test), the bees showed a statistically significant preference for water solution vs the amino acid in water solution (*Figure 10*).

In sucrose, the bees seemed to consume more of the lower concentration amino acid solution (mean: 6.1 mm at 120 minutes) in comparison to the higher concentration (mean: 3.1 mm at 120 minutes). However, at both concentrations, there was more sucrose consumed than amino acid solution (*Figure 11*). At the lower concentration, bees consumed an average of 8.5 mm of sucrose in comparison to 6.1 mm of amino acid in sucrose at 120 minutes. At the higher concentration, bees consumed an average of 7.4 mm of sucrose in comparison to 3.1 mm of amino acid in sucrose at 120 minutes. At 120 minutes, a Wilcoxon Signed Ranks Test showed that bees favored the sucrose solution over the low concentration amino acid in sucrose solution ($P=0.002$). Similarly, there was statistical significance at the higher concentration as well, with the bees preferring the sucrose to the amino acid solution (*Figure 12;* $P<0.001$).

Analysis 2: Did bees show a different response to essential vs. non-essential amino acids?

In water, the bees consumed more of the non-essential amino acids (mean: 1.3 mm at 120 minutes) in comparison to the essential amino acids (0.95 mm at 120 minutes) at the lower concentration of 0.01M. At the higher concentration of 0.1M, the bees also consumed more of the non-essential amino acids (mean: 0.78 mm at 120 minutes) in comparison to the essential amino acids (0.55 mm at 120 minutes). When comparing the two concentrations, bees seemed to prefer the essential amino acids at the lower concentration (mean: 0.95 mm consumed at 120 minutes) than at the higher concentration (0.55 mm consumed at 120 minutes). Similarly, the non-essential amino acids were favored at the lower concentration (mean: 1.25 mm consumed at 120 minutes) in comparison to the higher concentration (0.78 mm consumed at 120 minutes) (*Figure 13*). Despite the higher consumption of non-essential amino acids versus essential amino acids, there was no statistically significant difference between the two groups at 120 minutes, using the Mann-Whitney Rank Sum Test. At the lower concentration comparing essential vs non-essential amino acids, the P-value was 0.482. At the higher concentration, there was almost statistical significance in favor of the non-essential amino acids, as the P-value=0.054 (*Figure 14*).

In sucrose, the non-essential amino acids were preferred over the essential amino acids at the lower concentration of 0.01M and the higher concentration of 0.1M. At the lower concentration, the bees consumed an average of 8.9 mm of nonessential amino acids in comparison to 3.5 mm of essential amino acids at 120 minutes. At the higher concentration, the bees consumed an average of 3.51mm of nonessential amino acids in comparison to 2.6 mm of essential amino acids at 120 minutes. When comparing the two concentrations, the bees consumed more of the essential amino acids at the lower concentration (mean: 3.5 mm at 120 minutes) than at the higher concentration (mean: 2.6 mm at 120 minutes). This is also true of the non-essential amino acids as the bees showed a strong preference for the non-essential amino acids at the lower concentration (mean: 8.9 mm at 120 minutes) than at the higher concentration (mean: 3.5 mm at 120 minutes) (*Figure 15*). A Mann-Whitney Rank Sum Test showed a statistically significant difference at 120 minutes, at the lower concentration of 0.01M, when comparing the essential vs non-essential amino acids consumed. The bees had a preference for the non-essential amino acids (P<0.001). There was not however statistical significance at the higher concentration of essential vs non-essential amino acids (P=0.128). (*Figure 16*).

Discussion

There are a few main questions that this research answers. First, it seems that overall, concentration is an important factor in amino acid preference in bumble bees. In general, at a lower concentration, bumble bees did not show a preference for amino acids over water, but at a higher concentration, bees preferred water over the amino acid in water solution. This suggests that bumble bees do have the ability to differentiate between amino acid presence in nectars and that additionally, they might seek out nectars with lower amino acid contents, as high concentrations of amino acids are aversive.

Another finding indicates that the bumble bee's response to the amino acid depends on the presence of sucrose. At both high (0.1M) and low (0.01M) concentrations, the bees preferred the sucrose solution to the amino acid in sucrose solution. However, as noted earlier, the same was not true in water solutions, where bees did not show a preference between water and amino acid in water at a lower concentration. This finding may be due to several factors. It may be possible that the amino acids are changing the composition of the sucrose solution to be one that the bumble bees find distasteful. Additionally, it is important to consider the possibility that the bees were not interested in the amino acid solution because they were already satiated in relation to amino acids. After all, bees had only been starved of pollen for approximately 12-24 hours. It may be that the bumble bees consume a smaller amount of the higher concentration amino acid solution and then an internal sensor tells the bee that it no longer needs that particular amino acid.

A surprising finding was that bees did not show a preference for essential amino acids, phenylalanine and threonine, when compared with nonessential amino acids, proline and glycine. This is different from the finding made by Inouye et al. (1984) that showed that honey bees were unable to detect the difference in essential and nonessential amino acids. In this case, bees showed a preference for nonessential amino acids in sucrose over essential amino acids in sucrose. Similar to Inouye et al. (1984), the bees that were given essential and nonessential amino acids in water did not show a preference. This suggests that bumble bees do not possess the ability to determine what is most necessary for their diet, growth, and survival. They may in fact be collecting nectar randomly, or perhaps other elements within the nectars (e.g. sugars and secondary metabolites) may be what determine whether a bee chooses to collect it or not (Inouye et al., 1984).

This research provides valuable knowledge for the long-term survival of bumble bees as well as the conservation of plants that benefit from bumble bee pollination. For example, in a study completed in Britain, it was determined that over the years 1930-1969, 1987-1999, and 1978-1998, there was a major decline in insect-pollinated plants (Carvell et al., 2006). Specifically, there was a greater reduction in plants pollinated by bumble bees, and even worse, those plants that are being removed are important sources of protein for bees (Carvell et al., 2006). Thus, bumble bee populations are declining internationally which in turn will have a cyclic effect on the number of plants in the world. In order to avoid this ecological disaster, it seems that it may be helpful to grow species of plants that provide more nonessential amino acids. Perhaps as a next step, scientists or conservation biologists should study the pollens present in different plants to determine what would be best to plant.

As this was a study focused on only two essential and nonessential amino acids, there are many different future directions. Since my research showed that the lower concentration had a more positive result than the higher concentration, I would pursue a concentration of 0.001M to see if there was a better indication of presence at this level in the future. The concentration values that I chose were based on those found in honey bees and there were various concentrations presented by various studies (Nicolson, 2011; DeGroot, 1953; Inouye et al., 1984). Thus, it could be that the concentrations of amino acid in solution were too high, in that they were in the aversive end of the range. Additionally, I would test more of the essential and nonessential amino acids. Since the amino acids I obtained for testing were based on honey bee research, there is a possibility that other amino acids will show different preference trends. The more amino acids that are tested, the more comprehensive our understanding of bumble bee nutrition will be. A final future direction of interest would be to run this experiment on bees that have been pollen starved for longer than 12 hours. Since pollen consists of proteins, which are made from amino acids, it is possible that the bees were not driven enough to consume certain solutions because they had been provided it 12-24 hours earlier. However, this research provides a good starting point for better understanding bumble bee nutrition and their ability to detect the presence of certain amino acids.

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Figures

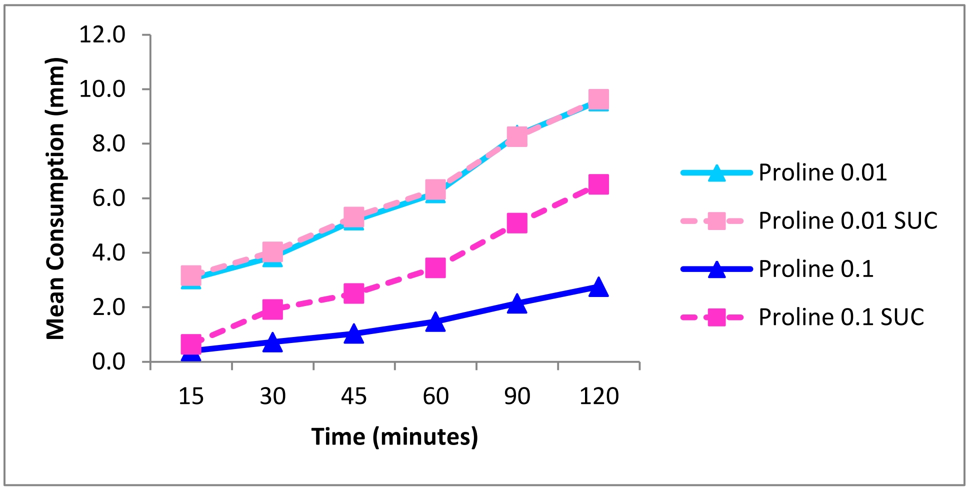

Figure 1. Proline at 0.01 and 0.1M in Sucrose

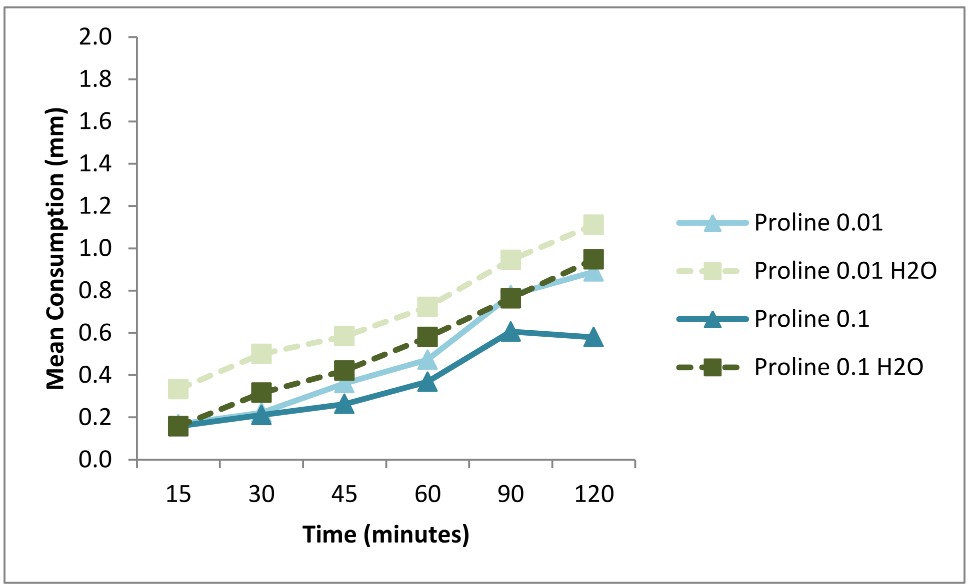

Figure 2. Proline at 0.01 and 0.1M in Water

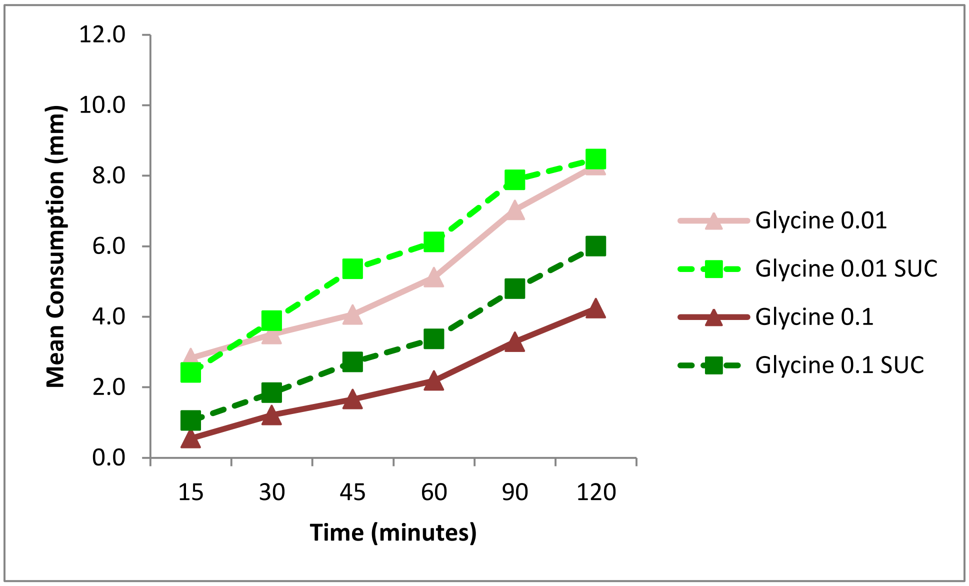

Figure 3. Glycine at 0.01M and 0.1M in Sucrose

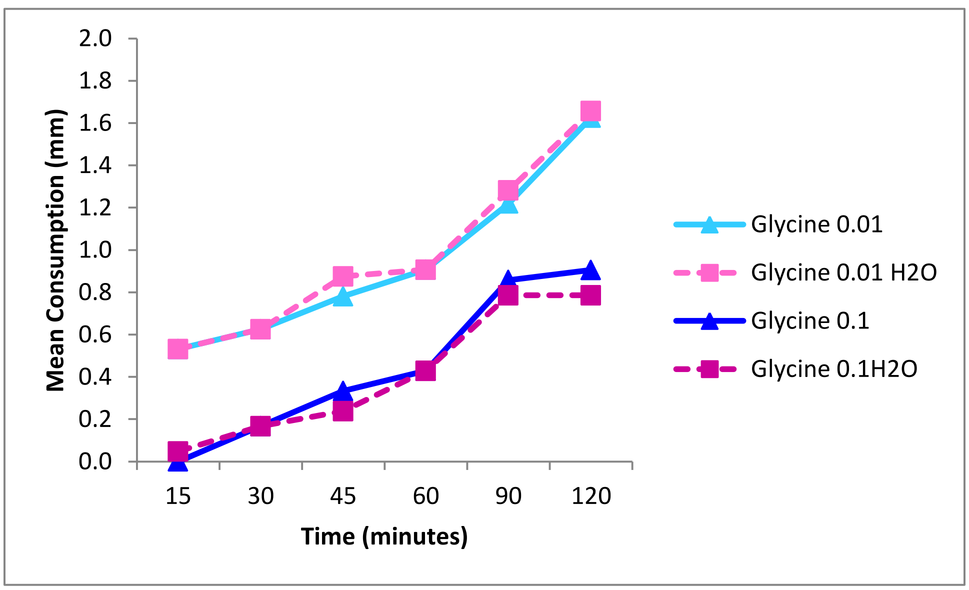

Figure 4. Glycine at 0.01M and 0.1M in Water

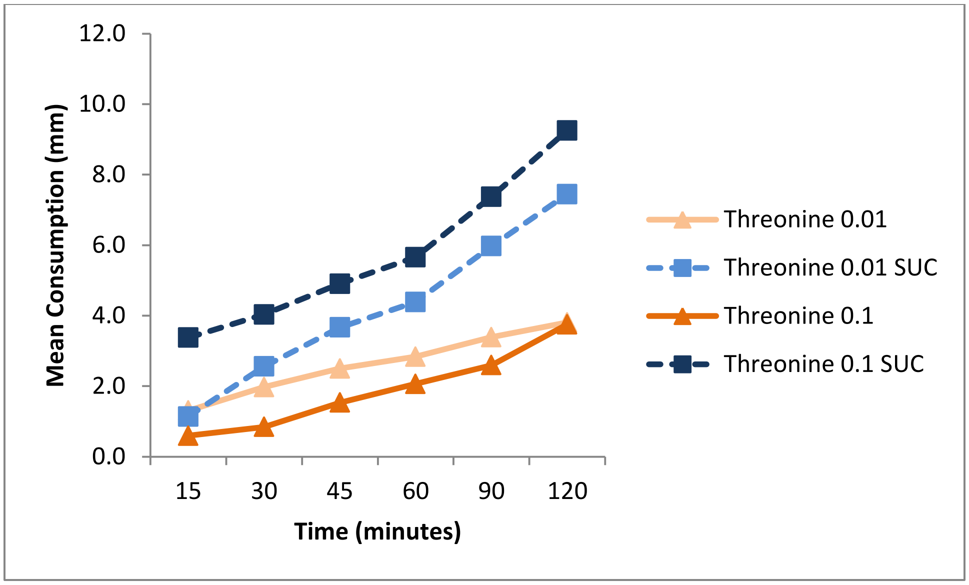

Figure 5. Threonine at 0.01M and 0.1M in Sucrose

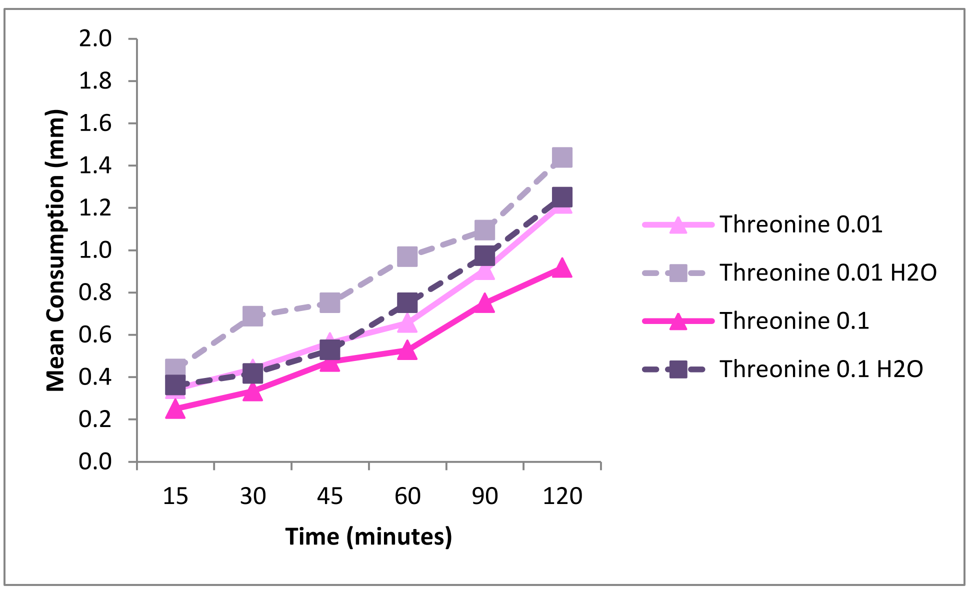

Figure 6. Threonine at 0.01M and 0.1M in Water

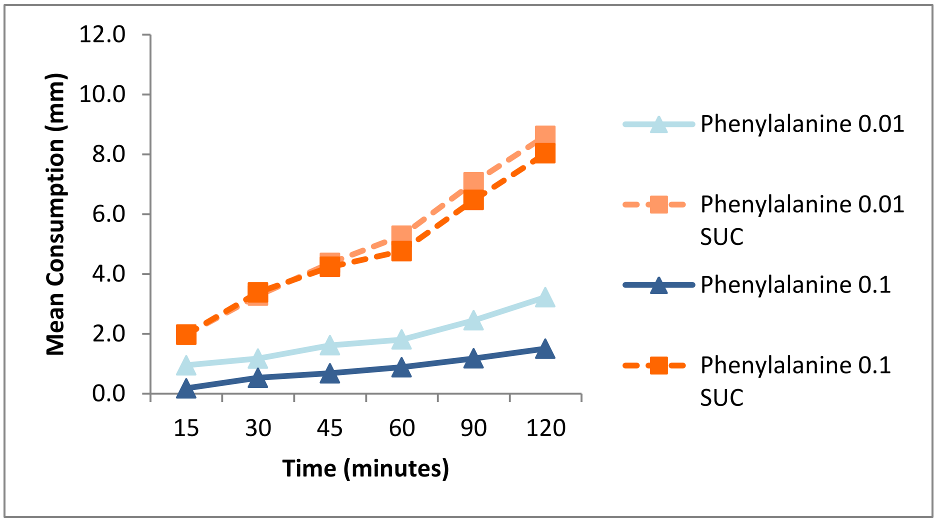

Figure 7. Phenylalanine at 0.01M and 0.1M in Sucrose

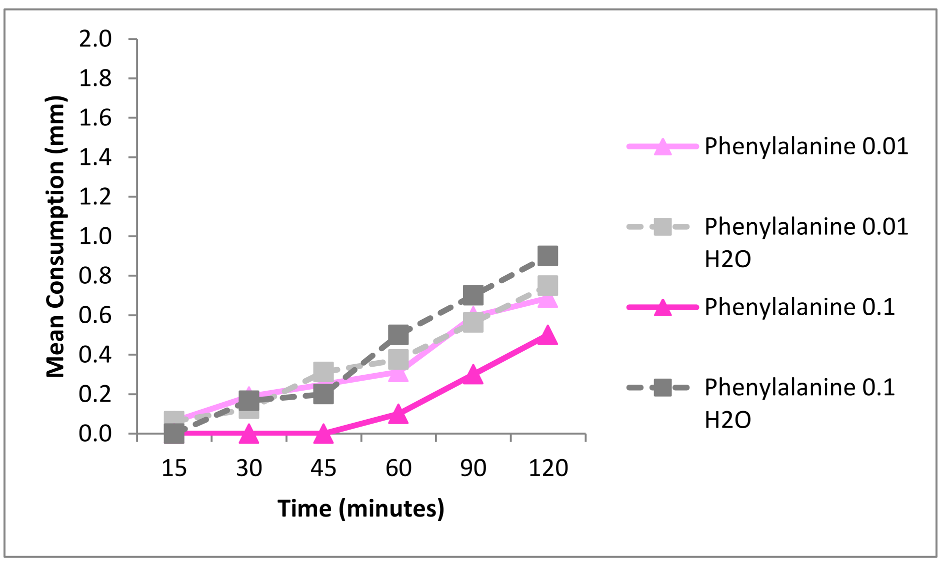

Figure 8. Phenylalanine at 0.01M and 0.1M in Water

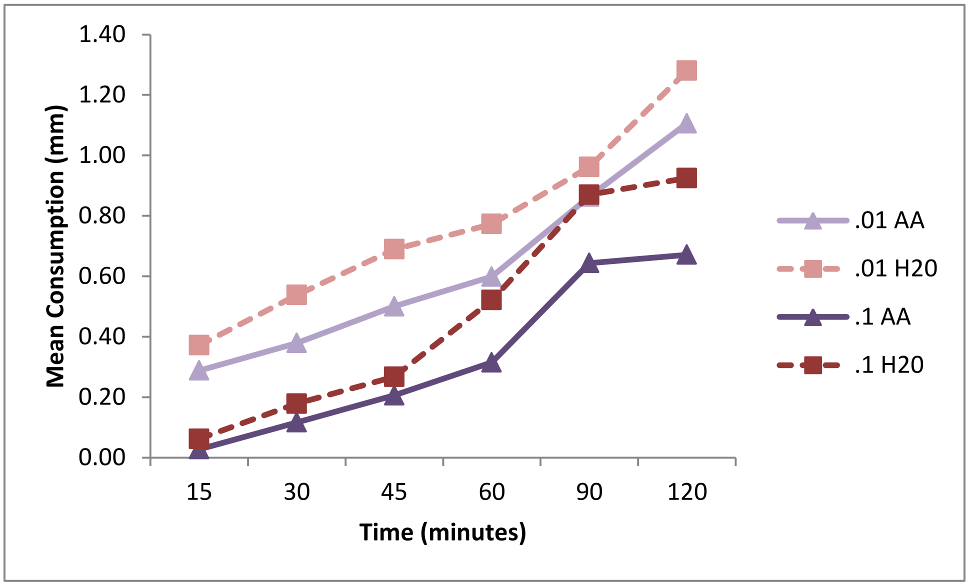

Figure 9. All Amino Acids vs Water at 0.01M and 0.1M Concentrations

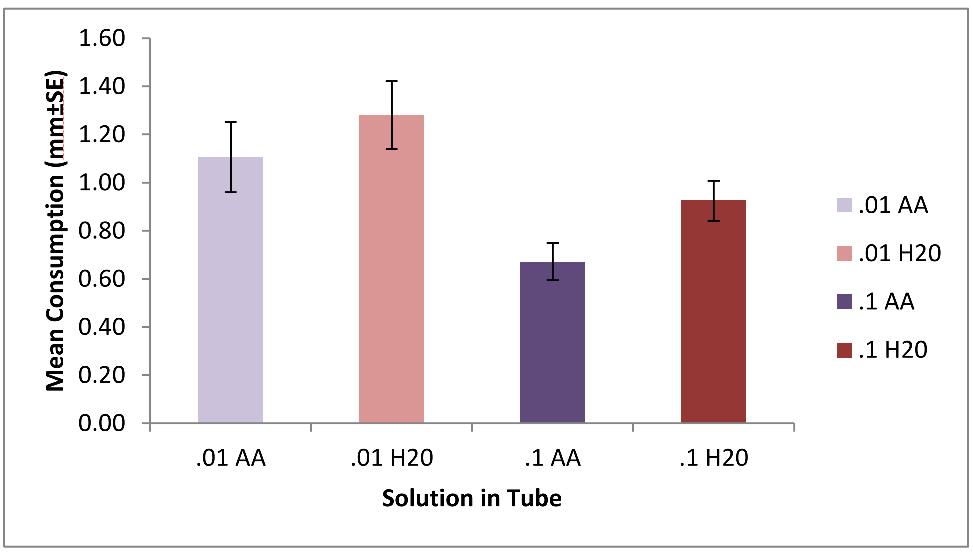

Figure 10. All Amino Acids vs Water at 0.01M and 0.1M Concentrations at 120 Minutes

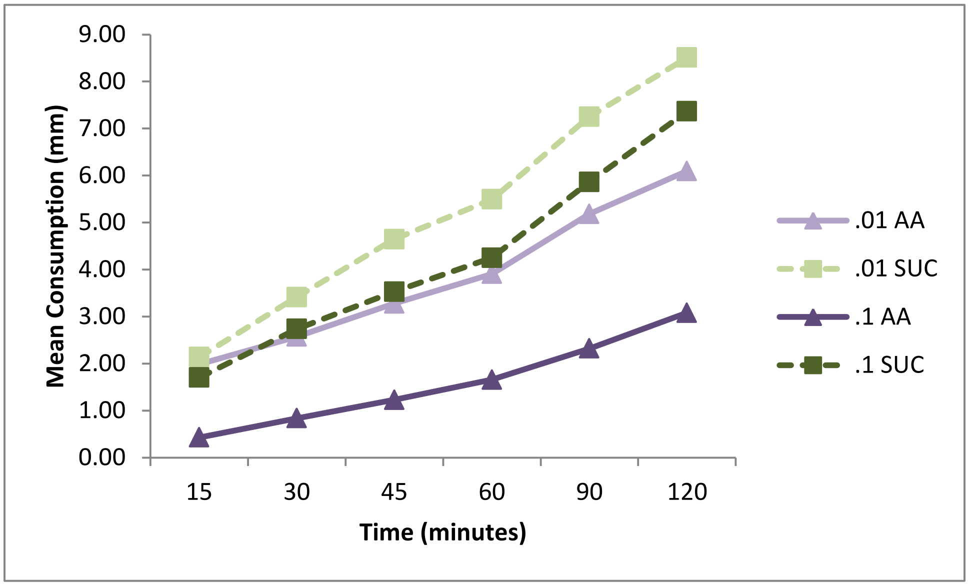

Figure 11. All Amino Acids vs Sucrose at 0.01M and 0.1M Concentrations

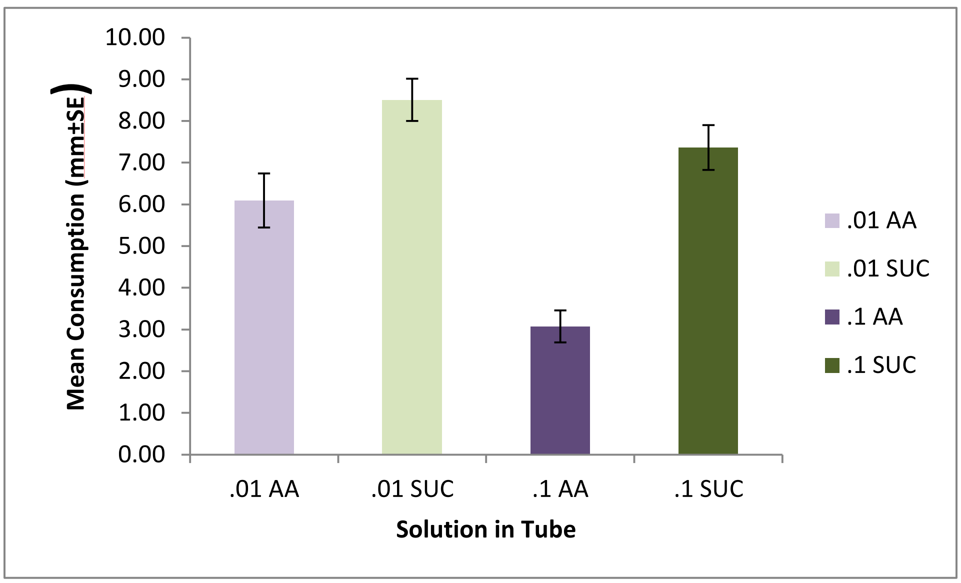

Figure 12. All Amino Acids vs Sucrose at 0.01M and 0.1M Concentrations at 120 Minutes

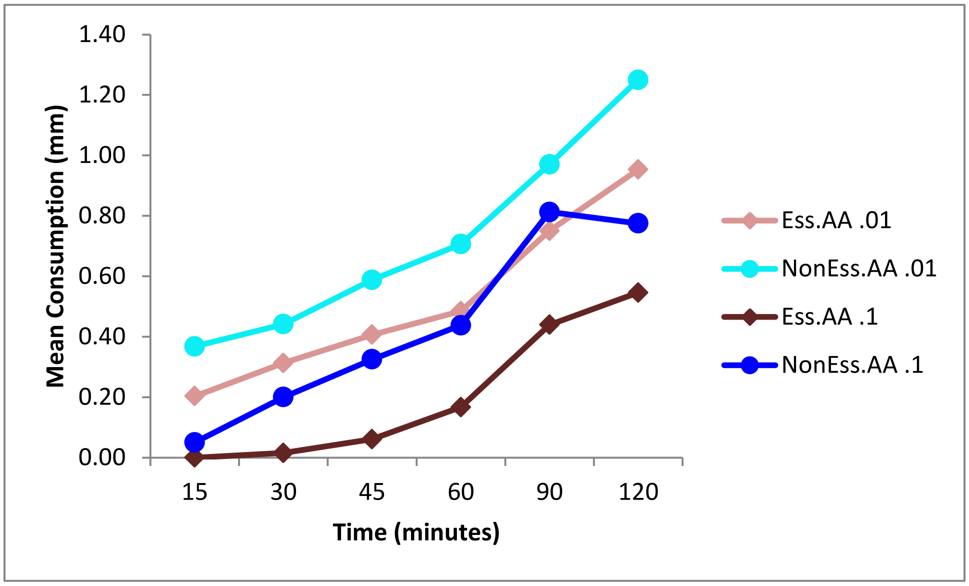

Figure 13. Essential vs Non-Essential Amino Acids in Water at 0.01M and 0.1M Concentrations

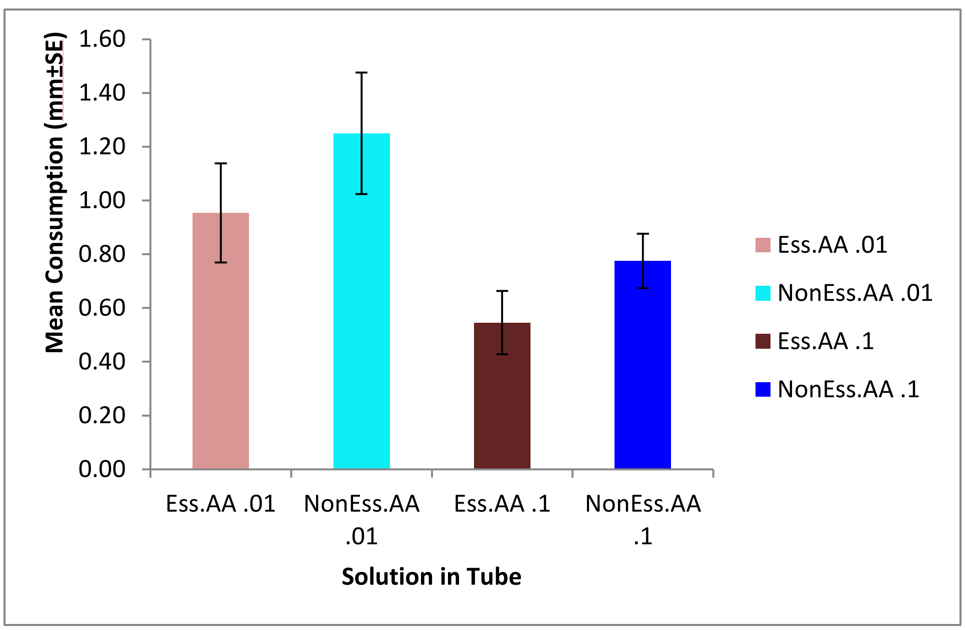

Figure 14. Essential vs Non-Essential Amino Acids in Water at 0.01M and 0.1M Concentrations at 120 Minutes

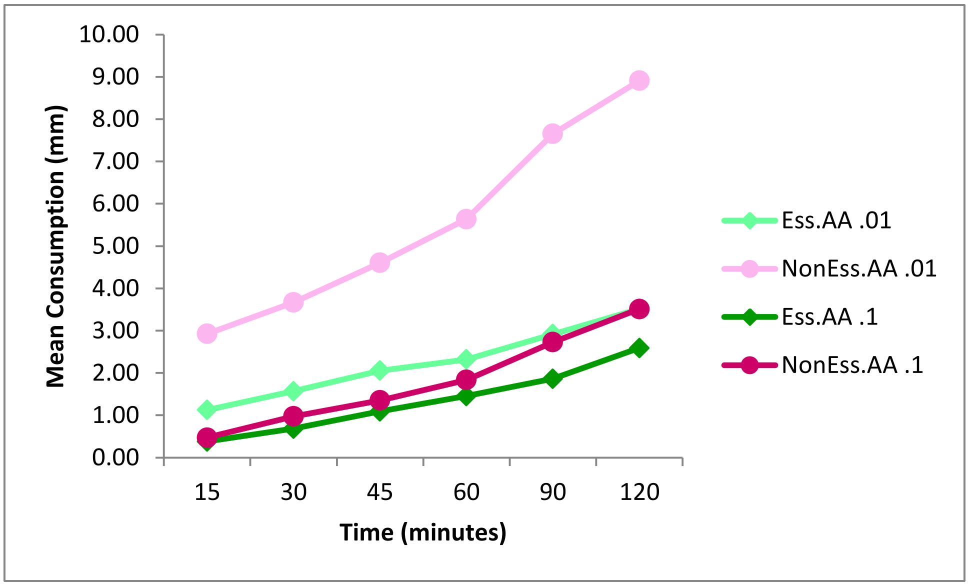

Figure 15. Essential vs Non-Essential Amino Acids in Sucrose at 0.01M and 0.1M Concentrations

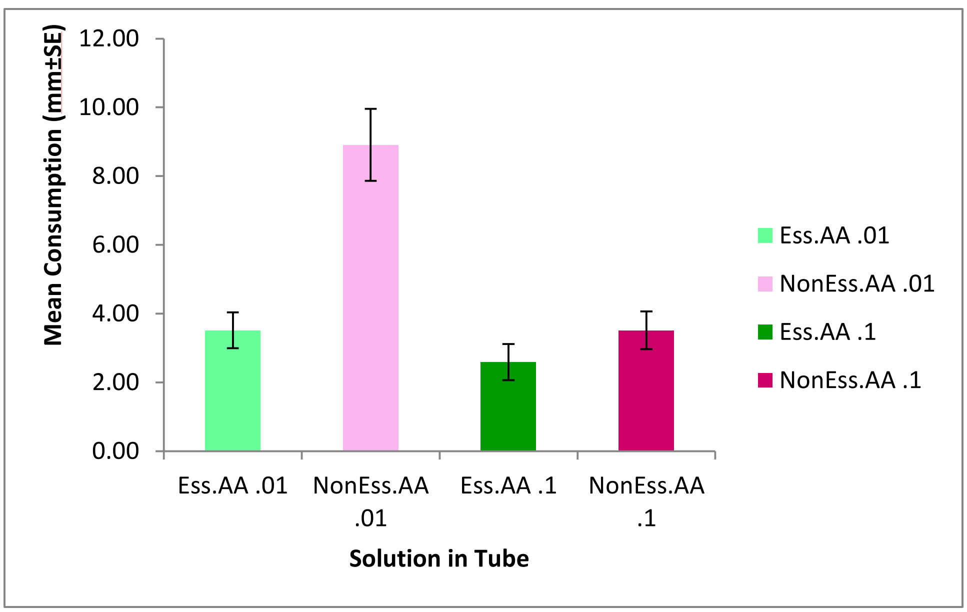

Figure 16. Essential vs Non-Essential Amino Acids in Sucrose at 0.01M and 0.1M Concentrations at 120 Minutes