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Got Milk? An Experimental Analysis of the Absorbance of Casein Protein in Breastmilk and Infant Formula

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ABSTRACT

Objective*:* To test the absorbance of casein protein by comparing two infant feeding methods: formula and breastmilk, in a healthy six-month-old infant. *Hypothesis:* Casein protein will be better absorbed through the feeding of breastmilk in a healthy six-month-old infant. *Methods:* Conducted an in vitro experiment testing casein absorbance using pepsin to simulate an infant's stomach environment and a colorimeter test to get absorbance measurements. *Result:* There is a statistically insignificant amount of evidence that casein protein is better absorbed through the feeding of breastmilk and therefore, breastmilk can be used interchangeably with infant formula in strict regards to casein supplementation.

Introduction

Breastfeeding is a common practice all over the world, but as more women move into professional settings, it becomes difficult to maintain the constant and reliable nature of breastfeeding. With less infant bonding and more professional pressures, a mother might often find herself exploring other options for feeding her baby. Over the past decade, there has been a large and increasing demand for infant formulas. Some estimates predict that between the years 2020 and 2025, the baby food and formula market will grow exponentially, by $22.7 billion.[1] Due to this unprecedented demand, companies have had to explore new ways of vitamin and nutrient supplementation for babies that almost identically replicate the chemical makeup of breast milk. While the biological and chemical makeups of the two feeding methods are similar, infant formulas end up using synthetic substitutes to reach that goal which affects the health of the infant. Most formulas on the market still contain harmful ingredients for an infant's short and long-term development such as palm oil and various types of sugars. These ingredients are linked with higher levels of disease in adulthood such as heart attacks, cardiovascular incidents, and higher cholesterol levels.[2]

Breast milk, however, has properties that have been found to prevent those same diseases, such as obesity and Type 2 diabetes (associated with infant formula). According to Savino Francesco, a pediatric researcher at the Regina Margherita Children Hospital states, "many studies have provided new evidence on the term positive effects of breastfeeding, in particular protection against obesity and type 2 diabetes, suggesting that breast milk may have a role in the programming of later metabolic diseases [heart attacks, strokes, or Type 2 Diabetes]".[3]

Furthermore, the benefits of breast milk are not limited to preventing chronic disease. In some cases, breast-fed infants build up stronger immune systems. Bo Lönnerdal, a researcher of pediatric nutrition at the University of California Davis, explains, "Several proteins with antimicrobial activity contribute to the defense of breastfed infants against pathogenic bacteria and viruses".[4] This research suggests the overarching implications of the different feeding methods as breastmilk helps to grow all areas of the infant. The growth of an immune system is important during infant development since babies are born with very weak immune systems. Breastmilk is able to facilitate that growth, unlike infant formula. In addition, breastfeeding encourages the growth of a bond between a mother and child while also supplying significant nutrients that play a large role throughout the duration of the child's life.[5] The bond established with the mother and child is mutually beneficial as it allows the mother to grow emotionally with her child while the child is nurtured and fed nutrients.

Literature Review

The debate between breastmilk and infant formula is a long-standing discussion in both the medical and social community. While breastmilk contains more nutrients that help to sustain an infant in the long term, infant formula is more convenient for new mothers. The scientific community realized this struggle and made efforts to increase nutrient supplementation in infant formula by genetically engineering modern-day infant formulas to be as similar to breastmilk as possible. According to a paper by Camilia R. Martin, the director of translational research within the Department of Neonatology at Harvard Medical School, titled "Review of Infant Feeding: Key Features of Breast Milk and Infant Formula", formulas are engineered to be as similar to breast milk as possible. Some of the elements are very similar such as proteins, fats, minerals, vitamins, and potentially bioactive agents.[6] However, even as these two feeding methods are biologically similar, that does not necessarily mean that they should be used interchangeably. A case study performed by Dr. Alvaro Flores and Dr. Yudy Persaud, doctors of pediatrics and immunology, examined the effect of casein and whey (the two most potent proteins in infant formula and breastmilk) on a child's allergic anaphylactic reactions. The results show that casein and whey protein concentrations in cow's milk formulas account for a total of 80% of dairy-related allergic reactions in young children.[7] As most allergies develop in childhood, some professionals believe that infant formula poses more risks than convenience benefits—further associating the uses of infant formula to risks within the medical community.

There are also more short-term acute risks associated with infant formula. According to Dartmouth-Hitchcock Hospital and Research Institute, "Formula is harder to digest for a new baby. It stays in the stomach longer than breast milk, which may cause your baby to feed less often".[8] This phenomenon can be explained by the fact that formulas are often harder to digest because of the casein and whey protein that come from synthetic sources or, in this case, cow's milk-based formulas. Due to the higher concentrations of foreign casein (cow's milk-based) in infant formulas, there are often more colic complications (upset stomach and other gastric discomforts) with formula-drinking babies, as noted by Dartmouth Hitchcock Hospital and Research Institute.

Infant formulas use a cow-based casein protein complex that is made up of both casein and whey protein. Casein proteins (used in formulas and breastmilk) are harder for infants to digest than their competitor, whey protein. Casein and whey are proteins that are found in milk and are essential to muscle development. Casein provides a more long-term, sustainable protein while whey provides quick, sugar-based protein. Whey is more common in the earlier stages of development, but as the child's muscles grow, they rely more heavily on casein.

In a paper by David C. Dallas, an employee of the University of California Davis' Food Science Department, on protein needs for premature infants, research suggests that breast milk is the better source of protein supplementation because the nutritional profile of the milk changes over time as the baby develops. The ratio of casein to whey suggests that more protein (whey, since the child is young) is contained in the breast milk of a preterm infant's mother in order to help the child develop and fight adverse complications and fasten development for a premature infant. This concept holds true throughout development. For example, when the child is younger, there are higher levels of whey protein in the mother's milk for easier digestion in weaker stomachs. As the child gets older, the concentrations of casein and whey continue to even out until they are at equal concentrations at around six months. And when the child gets older than six months, there is more casein present in breastmilk because their stomachs are stronger and it is crucial to the muscle development that happens as they age.[9] While Dallas' research focused on the difference in the protein contents in premature versus maturely born infants, my experimental analysis focused on the age range of six-month-olds. Although Dallas' information is presented with respect to birth time, and therefore preterm age, there is no academic indication of a change in protein absorbance patterns as a child develops past the preterm stages in infant formulas. This tailoring of the protein content is seen throughout all stages of infant development but has never been studied in the six-month age cohort, where casein and whey concentrations are around equal. Rather than generic infant formulas that need to be replaced at each stage of development, breastmilk's casein and whey concentrations are able to naturally adapt to the rate at which the child is growing by supplementing different amounts of those proteins.

This ratio is very important to infant development. Dr. Martin also explains the importance of this ratio and the biological mechanism that causes it to change during lactation stages. "The composition of human breast milk is dynamic and changes over time, adapting itself to the changing needs of the growing child….The whey/casein ratio in human milk fluctuates between 70/30 and 80/20 in early lactation and decreases to 50/50 in late lactation".[6] Infant formula does not have this unique property and cannot adjust to the needs of the infant naturally. To compensate, parents have to buy different formulas as the child ages that try to replicate the effects of breast milk on development, but that choice often comes with unforeseen risks. Dr. Minghua Tang, associate professor of pediatrics and nutrition at the University of Colorado explains, "Compared with human milk, infant formula tends to have a higher protein content and is associated with greater weight gain and later-in-life obesity risk".[10] Dr. Tang's research indicates a similar idea from that of Sowmya Kadandale, which indicated that infant formula is associated with higher levels of metabolic disease later in life. Both researchers agree that infant formulas are not able to provide those key protein levels in the infant-specific amount. The debate regarding the two methods is not about the protein concentrations, but more towards the supplementation of a variety of nutrients that are necessary for infant development such as fats, sugars, and vitamins. This inquiry, however, will look solely at protein concentrations, but will focus on the supplementation aspect by studying the absorption of said proteins. Having a larger amount of protein, casein and whey specifically, does not necessarily mean that it will be absorbed better by the child. Therefore, in order to control these concentrations, one needs to have around equal concentrations of whey and casein to isolate the results of a certain protein. By using generic infant formula for 6-month-olds, this experiment will be able to limit possible confounding variables found in fortified formulas or formulas meant for younger infants since they would have uneven casein to whey ratios. The 60/40 casein to whey ratio found in both infant formula for a 6-month-old and breast milk for a six-month-old was studied.

Although some sources reason that casein-based formulas are the most beneficial due to the longer and more sturdy protein supplementation, there are some drawbacks of feeding high levels of casein to younger infants. For example, high levels of casein are not suited for young infants due to their underdeveloped stomachs.[11] This can lead to those previously mentioned colic complications. However, as infants mature, they adapt to the protein as they depend on it more for muscle development. As they learn to develop motor skills, they rely on those muscle groups more and the body naturally regulates the protein digestion capabilities. The ratio between casein and whey proteins changes during development to make sure that the infant is getting enough proteins at each stage.

In order to test this casein to whey ratio and its absorbance measure, a technique known as a spectrophotometric enzyme assay was used. This technique separates the casein protein from milk, unfolds the layers of the biological protein, and tests the absorptivity using different wavelength measures. This will be able to replicate the conditions of the stomach of an infant in this age range by using potent digestive enzyme concentrations that are most similar to that of a six-month-old infant. This method is very sensitive due to the delicate nature of the pH, wavelengths, and temperature values for the experiment. The temperature value, specifically, is important to regulate the breakdown of casein protein. There are different opinions on the temperature ranges of this enzyme assay. According to Dr. Balint Földesi, a biology and genetics researcher, some sources argue that lower temperatures would be better to prevent the protein from unfolding too much since heat tends to lead to higher levels of protein breakdown. Földesi, however, believes that the temperature would be best if it is at equal levels with the internal body temperature.[12] Regulating the temperature at a level that is equal to the internal body temperature is important in order to regulate the stomach environment that is being replicated in this experiment. In order to make the experiment settings as similar to the infant stomach environment as possible, the temperature chosen was the internal body temperature.

Casein and whey have been studied in various contexts in the aforementioned literature leads, yet there is no research on the absorbance of casein protein specifically in six-month-old infants by studying both breast milk and infant formulas at the same time. These studies focus on the extremes of the casein to whey ratio, either a large amount of casein or a large amount of whey. Some inquiries also focus on breast milk or infant formula, but never at the same time in regard to casein protein absorption. By equalizing the casein to whey ratio in a six-month-old's formula, this research can focus primarily on the casein absorptivity in six-month-old infants, instead of the premature infants on which this study had been previously conducted. In addition, the large gap in previous research regarding age cohorts—as only premature infants have been studied—makes it necessary to study casein, the sturdiest developmental protein, within the most crucial stage of muscle development. Therefore, this inquiry aimed to discover the efficacy and absorbance of casein protein on six-month-old infants.

Methodology Review

The purpose of this inquiry was to determine the efficacy and absorbance measures of casein protein in infant formulas as compared to breast milk. Both of these feeding methods (breast milk and infant formula) supplement casein protein differently to a developing infant. This protein is especially important during the six-month muscle development stage as casein provides sturdy muscle sustenance. In order to test these levels of absorbance, a quantitative science experiment was conducted. Even though there is research in this field and mothers who actively use each method (infant formula or breastmilk), it is not feasible to have multiple mothers research, test, and report casein protein concentrations and absorption rates. Having multiple mothers conduct the experiment on their own breastmilk would lead to unnecessary expenditures as each mother would need to buy the specific brand of formula used in this project. It could also lead to errors in information reporting as the procedures could be interpreted differently. Due to this limitation, this research was conducted experimentally to find results consistent across the field and localizing the costs to just me, the experimenter. In addition, the breastmilk used in this project came from a mother who followed a dairy-free diet. This will allow any extraneous dietary casein protein from her diet to be isolated so that the casein studied will come solely from the breastmilk. Casein protein is potent in dairy products as well. By ruling out this confounding variable of dietary casein, the experiment focused on the mother-made casein protein. The casein protein found in dietary dairy products can be transferred to breastmilk since the nutrients in breastmilk are either naturally produced by the mother or come from her dietary intake. Dietary casein could also be picked up by the colorimeter, the scientific device that tested absorbance by measuring the amount of light entering a certain substance. This experiment focused on the absorbance rates of casein protein in breast milk, and its competitor, infant formula. By studying the different types of supplementations, the independent variable, the experiment tested these absorbance rates, the dependent variable. This quantitative experiment pulled values from the colorimeter which indicated the numerical values of absorbance directly.

Procedure

This experiment contained a two-part procedure. The first part involved the extraction of the casein protein from both milk samples, the breast milk and the infant formula in order to test their absorbances. After mixing one serving scoop of the powdered infant formula (a generic brand with a 60/40 whey to casein ratio) with distilled water and allowing the frozen breast milk sample to reach room temperature, the casein extraction process began.

Figure 1. Both samples procured at room temperature before the addition of hydrochloric acid (breastmilk pictured on the left and infant formula pictured on the right).

In order to extract casein protein, five milliliters of hydrochloric acid were added to each sample to curdle the milk and therefore, change the pH to 4.6, the value noted for proper casein absorbance readings, according to Raluca Stefanescu, a Faculty of Chemistry at University Iasi.[13] This process changed the chemical composition of the milks and allowed the protein to separate from the samples in a solid form. The samples were then left to sit for 10 minutes to allow the solids to form. These solids needed to be cleaned in order to rinse off any excess whey protein that separated after the addition of the acid. After washing the solids in an ice bath and draining the excess whey protein off using a cheesecloth, the casein solids were ready for the next steps of the procedure.

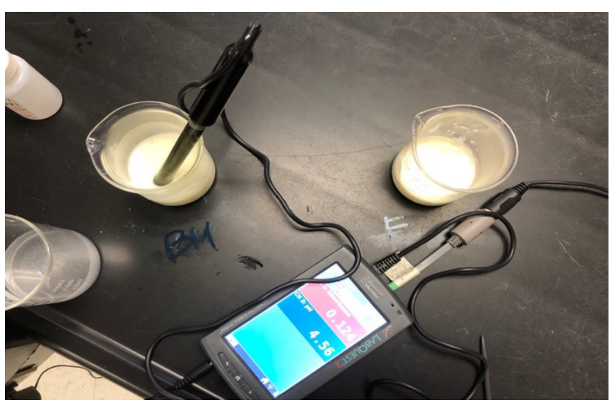

Figure 2. Samples being tested for a pH of 4.6 (as seen on the blue row of the meter's screen) after the addition of 5mL of hydrochloric acid to each sample

The second part of the procedure involved testing the absorbance rates using a spectrophotometric analysis after finishing the casein solid solution procurement. In order to prepare the milk proteins for testing, the enzyme pepsin was added to the solution. This enzyme in particular will be used because of its parallel uses in the human stomach. Pepsin is the main enzyme used in stomach digestion, making it fit to replicate the protein breakdown factors most accurately of an infant's gastrointestinal system. Twenty milligrams of pepsin were used in order to replicate the amount of a typical supplement for digestive purposes.[14] After adding 3.5 mL (the standard cuvette volume) of the protein solid mixture to the cuvette, the sample holding device of the colorimeter, the colorimeter ran the absorbance test. The colorimeter machine tested the amount of light that passed through the sample with the help of an enzyme to replicate the absorbance conditions of the stomach in an infant. The settings on the colorimeter were set at 280 nanometers, the wavelength that was most widely picked up by proteins because of the tryptophan content, an amino acid that builds the casein protein's biological structure. Since tryptophan is the most widely used amino acid in casein formulation, it will be picked up the most during the experiment. The 280-nanometer parameter was also used in Stefanecu's paper to study casein absorbance, so it was used as a control variable to ensure that the testing method was academically valid.

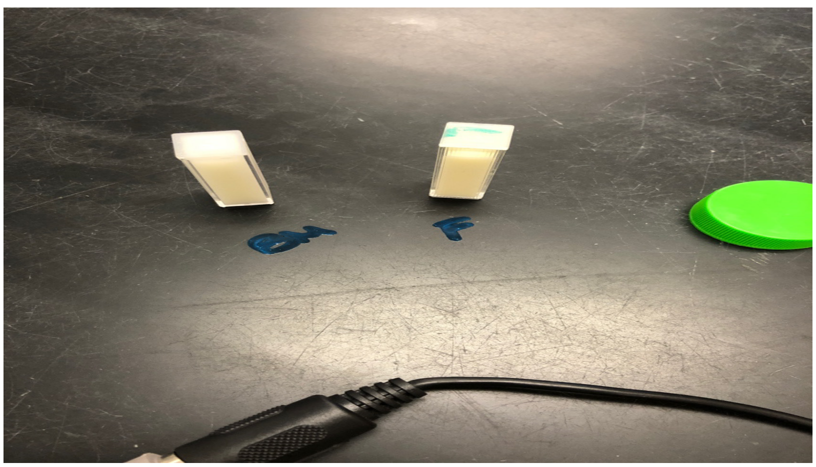

Figure 3. Samples placed into the cuvette devices and about to be run through the colorimeter—breastmilk sample on the left, formula on the right.

Quantitative Data

In order to test the casein protein absorption rates in infant formula versus breast milk, a quantitative experimental analysis was conducted. The quantitative data (absorbance level measured in Absorbance Units) was compiled over five consecutive trials of the experiment to make sure the absorbance factors were constant. Barring any stark differences in absorbance measures, the experiment was to be retried, thus the multiple trials for consistency.

Table 1. Absorbance Rates of Casein in Breastmilk and Infant Formula
This table organizes the quantitative measurements in Au (Absorbance units). All five trials are presented with the averages used for statistical analysis denoted on the bottom row.

Absorbance Rate	Infant Formula	Breast Milk
Trial 1	0.851 Au	0.831 Au
Trial 2	0.849 Au	0.829 Au
Trial 3	0.850 Au	0.830 Au
Trial 4	0.850 Au	0.828 Au
Trial 5	0.851 Au	0.829 Au
Average	0.8502 Au	0.8294 Au

The absorbance values noted in this table show how well the casein protein is absorbed. In Stefanescu's paper, the absorbance value for casein was 0.972 Au, so the closer that the average value is to that number, the better it was absorbed. The average absorbance level for infant formula was 0.8502 Au whereas the average for breastmilk was 0.8294 Au. The average absorbance was closer to the control for the infant formula than breastmilk. However, this finding does not necessarily mean that infant formula has a better absorbance of casein than breastmilk. A statistical analysis was conducted to test the conclusions draw, from these findings.

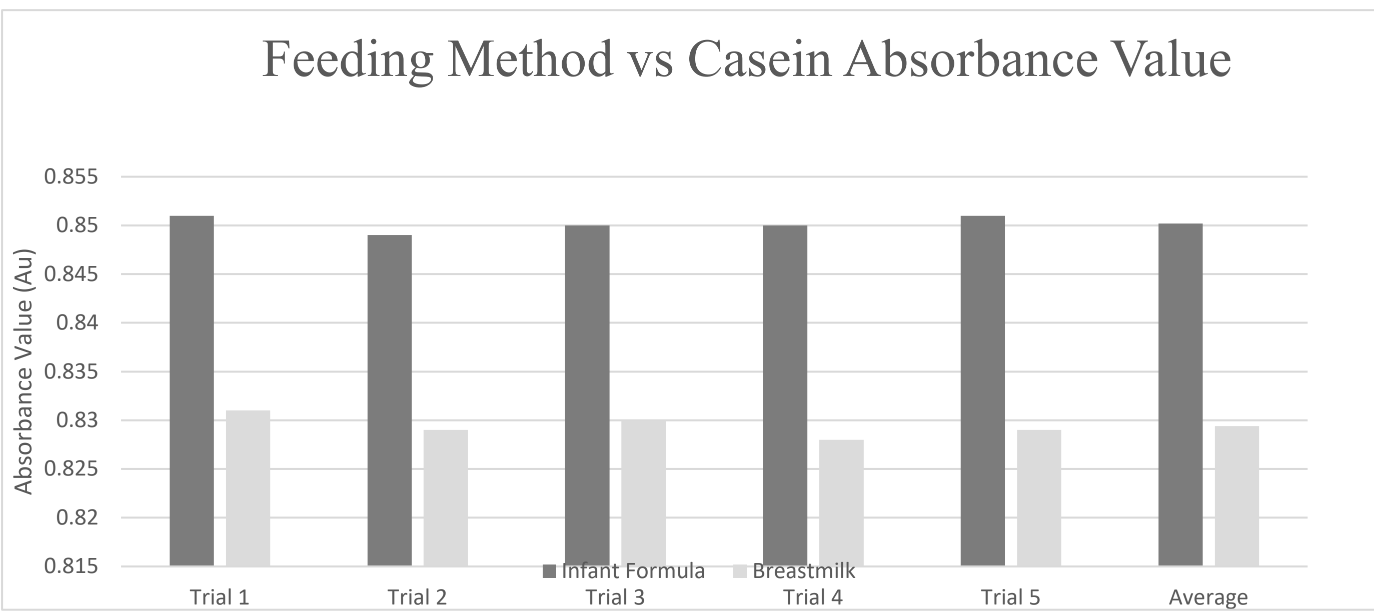

Figure 4. This bar graph plots the data for each specific trial. The dependent variable (Au) is plotted on the y-axis and the independent variable (feeding method) is plotted on the x-axis. The graph includes error bars for an in-depth statistical analysis with a p-value.

The data analysis looked at the absorbance rate numbers in relation to Stefanecu's control value (0.972 Au) to determine which of the two-milk options supplement casein protein better, or in other words, which one has casein protein absorbed better by the stomach. As shown in the graph above, the error bars for the plotted absorbance levels overlap through every trial, including the averages for all five trials. This means that the difference in absorbance levels is statistically insignificant. The overlapping of the error bars indicates that the p-value associated with is greater than 0.05, leading to a statistical insignificance. To further explain the results, a chi-square analysis was conducted based on the information in the data table to situate this experiment's findings within the greater body of knowledge presented in this field.

Chi-Square Statistical Analysis

A Chi-Square analysis was performed to test the significance of the results plotted in the data table. If the computed chi-square is less than the critical value, then the null hypothesis is to be accepted. If the computer chi-square value is greater than the critical value, then the null hypothesis is to be rejected.

Null Hypothesis: There is no statistically significant difference in casein absorption between breastmilk and infant formula in a healthy six-month-old infant. The figures were drawn from the averages of all five trials for breast-milk and infant formula separately and then compared to Stefanescu's control result of 0.972 Au.

Equation 1:

$$[(0.8502-0.972)^2/0.972] + [(0.8294-0.972)^2/0.972] = 0.015263 + 0.020921 = \mathbf{0.036184}$$

Chi Square Value: 0.036184

Critical Value: 3.84

$0.036184<3.84$

ACCEPT the null hypothesis

Results Discussion

The results showed that the difference in absorbance levels between infant formula and breast milk are statistically insignificant in comparison to themselves and observed values from other similar research processes through the completion of a chi-square and error bar statistical analyses based on p-value predictions. The chi-square analysis compared the observed absorbance value to the expected absorbance value. For reference, according to Raluca Stefanescu, the absorbance rates for casein protein were 0.972 at 280nm, using a similar ultraviolet spectroscopy method. When the calculation for this experiment was made (0.036184), it was then compared to the critical value. This critical value was 3.84 since there were two options for a change in the independent variable (degrees of freedoms set by this method of statistical analysis) and confidence intervals were set at 95%. If the calculated value was lower than the critical value, then the hypothesis would be null, the case in this experiment. Therefore, according to this statistical chi-square analysis, this experiment fails to reject the null hypothesis. Whether a mother uses infant formula or breast milk to supplement feeding, casein absorption is similar enough to have these methods be used interchangeably in regard to casein supplementation for 6-month-old infants with no other specific health concerns or conditions.

During the experiment, there were also a few findings that could have led to slightly higher absorbance levels of casein in the infant formula trials. As seen below, there was more casein separation, noted by the grainy solids, in the infant formula before the absorbance levels were tested. While it was originally thought that both the infant formula and the breast milk would have equal levels of casein solid separation, this discrepancy could have definitely led to the differences in absorbance levels measured in the colorimeter. Since this separation of casein after the addition of the pepsin enzyme was higher and more pronounced in the infant formula, it is only natural that casein levels were picked up in larger quantities when absorbance was measured, as there was a larger concentration of the hydrolyzed (broken down) casein protein.

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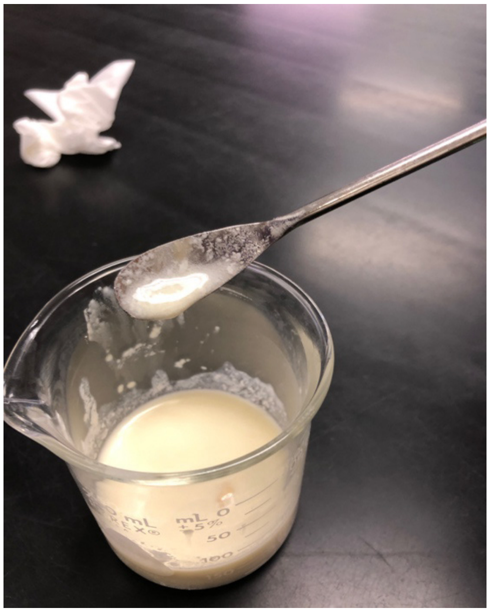

Figure 5. More casein solid separation before absorbance measurements (infant formula) noted by grainy texture

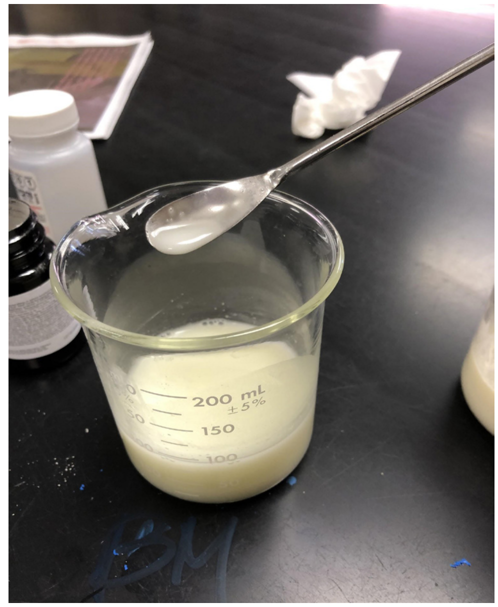

Figure 6. Less casein solid separation before absorbance measurements (breastmilk) noted by less grainy texture

Although there were conclusive results regarding significance, there are limitations to this data. For one, the standard high-school chemistry colorimeter used only had three wavelength settings as opposed to a scientific colorimeter with manually adjustable wavelengths to test from multiple values of the ultraviolet light spectrum. From the three options (470nm, 565nm, and 635nm), the 470-nanometer option was used as it was closest to the expected test parameter of 280 nanometers, noted in Stenfanescu's paper. This led to another potential independent variable that skewed the results, possibly leading to the statistical insignificance as noted with the chi-square analysis. Due to this limitation, it is possible that there were amounts of tryptophan not picked up by the colorimeter. The amino acid tryptophan is the biological building block for casein protein. Due to the stark differences in experimental procedure, it is possible that lower levels of tryptophan were measured in the colorimeter, explaining the lower average absorbance rates in comparison to the control mentioned in the accredited literature. In addition, even though Stefanescu's research focused on full-fat cow's milk, the protein tested was casein in both his research and this experiment, leading to the fact that valid conclusions can be made from his set of data and placed onto this one even though breast milk and infant formula are not chemically identical to cow's milk. The casein protein, though, is chemically and biologically identical in cow's milk, infant formula, and breastmilk, making the testing process universal. In addition, Stefanescu used acetic acid which was able to raise the pH into the 4.6 range for casein solid extraction in the first part of the procedure. While hydrochloric acid was used alternatively in this experimental analysis, it resulted in the same pH of 4.6 which was consistent across all five trials. The primary role of the acid was to bring the pH into that range, so Stefanescu's result was yielded. However, hydrochloric acid is a stronger acid [15] meaning that the casein protein could have denatured (broken down or turned into solids) further throughout the experiment yielding absorbance numbers that lead to the comparative discrepancies with Stefanescu's results. If the protein was more broken down, there would be less available to test in the colorimeter for absorbance values. A smaller concentration of protein could have led to the smaller absorbance values.

However unparalleled these results are from the control statistics; the outcome was not entirely unexpected due to Martin's findings in the literature review. The fact of the matter is that breast milk and infant formula are engineered to be very similar to each other in almost all aspects, leading to the similarities in casein absorbance as well as numerous factors not explored in this current research. In regard to casein absorption for 6-month-olds, these findings will allow mothers who have casein absorbance and muscle development concerns to adopt either method of feeding. However, there are a few exceptions to this conclusion. Some parents will prefer to continue using breast milk as the primary feeding method for a wide variety of reasons. For example, some mothers tend to have their children follow vegan or vegetarian diets from birth meaning that most of the cow protein-based infant formulas are not an option for their children. In addition, some children also have health complications that can potentially be further exacerbated with the feeding of synthetic infant formulas as seen in the allergy case study report done by Drs. Flores and Persaud. To contrast, some mothers might also prefer to follow a strict formula-based diet for their child because of common issues such as body image and professional convenience.

In regard to the gap of findings within the examined prior literature, there are a few findings that remain consistent and others that diverge. In comparison to the numbers reported in Stefanescu's paper, the casein absorbance levels in this research process differed with an average of 0.1218 and 0.1426, for infant formula and breastmilk respectively. While these similarities are not universal in all nutrients in breastmilk and infant formula, this study focused solely on casein protein which shows absorbance similarities in a healthy six-month-old infant.

For future research, the experimenter should have access to a few more items and options to make the results more accurate and replicable. For example, research should have access to a colorimeter with either manual input wavelengths or wavelength measurements closer to the 280-nanometer value needed to test and accurately report the casein protein absorbance rates. In addition, it would be more useful to test breast milk from both dairy-free and dairy-fed donors. By testing both, the researcher would be able to develop a stricter control variable by also analyzing how largely the casein in a dairy-fed diet impacts casein concentration in breast milk. It would also be beneficial to the existing body of knowledge to study non-dairy infant formulas. For example, some parents opt for soy-based infant formulas which have casein contents that have not been studied within the 6-month-old cohort in this research.

Even as the findings of this experiment were calculated as insignificant, there are other elements of this research inquiry that add to a new understanding for parents concerned about casein absorption. It has been discussed that casein concentration in breast milk is able to naturally adapt as the child ages. As noted in Martin's research, the casein content is able to change along with the whey ratio that is best suited for supplementing protein at each given stage in an infant's first year development, the typical period of time a child is breastfed. Due to this evolutionary adaptation, it is still safest, regarding protein needs, for a child to be breastfed. Because even though both feeding methods are able to supplement casein the same, breastmilk can still naturally regulate the protein needs of the infant, unlike infant formula. For example, infant formulas tend to have higher protein contents than breastmilk for any given stage in development. And while higher protein content might seem like the right choice for a developing infant, it is not always the case. As previously mentioned in Tang's paper, higher protein content in infant formulas is associated with higher risks of diabetes and other eventually chronic conditions. Breastmilk, however, can provide casein without the risks of these health conditions in the future.

Conclusion

This research inquiry has presented findings that are significant to the field of infant nutritional supplementation with the help of the body of knowledge presented by the accredited literature in this field. Although these results were calculated as insignificant, these findings are corroborated by other researchers in the field. As seen in previously mentioned research, infant formulas are often a biologically similar substitute to breast milk concerning various nutrients and this inquiry further solidified that notion in regards to casein protein, specifically. In conclusion, the distinction between the absorbance of casein protein in infant formula versus breast milk for an otherwise healthy six-month-old infant is statistically insignificant even though there are other factors that parents take into account when choosing a feeding method for their child. Even as this inquiry showed higher absorbance numbers for infant formula, the difference in supplementation is almost nil, showing that the feeding methods can be used interchangeably. Whatever the choice of the parent may be, breastmilk is still the safer option when looking at the entirety of the scientific debate. This inquiry has led to a more comprehensive approach and design to the study of casein protein absorbance in infant formula as compared to breast milk within the scientific community.

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