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Comparative Analysis of Arginine Content of Three Commercially Available Baby Formula in the Philippines using Multiple Point Standard Addition Method

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Abstract. L-arginine is an essential amino acid required for growth and development of infants and should be present in sufficient amounts in the infant's diet to prevent the occurrence of growth defects. A minimum of 309 mg of arginine per serving of infant formula has been identified. This study quantifies the amount of arginine present in three baby formulas available in the market, labeled Sample 1, Sample 2, and Sample 3, to determine whether they meet the minimum requirement. The three sample solutions were subjected to UV spectrophotometry at 657 nm; using Sakaguchi Reaction to color the mixture and Multiple Point Standard Addition Method to account for the matrix effect. The study will also determine the feasibility of the proposed method in quantifying arginine present in commercially-available food products. Based on the recommended procedure for preparation of each infant formula, results show that there is 16.0258 mg of arginine per serving of Sample 1, 125.7020 mg of arginine per serving of Sample 2, and 171.8751 mg of arginine per serving of Sample 3. This shows that there is insufficient arginine present in each of the three milk samples tested. The study also confirms the viability of Multiple Point Standard Addition as a method for quantifying arginine content in food products.

Keywords: Arginine, baby formula, breast milk, proteins, amino acids

1. Introduction

1.1. Background of the Study

Based on the National Statistical Coordination Board, the Philippines experienced an increase from 60.703 million in 1990 to 88.574 million in 2007 [1]. This increase in population led to the increase in the market of baby food and infant formula milk as they become the main source of nutrition of infants due to the shift from breast-feeding to bottle-feeding in modern households.

Infant formula is believed to contain proteins, fats, linoleic acid, vitamins, minerals, and calcium, among others. Parallel to the shift away from breastfeeding to infant formulas, nutrition scientists continue to analyze human milk and attempt to make infant formulas that closely match its composition.

One of the essential amino acids for the growth and development of the fetus and the neonate is arginine. This becomes nonessential in healthy adults since it is a component in the urea cycle [2]. It has been reported that at a very low serum arginine level, a peculiar rash and cessation of growth was observed in a child with urea cycle defects [3].

Continued growth failure was observed in the subject during the two-week period without arginine supplementation, coupled with an observed drop in the serum arginine level from 0.048 mmole/mL to 0.034 mmole/mL [3]. Upon addition of 400 mg of arginine in the subject's diet, there was resumption of normal growth. This supports that arginine is essential for the growth and development of the neonates and for persons with urea cycle defects.

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The instances of growth disorders among children goes back to question if these children were given the right type of nutrition during infancy. The leading brands of milk are the focus of the study as they are the ones frequently bought in the market. Infant formulas, to provide proper nutrition to infants should contain all proteins, vitamins, and minerals needed for proper growth and development.

Tomlinson, C. found that human infants require a total of 206 mg of arginine per kg of the infant per day [4]. Given that the average weight of infants at 6 months is 7.5 kg [5], the daily requirement of infants is at least 1545 mg of arginine. Taken at an average of a minimum of 5 servings per day, each serving should therefore contain at least 309 mg of arginine to supply the basic arginine requirement for infant growth.

1.2. Objectives

This proposed research study intends to determine if commercially-available baby formulas contain a sufficient amount of arginine for proper growth and development. Specifically, it aims to determine, quantitatively, the amount of arginine present in three selected commercially-available baby formula. The study also aims to develop a feasible method of quantifying arginine in commercially-available food products without compromising its contents.

1.3. Hypothesis

Infant formula milk readily available in the market does not contain the minimum required 309 mg of arginine per serving.

Multiple Point Standard Addition coupled with Sakaguchi Reaction, is a feasible method for quantifying arginine present in commercially-available food products.

1.4. Scope and Limitations

This proposed study is intended to take a quantitative approach in determining the amount of arginine in three commercially-available baby formulas for babies 6 to 12 months specifically, the study shall focus on the nutritional requirement of an average six-month-old infant. A quantitative test will be performed if arginine is confirmed to be present through a preliminary qualitative test. The study shall only quantify arginine, and will not aim to determine the optimum levels of concentration, nor the minimum and maximum therapeutic concentrations of the said amino acid. The study shall only test for arginine; no other amino acid will be included in this study.

1.5. Significance of the Study

Infants have a relatively large dietary requirement for the amino acid arginine. Arginine is known to be essential in the prevention of Necrotizing Eneterocolitis (NEC), hyperammonemia, neonatal pulmonary disease, and neonatal respiratory distress. It also plays a vital role in metabolism by priming the urea cycle and activating carbamoyl phosphate synthase, which leads to arginine biosynthesis [6]. Clinical manifestations of urea cycle defects begin to manifest 2–6 years old so infants require arginine supplementation up to 2 years of age [7]. It is therefore important that formula milks available in the market be examined for their arginine content.

2. Methodology

The selected Infant formulas for babies aged 6-12 months were tested for arginine content. Employing simple random sampling [8], one box per brand of baby formula was acquired.

Qualitative test using Sakaguchi Reaction was performed prior to the quantitative test to confirm the presence of arginine.

Interfering species include ammonia, histidine, tyrosine, tryptophan, creatine, and urea [9]. Multiple point Standard Addition Method allows the determination of the amount of arginine present in the sample, while taking into account the possible effects of the said species in the sample solution during absorbance determination via UV Spectrophotometry [10].

2.1. Milk Sample

Infant formulas were individually reconstituted as indicated on the packaging label. Each sample solution was labelled Sample 1, Sample 2, and Sample 3, respectively, and was filtered prior to the preparation of the solutions of different concentrations.

2.2. Preparation of Solutions [10] and Analysis of Arginine by Sakaguchi Reaction [11]

10.0 mL of each sample solution was placed in five separate 25.0-mL volumetric flasks. Then, a series of increasing volumes of the 20mg/100mL arginine standard solution was added: 0.0 mL, 2.0 mL, 4.0 mL, 6.0 mL, and 8.0 mL. 1.00 mL of 10% sodium hydroxide and 1.00 mL α-naphthol were then added and mixed thoroughly. 0.50 mL of 0.40% sodium hypobromite is added to develop the color. The solutions were then diluted with distilled water to volume.

4.0 mL of untreated sample solution was diluted with water to 10.0 mL and was used as blank for each of the three samples. The mixture was kept in a water bath at 0 ℃ until UV determination.

2.3. Quantitative Determination using UV Spectrophotometer [11]

Arginine concentration is determined by subjecting the sample to UV spectrometry at the wavelength of maximum absorbance (657 nm). All reagents and sample solutions were cooled on an ice bath until determination.

3. Results

23.0 mg of arginine standard was weighed and dissolved and diluted to 100.0 mL with water. This gives a concentration of 0.23 mg/mL and is labelled as the standard solution.

Sample solutions were measured using UV Spectrophotometry at 657 nm. The results gathered are as follows:

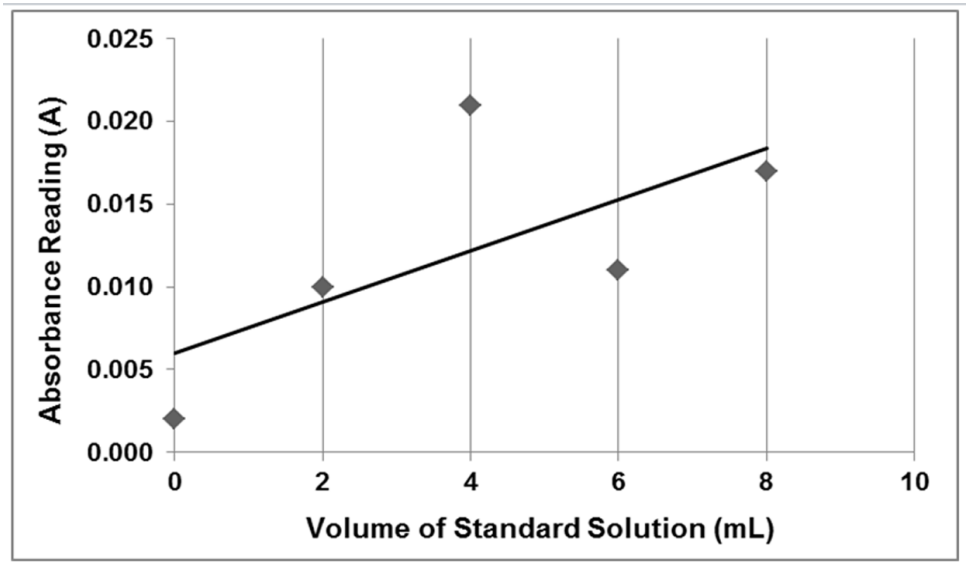

Figure 1. Linear Regression of the absorbance readings (y-value) against the volume of arginine standard solution in mL (x-value) for Sample 1

Using linear regression, the equation of the best-fit line for Sample 1 was determined to be y = 0.0016x + 0.006. A concentration of 0.0890 mg of arginine per mL of sample solution was obtained using the equation $C_x = \frac{bC_s}{mV_x}$, where Cx = concentration of the sample solution in mg/mL

Cs = concentration of the standard solution in mg/mL

b = y-intercept of the best-fit line

m = slope of the best-fit line

Vx = volume of the standard solution added to the mixture in mL

Using linear regression, the equation of the best-fit line for Sample 2 was determined to be y = 0.0108x + 0.3264. Using the equation $C_x = \frac{bC_s}{mV_x}$, a concentration of 0.6983 mg of arginine per mL of sample solution was obtained.

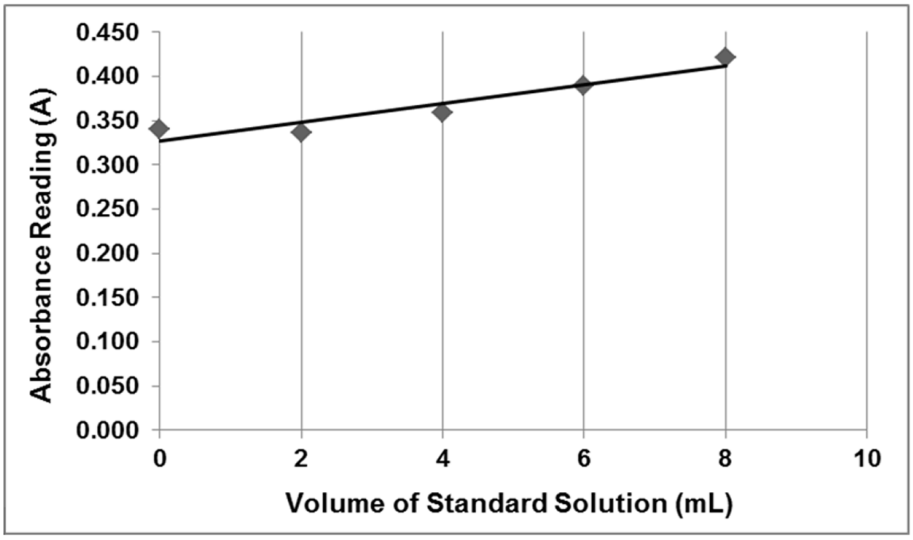

Figure 2. Linear Regression of the absorbance readings (y-value) against the volume of arginine standard solution in mL (x-value) for Sample 2

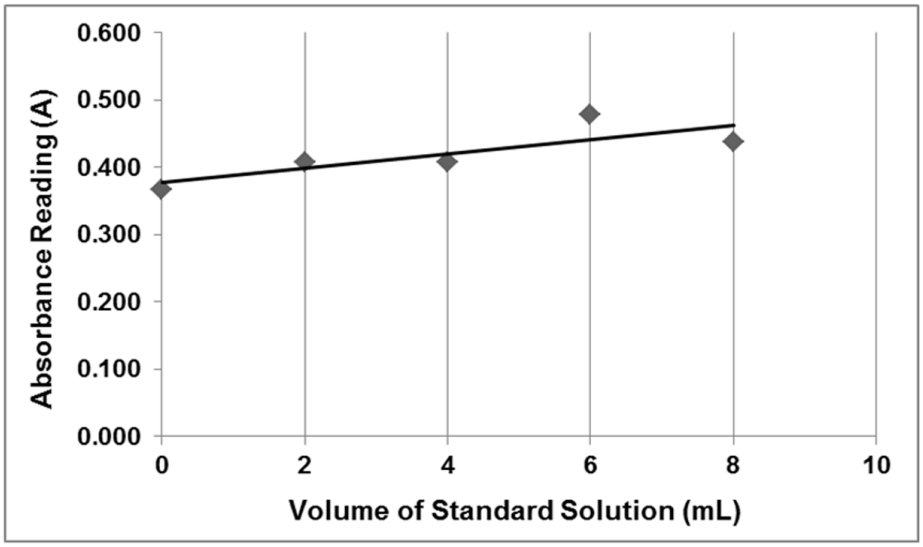

Figure 3. Linear Regression of the absorbance readings (y-value) against the volume of arginine standard solution in mL (x-value) for Sample 3

Using linear regression, the equation of the best-fit line for Sample 3 was determined to be y = 0.0106x + 0.3772. Using the equation $C_x = \frac{bC_s}{mV_x}$, a concentration of 0.8185 mg of arginine per mL of sample solution was obtained.

In summary, the results of the study show that sample 1 contains 0.0890 mg/mL arginine, sample 2 contains 0.6983 mg/mL arginine, and sample 3 contains 0.8185 mg/mL arginine. Each serving of sample 1 contains 16.0258 mg of arginine, while 125.7020 mg of arginine is present per serving of sample 2 and 171.8751 mg for each serving of sample 3.

4. Discussion

From the data gathered, sample 1 contains the least amount of arginine per serving at 16.0258 mg. Sample 2 follows at 125.7020 mg of arginine per serving. Sample 3 contains the highest amount of arginine per serving at 171.8751 mg.

All three samples contain an amount of arginine that is insufficient to meet an infant's daily requirement if the infant is maintained strictly on a baby formula diet. With sample 3, which contains the highest amount of arginine per serving, an infant weighing 7.5 kg would require 9 feedings per day, or one feeding every 2.67 hours (160 minutes) to meet the 1545 mg daily requirement of arginine and ensure proper growth and

development. Sample 2 would require 13 feedings per day, or one feeding every 1.95 hours (117 minutes) and Sample 1 would require 97 feedings per day, or one feeding every 0.25 hours (15 minutes).

5. Conclusion

There is insufficient arginine present in the three baby formula used in this study. The recommended amount of serving of the milks tested, which are locally available, does not provide ample amounts of arginine required for an average 6-month-old infant. It was also determined that Multiple Point Standard Addition Method coupled with Sakaguchi Reaction is a viable method in quantifying arginine content in commercially-available food products using UV Spectrophotometry.

6. Recommendation

It is recommended that further studies be conducted on other commercially available baby formula available to determine its arginine content. It is also recommended that guidelines be set by the Food and Drug Administration (FDA) regarding nutritional content of baby formula to ensure infant nutritional requirement is properly met.

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8. References

[1] National Statistical Coordination Board. Statistics: Population [online]. 2011. Available from: www.nscb.gov.ph [Accessed 18 August 2011].

[2] G. Wu, F.W. Bazer, T.A. Cudd, C.J. Meininger, and T.E. Spencer. Maternal Nutrition and Fetal Development [online]. *American Society for Nutritional Sciences: The Journal of Nutrition* 2004, 132: 2169-2172. Available from http://jn.nutrition.org/content/134/9/2169.full.pdf+html . [Accessed 17 August 2011].

[3] J.J. Kline, G. Hug, W.K. Schubert, and H. Berry. Arginine Deficiency Syndrome: Its Occurrence in Carbamyl Phosphate Synthetase Deficiency [online]. *American Medical Association: American Journal of Diseases of Children* 1981, 135: 437-442. Available from http://archpedi.ama-assn.org. [Accessed 18 Aug 2011].

[4] C. Tomlison. Arginine metabolism in human infants [online]. *Sick Kids*, 2009. Available from: http://www.sickkids.ca/images/Research/Physiology-and-experimental-medicine/20022-Chris%20T%20PEM%20PP%20June%202009.pdf. [Accessed 11 October 2011].

[5] MedIndia. Ideal Weight for Infants [online]. 2011. Available from: http://www.medindia.net/patients/calculators/ideal_weight_infant.asp. [Accessed 11 October 2011].

[6] P. Thureen, and W. Hay. Neonatal Nutrition and Metabolism, 2nd edition. Cambridge University Press, 2006.

[7] I. Görker, and Ü. Tüzün. Autistic-like findings associated with a urea cycle disorder in a 4-year-old girl [online]. Department of Child and Adolescent Psychiatry, Faculty of Medicine, Istanbul University, Istanbul, Turkey: J Psychiatry Neurosci 2005, 30 (2): 133-135. Available from www.ncbi.nlm.nih.gov [Accessed 04 November 2011].

[8] B. Johnson, and L. Christensen. Educational Research: Quantitative, Qualitative, and Mixed Approaches. California: Sage, 2010.

[9] C.J. Weber. A Modification of Sakaguchi's Reaction for the Quantitative Determination of Arginine [online]. *The Journal of Biological Chemistry* 1930, 86: 217-222. Available from: http://www.jbc.org/content/86/1/217.full.pdf+html. [Accessed 17 August 2011].

[10] D.L. Zellmer. Standard Addition [online]. 1998. Available from: http://zimmer.csufresno.edu/~davidz/Chem106/StdAddn/StdAddn.html [Accessed 11 October 2011].

[11] H. Li, X.H. Liang, L.D. Feng, Y.L. Liu, and H. Wang. Simple and Fast Method for Arginine Determination in Grape Juice [online]. *Journal of Food and Drug Analysis* 2007, 16: 3, 53-58. Available from: food.nwsuaf.edu.cn/sfzx/uploadfile/lunwen/2.pdf [Accessed 13 August 2011].