The amino acid composition of human milk - towards determining the amino acid requirements of the human infant

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This thesis is dedicated to God,
for without him it would not exist,
both in concept and in completion.

ABSTRACT

The overall aim was to determine the digestible amino acid composition of human milk. The gross amino acid composition of human milk was corrected for digestibility using true ileal amino acid digestibility coefficients for human milk determined in the piglet. The digestible amino acid composition of human milk was used to evaluate protein quality in a commercial infant formula.

In the first part of the study the piglet was evaluated as a model animal for studying aspects of protein digestion in human infants. Three-week-old male piglets and three-month-old male human infants were bottle-fed an infant formula over a 17 day balance study which included a 10 day total faecal collection period. Small but statistically significant differences between the piglets and infants were found for the apparent faecal digestibility of dietary dry matter, organic matter and total nitrogen. The faecal digestibilities for most of the amino acids, however, were not significantly different between the species. It was concluded that the digestion of protein, to the end of the gastrointestinal tract, appeared to be similar in the two species.

For application of an ileal digestibility assay, it is necessary to assume that amino acids are not absorbed in significant amounts posterior to the ileo-caecal junction. An experiment was conducted, therefore, to determine whether lysine and/or methionine, two dietary essential amino acids, were absorbed in nutritionally significant amounts from the large intestine of the three-week-old piglet. Piglets, surgically prepared with simple catheters which allowed infusion into the proximal colon, were given one of two milk-formula diets which were deficient in either lysine or in the sulphur amino acids, yet were balanced for all other amino acids. An isotonic solution containing the respective deficient amino acid or physiological saline was infused via the catheter at each feeding. Total daily excretions of urinary urea and total nitrogen were determined. There were no significant differences in urinary nitrogen metabolite excretion for piglets infused with amino acids compared with those infused with saline. Lysine and methionine did not appear to be absorbed in nutritionally significant amounts from the proximal colon of the milk-fed piglet.

Two experiments were conducted to develop a method for accurately determining the amino acid composition of human milk. In the first, a non-linear model, that describes the simultaneous processes of amino acid yield and decay that occur during acid hydrolysis of a protein prior to amino acid detection, was used to regress data derived from multiple hydrolysis intervals. Most of the amino acids

underwent some degree of loss during hydrolysis. Of particular note was the loss rate for cysteic acid, which was greater than that found for serine. Using the routine duplicate sampling system, a non-linear regression including 10 hydrolysis intervals resulted in a mean amino acid recovery of 100% and provided an acceptable compromise between accuracy and the cost of analysis. In the second experiment, the non-linear model was modified to account for samples, such as human milk, having amino acids in free form prior to hydrolysis. The original and new models were compared. A biological sample (human milk) was hydrolysed in acid for multiple hydrolysis intervals. As in the previous experiment, most of the amino acids (and in particular, cysteic acid) underwent some degree of loss during hydrolysis. It was concluded that using the original model to analyse data obtained from hydrolysis of a sample containing protein and free amino acids will not lead to the introduction of any large bias in the determination of amino acid composition. The modified model, however, is more accurate for application where a sample contains both protein-bound and free amino acids.

In the penultimate experiment of the study, human milk was collected from women in their 10th-14th weeks of lactation, and was analysed for amino acids with correction for losses of amino acids during acid hydrolysis, using the model parameters determined in the earlier experiment. The mean amino acid composition of the human milk was similar to previously reported estimates, though the cysteine content of the human milk was 20% higher than the mean literature estimate. True (corrected for endogenous amino acid excretion) ileal amino acid digestibility coefficients for human milk, determined in three-week-old piglets fed human milk, ranged from 81-101% with threonine (86%) being the least digestible essential amino acid. The overall digestibility of amino acid nitrogen was 95%. When the true ileal digestibility values were used to correct the amino acid composition of human milk, the pattern of amino acids absorbed from human milk was different compared to the currently recommended dietary pattern of amino acids for the infant.

In the final study, true ileal amino acid digestibility coefficients for a commercial infant formula were determined using the three-week-old piglet. Coefficients ranged from 95% for lysine to 103% for arginine, indicating near-complete digestion of the protein in the infant formula. The profile of absorbed amino acids for the infant formula was compared with that for human milk to evaluate protein quality in the infant formula. It was concluded that the protein in the formula was of high quality.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES