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International Journal of Food Sciences and Nutrition,
Month 2007; 00(0): 1–12

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Amino acid composition determined using multiple hydrolysis times for three goat milk formulations

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Abstract

The amino acid composition of goat milk formulations with varying protein and carbohydrate concentrations were determined. Proteins in goat milk infant formula, goat milk growing-up formula and goat whole milk powder were hydrolysed using multiple hydrolysis time intervals. A least-squares non-linear regression model was used to predict the free and protein bound amino acid concentrations. The amino acid composition of goat infant formula was compared with human milk reference values. There was good agreement between the multiple hydrolysis and single 24-h hydrolysis methods for approximately one-half of the amino acids. Tryptophan, aspartic acid, threonine, tyrosine, isoleucine, valine, serine and alanine contents were underestimated by 10.6, 5.6, 5.6, 4.7, 4.4, 3.7, 3.7 and 3.6%, respectively, by the single 24-h hydrolysis. The study provides accurate reference data on the amino acid composition of goat milk powders. Goat milk infant formula has amino acids in amounts similar to human milk reference values, when expressed on a per-energy basis.

Keywords: *Amino acids, goat, milk, infant formula*

Introduction

Amino acids are vital nutrients for growth and the maintenance of health in humans. This is particularly true for infants, who rely on milk to meet their nutrient needs. Knowing the amino acid composition of infant formula is therefore critical to ensuring the amino acid requirements of the infant are met. Very few data are available on the amino acid composition of goat milk (D'Urso 2000; Davis et al. 1994) or goat milk infant formula (Rutherfurd et al. 2006). Goat milk is widely consumed in many European countries, the Middle East and Bangladesh (Haenlein 2004). Goat whole milk powders are manufactured in several European countries, the USA, Australia and New Zealand, and goat milk infant formula is in use in several countries, including Australia, New Zealand, Taiwan, Korea, Russia and China.

When determining the amino acid content of foods, the amino acids are first released from the proteins by acid hydrolysis of the peptide bonds. This is usually

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ISSN 0963-7486 print/ISSN 1465-3478 online
DOI: 10.1080/09637480701705424

conducted for 24 h at 110°C in 6 M HCl. However, not all amino acids are stable under hydrolytic conditions and peptide bonds involving hydrophobic residues are difficult to break even under the extreme hydrolysis conditions (Rutherfurd and Moughan 2000). The routine 24-h analysis is a compromise to minimize the degradation of acid-labile amino acids while maximizing the hydrolysis of resistant peptide bonds, but it may not be accurate for a number of amino acids.

Multiple hydrolysis times have been used to improve the accuracy of amino acid analysis. Rowan et al. (1992) used the maximum amino acid concentration obtained from multiple hydrolysis times to calculate the amino acid composition of diets. This approach is flawed, however, in that the acid-labile amino acids may begin to be degraded before all the amino acids are liberated, underestimating these amino acids. Hirs et al. (1954) conducted multiple time hydrolysis and extrapolated the amino acid concentrations back to time 0 to obtain more accurate data for the acid-labile amino acids. While an improvement over the approach of Rowan et al. (1992), this method still leads to underestimation for some amino acids. Robel and Crane (1972) used a much wider range of hydrolysis times, including very short hydrolysis times. This, along with the degradation rates obtained from the longer hydrolysis times and using least-squares non-linear regression, allowed them to predict the original amino acid content in the protein prior to hydrolysis. This method has since been used to determine the amino acid composition of a purified protein (Darragh et al. 1996), human milk (Darragh and Moughan 1998), cat hair (Hendriks et al. 1998) and cow skim milk powder, whey and caseinate (Darragh and Moughan, 2005), indicating its suitability for different proteins and protein mixtures. However, other components, such as carbohydrates, may impact on the loss rates of amino acids (Darragh and Moughan, 2005). This has important implications for the amino acid analysis of fortified milk formulations for different age groups, since carbohydrate and protein vary considerably.

The objective of the present study was to use multiple hydrolysis time curves combined with least-squares non-linear extrapolation to accurately determine the amino acid composition in three different goat milk formulations containing varying proportions of protein and carbohydrate. The formulations were infant formula, for ages up to 6 months, growing up milk, which is a mineral and vitamin-fortified milk powder for ages from 12 months and above, and goat whole milk powder, a spray-dried powder made from fresh goat milk.

Materials and methods

Goat milk infant formula, goat milk growing-up formula and goat whole milk powder were manufactured by Dairy Goat Co-operative (N.Z.) Ltd (Hamilton, New Zealand). Three separate batches manufactured at different times of the season were pooled for each formula or whole milk powder to reduce variation in seasonality on amino acid content. The infant and growing-up formulae consisted of a mixture of whole goat milk and vegetable oils with additional lactose, vitamins and minerals, which was then spray-dried. The whole milk powder was spray-dried from whole goat milk alone. The crude protein, carbohydrate and total fat contents of the samples are presented in Table I.

Table I. Crude protein, nitrogen-free extractive (carbohydrate) and total fat of the goat whole milk powder and two goat milk formulas used in this study.

	Protein (%) (nitrogen (%))	Carbohydrate (%)	Fat (%)
Infant formula	10.5 (1.65)	56.8	27
Growing-up formula	18.3 (2.87)	48.4	26
Whole milk powder	25.0 (3.92)	35.5	28

Each sample is made up of a composite of three separate batches manufactured at different times of the season.

Chemical analysis

The total nitrogen content of the three goat milk powders was determined on a LECO analyser using the Dumas method (ISO 2002), and crude protein was calculated as the total nitrogen content multiplied by 6.38. Dry matter, ash and total fat were determined according to the methods described by AOAC (1995). Nitrogen-free extractive (an estimate of the carbohydrate) was determined as the difference between the total sample weight and the sum of the moisture, ash, crude protein, crude fibre and ether extract.

The powders were suspended in water such that the concentration was approximately 2 g powder/100 ml. Amounts of 0.46 ml infant formula, 0.27 ml growing-up formula and 0.19 ml whole milk powder (such that there was an equal amount of protein from each sample) were each aliquoted in duplicate into two series of hydrolysis tubes. One series underwent analysis for the 'acid-stable' amino acids and the other underwent analysis for the sulphur amino acids. It should be noted that no attempt was made to remove any fat from the samples. All tubes were then dried down using a Savant Speedvac Centrifugal Concentrator (Savant Instruments Inc., Farmingdale, NY, USA).

Analysis of the 'acid-stable' amino acids

The tubes used to determine the 'acid-stable' amino acids had 1 ml of 6 M glass-distilled HCl containing 0.1% phenol added before being degassed under vacuum (using a vacuum pump) and sealed by melting the narrowed neck of the sample tube. These tubes were then hydrolysed at 110°C in duplicate for 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 19, 21, 24, 30, 40, 52, 72, 95, 126 and 168 h. After hydrolysis, the tubes were cracked open and norleucine added as an internal standard before being dried down again. Once dry, the amino acids were dissolved by the addition of loading buffer (67 mM sodium citrate, pH 2.2, containing 0.1% (w/v) phenol) before being analysed using a Waters ion-exchange high-performance liquid chromatography system, utilizing post-column ninhydrin derivatization and detection using absorbance at 570 nm (440 nm for proline). Where appropriate, the weight of each amino acid was calculated using free amino acid molecular weights.

Sulphur amino acid analysis

The tubes used to determine the sulphur amino acids were treated with performic acid prior to hydrolysis to quantitatively oxidize the cysteine and methionine to the more stable compounds, cysteic acid and methionine sulphone. These tubes were prepared

in duplicate. They were firstly cooled in ice, before 1 ml ice-cold performic acid (9:1 ratio of 88% formic acid:30% hydrogen peroxide) was added. The tubes were then incubated on ice in a refrigerator for 16 h. After incubation, 0.15 ml hydrogen bromide was added to the tubes, which were then dried down. Once dry, the tubes underwent acid hydrolysis as described above.

Tryptophan analysis

Tryptophan was hydrolysed as described by AOAC (1990) using lithium hydroxide, except that hydrolysis was for 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 19, 21, 24, 30, 39, 50, 72, 95, 117 and 140 h. The free tryptophan molecular weight was used to calculate the tryptophan weight.

Prediction of amino acid concentration

The amino acid concentrations for each amino acid for each goat milk powder were plotted against hydrolysis time. The equation used to fit curves to each plot is:

$$B(t) = \frac{A_o h(e^{-lt} - e^{-ht})}{h - l} + B_o(e^{-lt})$$

where $B(t)$ was the amino acid concentration at time t, B_o is the free amino acid concentration determined prior to hydrolysis, h is the hydrolysis rate (proportion of bound amino acid hydrolysed per hour), l is the loss rate (proportion of bound amino acid destroyed per hour) and A_o is the actual amino acid content of the protein within the samples. A_o, h and l for each powder was derived for each amino acid using least-squares non-linear regression with the constraints that $B_o \geq 0$, $A_o > 0$ and $h > 0$ (Darragh and Moughan 1998).

Results

The overall mean difference between duplicates for all analyses, over all hydrolysis times, was 2.8% for the amino acids determined using acid hydrolysis only, 3.4% for cysteic acid and methionine sulphone, and 6.0% for tryptophan. The hydrolysis curves showing amino acid content plotted against hydrolysis time for the infant formula are shown in Figure 1. The hydrolysis curves for the growing-up formula and the whole milk powder were similar (data not shown). The mean R^2 value for all amino acids across all samples was 0.98. Proline content was the most variable amino acid content around the predicted line ($R^2 = 0.88$) when determined in the whole milk powder. Histidine was also variable ($R^2 = 0.91$) when determined in the infant formula. For all the other amino acids, across all three goat milk powders, least-squares non-linear regression provided a close fit for the hydrolysis time curves ($R^2 =$ 0.94–1.00).

The hydrolysis rate (h) and loss rate (l) were estimated for each amino acid for each goat milk powder, and these data are presented in Table II. With the exception of histidine, the hydrolysis rates ranged from 0.11 for tryptophan in the infant formula to 0.74 for glycine in the whole milk powder. The hydrolysis rates for histidine (5.2–17.8) and cysteine (cysteic acid) (1.2–2.8) for all three goat milk powders were unrealistic. Furthermore, and in contrast to all the other amino acids, there was no

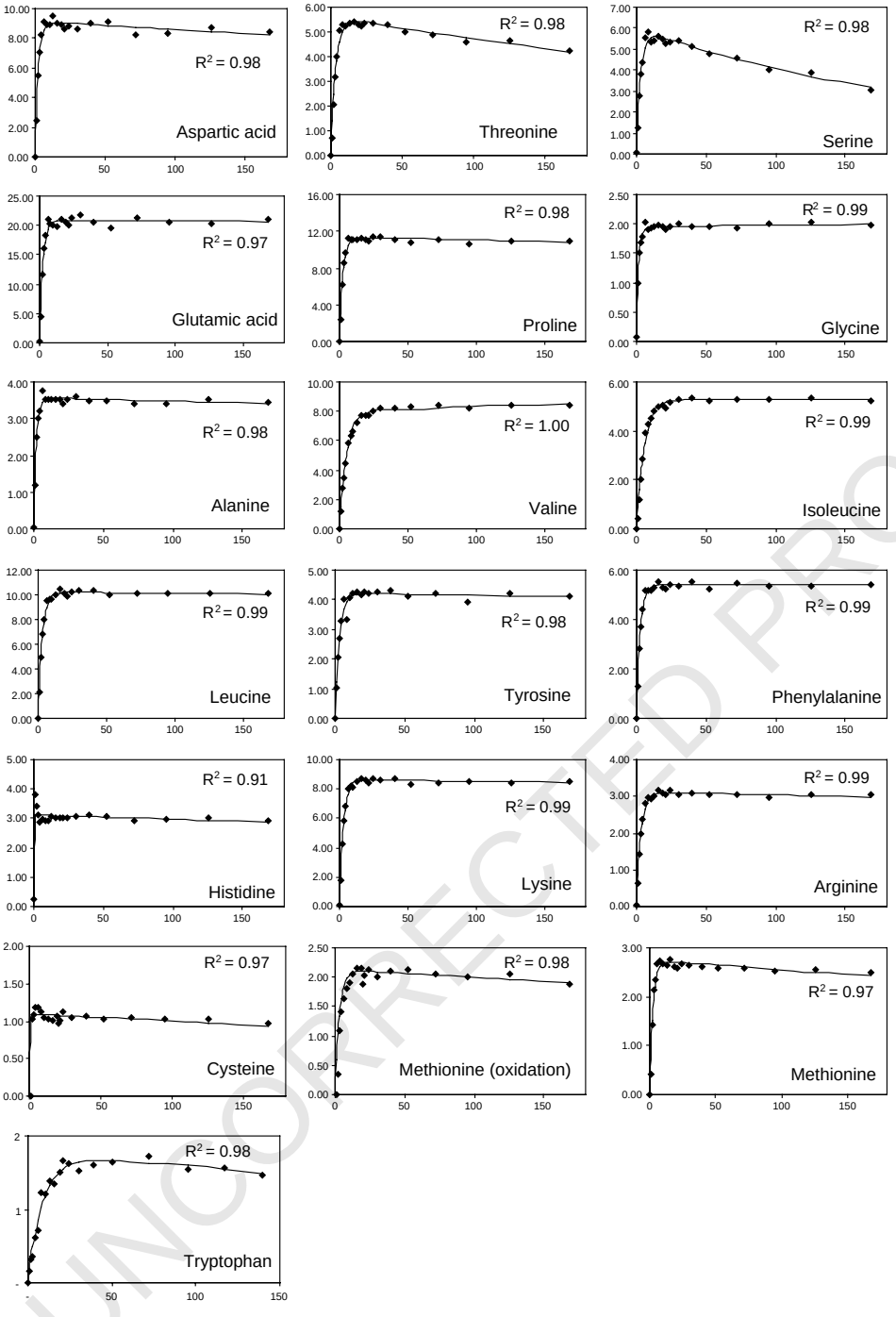

Figure 1. Effect of hydrolysis time (*x* axis, h) on the yield of amino acids (*y* axis, mg/g formula) in goat milk infant formula. The mean amino acid yield (duplicate) (◆) is plotted along with the line of best fit predicted from A_o, h and l for that data-set.

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Table II. Estimated hydrolysis rate (h) (proportion of bound amino acid hydrolysed per hour) and loss rate (l) (proportion of bound amino acid destroyed per hour) for each amino acid in three goat milk powders during hydrolysis.

	Hydrolysis rate h[a]			Loss rate l[a]		
	Infant formula	Growing-up formula	Whole milk powder	Infant formula	Growing-up formula	Whole milk powder
Aspartic acid	0.464	0.459	0.497	0.0006	0.0009	0.0009
Threonine	0.286	0.283	0.291	0.0018	0.0018	0.0016
Serine	0.347	0.336	0.350	0.0038	0.0036	0.0032
Glutamic acid	0.429	0.431	0.478	0.0001	0.0000	–0.0001
Proline	0.418	0.431	0.390	0.0003	–0.0003	0.0016
Glycine	0.694	0.623	0.740	–0.0002	–0.0005	0.0001
Alanine	0.554	0.540	0.607	0.0003	0.0000	0.0006
Cysteine[b]	2.750	1.373	1.220	0.0010	0.0006	0.0010
Valine	0.196	0.194	0.210	–0.0004	–0.0005	–0.0004
Isoleucine	0.184	0.183	0.179	0.0000	–0.0002	0.0002
Leucine	0.347	0.345	0.359	0.0001	0.0000	0.0000
Tyrosine	0.336	0.327	0.349	0.0002	0.0004	0.0003
Phenylalanine	0.381	0.366	0.393	0.0000	0.0002	0.0000
Histidine	17.080	5.228	17.880	0.0005	–0.0001	0.0001
Tryptophan	0.112	0.127	0.141	0.0010	0.0010	0.0007
Lysine	0.350	0.351	0.378	0.0002	–0.0002	–0.0002
Arginine	0.322	0.285	0.326	0.0003	–0.0001	0.0003
Methionine[c]	0.409	0.425	0.488	0.0007	0.0003	0.0003
Methionine[d]	0.333	0.276	0.251	0.0007	0.0003	0.0003

[a]Determined using least-squares non-linear regression of amino acid concentration plotted against multiple hydrolysis times.
[b]Determined as cysteic acid after performic acid oxidation followed by acid hydrolysis.
[c]Determined as methionine after acid hydrolysis.
[d]Determined as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

increase in histidine concentration and the cysteine concentration in the infant formula over the first 8 h of hydrolysis. Histidine concentrations after 1 h of hydrolysis were similar to those observed after longer hydrolysis periods (2–168 h).

When the loss rate was averaged across all powders, the least stable amino acid was serine ($l = 0.0035$), followed by threonine ($l = 0.0017$), tryptophan and cysteic acid ($l = 0.0009$), and aspartic acid ($l = 0.0008$). Interestingly the loss rate for methionine (0.00043) when determined without performic acid oxidation was similar to the loss rate of methionine sulphone (0.00042) after performic acid oxidation prior to acid hydrolysis.

The estimated total amino acid content ($A_o + B_o$) and the amino acid content after 24 h of hydrolysis for each amino acid for the infant formula, growing-up formula and whole milk powder after converting to a 100 g protein basis are presented in Table III. The 24-h hydrolysis values were the mean of two duplicate determinations—one performed several weeks prior to the commencement of this study, and the other was determined as the 24-h hydrolysis time point of the multi-time hydrolysis curves.

The greatest and most consistent difference between the 24-h and multiple hydrolysis interval methods across all powders was for tryptophan, averaging 10.6% (Table III). There was good agreement for glutamic acid, proline, glycine, cysteic acid, methionine (when measured without oxidation), leucine, phenylalanine, histidine, lysine and arginine, whereas aspartic acid, threonine, serine, alanine, valine, isoleucine, tyrosine and were all underestimated from between 3.6% and 5.6% by the single 24-h hydrolysis method relative to the multiple hydrolysis interval method.

There was some difference between powders in which amino acids were underestimated or overestimated by the 24 h value. For the infant formula, the 24 h value underestimated the total amino acid content of tryptophan (12.1%), threonine (8.6%), alanine (7.6%), aspartic acid (7.4%) and serine (6.9%). For the growing-up formula, tryptophan (8.6%), tyrosine (5.0%), threonine (4.4%) and aspartic acid (3.6%) were underestimated; and for whole milk powder, those underestimated were tryptophan (11.0%), proline (6.4%), aspartic acid (5.8%) and isoleucine (5.4%). Several amino acids were overestimated. For instance, methionine sulphone was overestimated by 12.8% in infant formula and by 17.1% in growing-up formula and whole milk powder. Cysteic acid was also overestimated by 5.2% in infant formula.

The average free amino acid contents (B_o) for the three samples are presented in Table IV. Free histidine and glutamic acid (glutamate + glutamine) were present at the greatest concentration, but glycine was found in the highest concentration when concentrations were expressed as a percentage of total amino acids. No free methionine, phenyalanine, tryptophan and proline were detected in any of the milk powders.

Figure 2 shows the amino acid composition of goat milk protein determined using multiple hydrolysis times compared with the average concentrations in human milk. The values for human milk are the mean (± standard deviation) from eight studies listed in Koletzko et al. (2005). One of these studies used multiple-time hydrolyses (Darragh and Moughan 1998) to determine the amino acid composition of human milk; the others, however, used 24-h acid hydrolysis. With the exception of cysteine, the amino acids in goat milk protein were within the range of the concentrations reported for human milk proteins.

Table III. Estimates of the amino acid composition (g/100 g protein[a]) determined using least-squares non-linear regression after multiple-time hydrolysis[b], compared with 24-h hydrolysis[c] values, for goat milk infant formula, growing-up formula and whole milk powders.

	Infant formula		Growing-up formula		Whole milk powder		Average (±SD)		
	Multiple time hydrolysis	24-h hydrolysis	Multiple-time hydrolysis	24-h hydrolysis	Multiple-time hydrolysis	24-h hydrolysis	Multiple-time hydrolysis	24-h hydrolysis	Difference (%)
Aspartic acid	8.67	8.03	8.72	8.40	8.46	7.97	8.62±0.14	8.13±0.23	5.6
Threonine	5.32	4.86	5.21	4.98	5.21	5.02	5.25±0.06	4.95±0.08	5.6
Serine	5.57	5.18	5.26	5.16	5.25	5.15	5.36±0.18	5.16±0.02	3.7
Glutamic acid	19.8	20.1	19.9	20.0	20.0	20.2	19.9±0.10	20.1±0.10	−1.0
Proline	10.8	10.7	10.5	10.5	12.7	11.8	11.3±1.19	11.0±0.70	2.9
Glycine	1.84	1.82	1.87	1.95	1.82	1.85	1.84±0.03	1.87±0.0	−1.6
Alanine	3.38	3.13	3.31	3.30	3.34	3.24	3.34±0.04	3.22±0.09	3.6
Cysteine[d]	1.04	1.10	0.93	0.94	0.92	0.87	0.96±0.07	0.97±0.12	−0.7
Valine	7.60	7.26	7.48	7.26	7.48	7.20	7.52±0.07	7.24±0.03	3.7
Isoleucine	5.00	4.75	4.92	4.79	4.98	4.71	4.97±0.04	4.75±0.04	4.4
Leucine	9.70	9.53	9.51	9.57	9.45	9.63	9.55±0.13	9.58±0.05	−0.3
Tyrosine	4.00	3.82	4.23	4.01	4.24	4.06	4.16±0.14	3.96±0.13	4.7
Phenylalanine	5.13	5.19	5.23	5.18	5.14	5.28	5.17±0.06	5.22±0.06	−1.0
Histidine	2.95	2.99	2.93	3.02	2.85	2.94	2.91±0.05	2.98±0.04	−2.5
Tryptophan	1.65	1.45	1.64	1.50	1.61	1.43	1.63±0.02	1.46±0.04	10.6
Lysine	8.21	8.19	8.22	8.35	8.03	8.39	8.15±0.11	8.31±0.11	−1.9
Arginine	2.96	2.79	2.89	2.91	2.74	2.89	2.86±0.11	2.86±0.06	0.0
Methionine[e]	2.59	2.62	2.60	2.62	2.57	2.56	2.59±0.02	2.60±0.03	−0.5
Methionine[f]	2.02	2.31	1.85	2.24	1.85	2.24	1.91±0.10	2.26±0.04	−18.7

[a]Protein was determined as nitrogen multiplied by 6.38.

[b]The total (protein bound, A_o+free, B_o) amino acids determined using least-squares non-linear regression of amino acid concentration after hydrolysis for a range of hydrolysis times.

[c]Mean of two determinations (each conducted in duplicate). One determination was performed during a preliminary study several weeks prior to the commencement of this study, and the other was determined as the 24-h hydrolysis time point of the multi-time hydrolysis curves determined in this study.

[d]Determined as cysteic acid after performic acid oxidation followed by acid hydrolysis.

[e]Determined as methionine after acid hydrolysis.

[f]Determined as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

Table IV. The average free[a] and total[b] amino acid composition of three different goat milk powders estimated from multiple-interval hydrolysis (mg/g protein[c]).

	Free amino acids	Total amino acids	Free amino acids (% of total)
Aspartic acid	0.31	86.17	0.36
Threonine	0.08	52.47	0.15
Serine	0.37	53.60	0.69
Glutamic acid	1.32	198.77	0.66
Proline	0.00	113.07	0.00
Glycine	0.77	18.43	4.18
Alanine	0.25	33.43	0.75
Cysteine[d]	0.04	9.63	0.42
Valine	0.20	75.20	0.27
Isoleucine	0.06	49.67	0.12
Leucine	0.08	95.53	0.08
Tyrosine	0.07	41.57	0.17
Phenylalanine	0.00	51.67	0.00
Histidine	1.79	29.10	6.15
Tryptophan	0.00	16.33	0.00
Lysine	0.43	81.53	0.53
Arginine	0.22	28.63	0.77
Methionine[e]	0.00	25.87	0.00
Methionine[f]	0.00	19.07	0.00

[a]The amino acid concentration present in the formulas prior to hydrolysis (B_o).
[b]The total (free, B_o + protein bound, A_o) amino acids determined using least-squares non-linear regression of multiple hydrolysis times.
[c]Protein was determined as nitrogen multiplied by 6.38.
[d]Determined as cysteic acid after performic acid oxidation followed by acid hydrolysis.
[e]Determined as methionine after acid hydrolysis.
[f]Determined as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

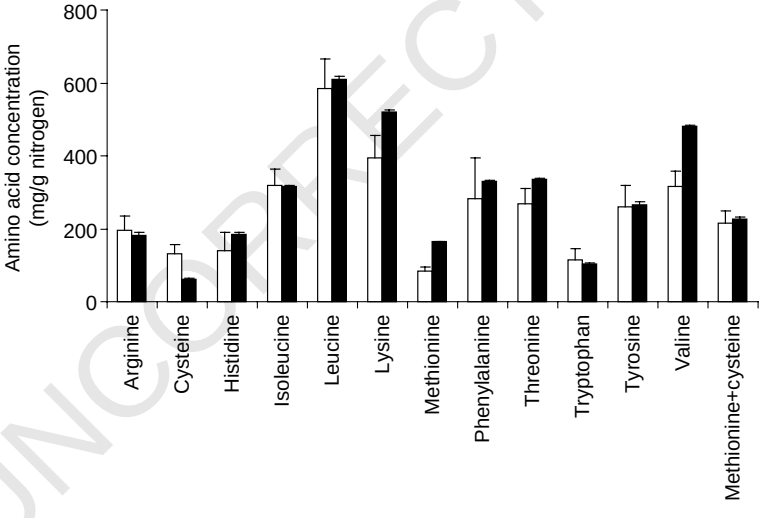

Figure 2. Comparison of the amino acid composition of human and goat milk protein. Values for human milk (white columns) are mean ± standard deviation of values from Koletzko et al. (2005). Values for goat milk (black columns) are mean ± standard deviation based on analysis of an infant formula, a growing-up formula and whole milk powder obtained using multiple-time hydrolysis.

Discussion

The hydrolysis rates observed in this study were similar to those observed with human milk (Darragh and Moughan 1998) and cow skim milk powder (Darragh and Moughan 2005). The exceptions were cysteic acid, for which the hydrolysis rate reported by Darragh and Moughan (1998) was unrealistic, and histidine, for which the rate calculated in this study was considerably higher than for any other amino acid. In the present study, histidine concentrations at the shorter hydrolysis times (<8 h) were higher than, or at least similar to, those observed for the longer hydrolysis times, similar to the findings of Robel and Crane (1972) and Darragh et al. (1996). This is contradictory to what was observed for the other amino acids where the concentrations increased from zero up to a maximum over 6–40 h. The reason for this phenomenon is not clear. It may be due to the presence of peptides, since on our high-performance liquid chromatography system we know of at least two dipeptides (carnosine and anserine) that co-elute with histidine. It appears that histidine hydrolysis may not follow the mathematical model used in this study.

The loss rates for most of the amino acids from goat milk were generally similar to those found for lysozyme (Robel and Crane 1972; Darragh et al. 1996), human milk (Darragh and Moughan 1998) and cow skim milk powder (Darragh and Moughan 2005). The exceptions were the loss rates for threonine, glutamic acid, cysteic acid, valine and histidine in human milk (Darragh and Moughan 1998), which were much higher than the loss rates for the same amino acids for goat milk powders or the cow skim milk powder (Daragh and Moughan 2005). The loss rate of cysteic acid, threonine and serine were also generally higher for lysozyme (Robel and Crane 1972; Darragh et al. 1996). These differences may be due in part to the variation in primary amino acid structures of the proteins in the different protein sources. It would appear from the present results that a 60% variation in carbohydrate content had little influence on the loss rates of amino acids from different formulations.

Overall, for approximately one-half of the amino acids determined in this study (glutamic acid, proline, glycine, cysteic acid, methionine measured without oxidation, leucine, phenylalanine, histidine, lysine and arginine), there was good agreement (<3% difference) between the amino acid content obtained from multiple hydrolysis intervals and the 24-h hydrolysis values. However, the contents of aspartic acid, threonine, tyrosine, isoleucine, valine, serine, and alanine determined by the 24-h hydrolysis method were all lower than the content estimate from multiple interval hydrolysis. The greatest disparity between the single-interval and multiple-interval hydrolysis value was for tryptophan, which was underestimated by an average 10.6% when using the single 24-h hydrolysis value. Overall, similar differences between methods were reported for lysozyme (Robel and Crane 1972; Darragh et al. 1996) and for human milk (Darragh and Moughan 1998).

The multiple-time hydrolysis is also useful in providing a measure of the free amino acids in milk, although the present analysis omitted taurine, which is naturally present at high concentrations in goat milk (Mehaia and Al-Kanhal 1992). In addition, for reasons outlined earlier, the present value for histidine should be viewed with caution. Glycine, glutamic acid (or glutamine), arginine and serine appear to be the most abundant free amino acids in goat milk and milk formula, similar to the case for human milk or cow milk infant formula (Donnovan and Lonnerdal 1989; Agostoni et al. 2000; Ferreira 2003).

The amino acid pattern of human milk is commonly used as a reference for the amino acid requirements of infants. The suitability of infant formula is then determined by comparing it with human milk. The recommendation is that the infant formula should contain the same amount of indispensable and conditionally indispensable amino acids on a per-energy basis as human milk (Koletzko et al. 2005). Based on the present analysis from multiple hydrolyses, and accepting that not all the studies examined by Koletzko et al. (2005) used multiple-time hydrolysis, goat milk infant formula contains a very similar amino acid pattern to human milk protein and would meet or exceed the recommended levels, except for cysteine (Table V). It is recognized, however, that some interconversion of methionine and cysteine can take place, so that the concentrations of methionine and cysteine can be summed, depending on the ratio of methionine and cysteine (Koletzko et al. 2005). While the sum of the concentrations of methionine and cysteine is greater than or equal to the sum of these amino acids in human milk irrespective of the method used to measure methionine, the methionine to cysteine ratio varied (Table V).

The sulphur amino acids cysteine and methionine undergo oxidation during acid hydrolysis and are usually subjected to oxidation with performic acid to cysteic acid and methionine sulphone, respectively, prior to hydrolysis. Interestingly, the levels of methionine sulphone were between 5% and 19% lower than for methionine determined by acid hydrolysis without prior performic acid oxidation in the three goat milk powders. However, the similar loss rates for methionine and methionine sulphone when determined across all three goat milk powders would suggest that the

Table V. Amino acid content of goat infant formula determined using multiple hydrolysis intervals analysis compared with the recommended amino acid pattern for infant formula.

	Goat infant fomula[a] (mg/100 kcal)	Recommended pattern[b] (mg/100 kcal)
Arginine	61	56
Threonine	109	77
Cysteine[c]	21	38
Valine	156	90
Isoleucine	103	92
Leucine	199	169
Tyrosine	82	75
Phenylalanine	105	81
Histidine	60	41
Tryptophan	34	33
Lysine	168	114
Methionine[d]	53	24
Methionine[e]	41	24
Methionine[d] + cysteine	74	62
Methionine[e] + cysteine	62	62
Methionine[d]:cysteine	2.5	0.7–1.5
Methionine[e]:cysteine	1.9	0.7–1.5

[a]Calculated based on 515 kcal/100 g formula (nitrogen conversion factor of 6.25 and 2.0 g protein/100 kcal formula).
[b]From Table 5 of Koletzko et al. (2005).
[c]Determined as cysteic acid after performic acid oxidation followed by acid hydrolysis.
[d]Determined as methionine after by acid hydrolysis.
[e]Determined as methionine sulphone after performic acid oxidation followed by acid hydrolysis.

method for removing oxygen from the sample and acid hydrolysis used in this study was effective. Methionine sulphone is susceptible to over-oxidation (Moore 1963), and it is possible that this had occurred in this study. Cysteic acid may also have been over-oxidized, although this amino acid is more resistant to over-oxidation (Moore 1963). A study investigating the effect of oxidation may therefore be warranted.

In conclusion, amino acid analysis of goat milk powders or formulae using a single-time-point hydrolysis (24 h) is not accurate for all amino acids. This conclusion highlights the importance of careful consideration of the method of analysis of the individual amino acids when making comparisons or setting regulatory standards for assessing the suitability of proteins for human nutrition, particularly for tryptophan and the sulphur amino acids.

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