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Content of non-protein tryptophan in human milk, bovine milk and milk- and soy-based formulas

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Variations in the content of total non-protein (protein-bound + free) and free tryptophan in human and bovine milk after delivery, soy 'milk', and adapted formulas based on bovine milk and soybean protein are reported. Colostrum contains much more of both forms of non-protein tryptophan than mature human milk and bovine milk, and the percentage of free tryptophan is also higher in human milk. Fresh commercially available bovine milk contains amounts of non-protein tryptophan similar to those of bovine milk 1 month after delivery. However, these levels are much lower than those observed in soy 'milk'. Both forms of non-protein tryptophan are also much higher in soybean than in bovine milk formulas, although there are some differences among the kinds of formulas. However, values are significantly lower than in colostrum. The differences in the content of non-protein tryptophan are discussed.

INTRODUCTION

Human milk is widely accepted as an ideal food for full-term infants, although artificial formulas based on bovine milk and soybean protein allow optimal growth and development for infants at term.

Examining the adequacy of bovine milk formulas in the growth of infants, Paiva (1953) observed that bovine milk promotes growth as does human milk. Fomon (1960) has also shown that the growth of infants fed bovine milk formulas is comparable to that of infants ingesting pasteurized pooled human milk during the first 6 months of life. Similar findings have been reported by Barness *et al.* (1963). Fomon (1959) and Fomon *et al.* (1964, 1973) also compared pooled human milk to a soybean formula for the ability to promote growth in infants and found no differences in weight gain.

Human milk contains much less protein but is much higher in total non-protein nitrogen (Lonnerdal *et al.*, 1976; Svanberg *et al.*, 1977) than bovine milk. Large differences between the two milks also concern protein and amino acids (Macy *et al.*, 1953; Fomon, 1974; Lee & Lorenz, 1978). Total non-protein nitrogen also consists of free amino acids (Ling *et al.*, 1961; Johnson, 1974) easily absorbed by newborn infants. The levels of free amino acids are higher in human than in bovine milk (George & Lebenthal, 1981). However, the content of free tryptophan in milk has not been determined for methodological reasons.

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Tryptophan is the only amino acid that is bound to circulating plasma albumin in quantities ranging between 80 and 90%. The small free fraction, the only one able to enter the brain, has great functional importance, since its availability appears to be vital in controlling synthesis of the neurotransmitter serotonin (Knott & Curzon, 1972; Tagliamonte *et al.*, 1973; Gessa & Tagliamonte, 1974).

This research was conducted to compare levels of total non-protein (protein-bound + free) and free tryptophan in human milk, fresh bovine milk, soy 'milk', and artificial formulas based on bovine milk and soybean protein.

MATERIALS AND METHODS

Materials

The following milks or formulas were analysed:

(a) Sixteen human breast milk samples obtained in the morning before nursing newborn infants from 16 healthy mothers of full-term infants, on days 2, 4, 6, 10, 20 and 30 after delivery.

(b) Twenty bovine milk samples, obtained from twenty cows on days 1, 2, 3, 4, 5, 6, 10, 20 and 30 *post partum*, before milking.

(c) Twenty randomized samples of fresh and UHT (Ultra High Temperature) long life bovine milk from commercial sources.

(d) Ten randomized samples of three types of fresh soy milk from commercial sources: 1. SOYALAC (Alpro, Izegem, Belgium); 2. SOYA MILK (Soya Health

Foods Ltd, Manchester, UK); 3. SOJA DRINK (De Vau-Ge, Lüneburg, Germany).

(e) Twenty randomized samples of various adapted bovine milk formulas from commercial sources: 1. PREAPTAMIL (Milupa, Colmar, France); 2. NAN (Nestlè, Vevey, Switzerland); 3. EULAC (Dieterba, Parma, Italy); 4. MELLIN-1 (Mellin Laitière de Cornouvaille, Quimper, France); 5. NATIVA'-1 (Guigoz, Vuadens, Switzerland); 6. ALFARE', a casein hydrolysate (Nestlè, Vevey, Switzerland).

(f) Eighty randomized samples of milk-free formulas based on soybean protein: 1. Two batches of 10 samples each of ISOMIL (Abbott, Zwolle, Holland); 2. HUMANA S.L. SINELAC (Humana Milchwerke, Westfalen, Herford, Germany); 3. MILUPA SOM (Milupa AG, Friedrichsdorf, Germany); 4. NEO SOYAL (Diadal, Opwijk, Belgium).

Dilutions of these formulas for the first 2 weeks of nutrition were prepared according to the manufacturer's instructions, for tryptophan determination.

Analytical procedure

The free form of tryptophan was obtained after ultrafiltration of 4 ml of the sample using an ultrafiltration cell (Amicon Model 12, Amicon, Oosterhout, Holland) with an XM-50 (Diaflo) membrane (Amicon, Oosterhout, Holland). Only the first 0·4 ml of filtrate was collected (Eccleston, 1973).

Total non-protein (protein-bound + free) and free tryptophan were measured on a combined HPLC-fluorescence system using the stop flow technique according to the methods of Bettero *et al.*, (1984) and Costa *et al.* (1987).

Standard solutions (10 μl) containing different concentrations of tryptophan (0·50–60·0 ng), 10 μl of milk, or 10 μl of formula solution to which increasing amounts of tryptophan were added, were injected directly by an automatic sampling device after filtration through a 45 μm millipore filter (Millipore S.A., Molsheim, France).

Recovery trials of added tryptophan to the milk samples were 98 ± 1·5% relative standard deviation (RSD). Measurements were carried out at a level of picomoles with a reproducibility of 0·92% RSD (n = 12) (Costa *et al.*, 1987).

Tryptophan characterization was made by fluorescence ratios, second derivative and synchronous emission spectra according to Bettero *et al.* (1987).

The Student's *t*-test was used to calculate the statistical significance of differences between averages.

RESULTS

Figure 1 shows mean values (mg/litre) and standard deviations of the two forms of non-protein tryptophan (protein-bound + free, and free) in human and bovine milk samples obtained during the first month after delivery. Human milk is much richer (P < 0·001) in non-protein tryptophan than bovine milk, above all in the first few days of lactation. Colostrum contains much more of both forms of non-protein tryptophan than mature human milk (P < 0·001). On day 2 *post partum*, the total non-protein and free tryptophan contents of human milk (11·09 ± 2·23 mg/litre and 7·97 ± 1·20 mg/litre respectively) are markedly higher (P < 0·001) than those of bovine milk on the same lactation day (1·44 ± 0·50 mg/litre and 0·39 ± 0·08 mg/litre). Ten days after delivery, tryptophan levels significantly (P < 0·001) decrease in both kinds of milk, except for the free form of tryptophan in bovine milk. On day 30 *post partum*, human tryptophan concentration continues to decrease, although more slowly: from 1·67 ± 0·28 mg/litre on day 10 to 1·32 ± 0·22 mg/litre on day 30 for total non-

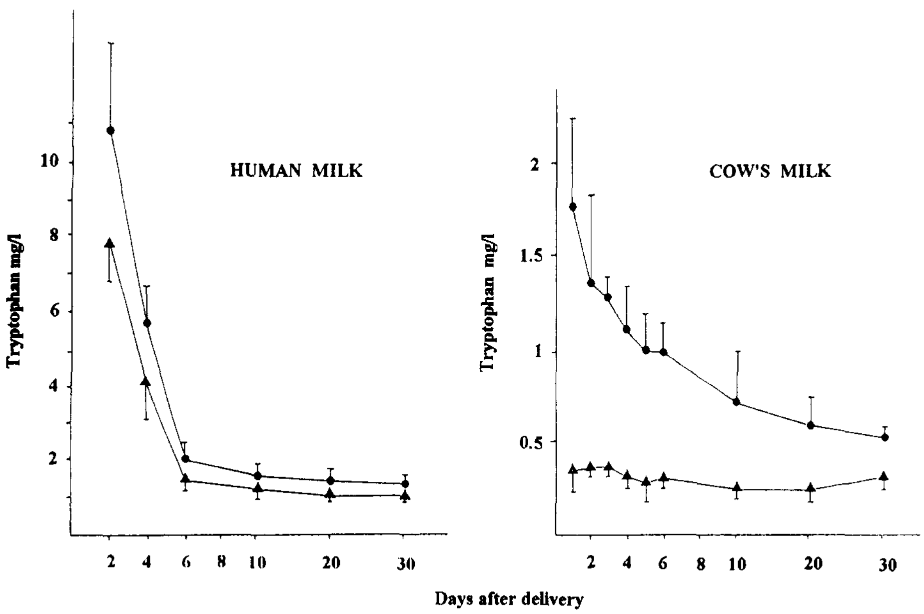

Fig. 1. Trends of mean levels (mg/litre ± SD) of total non-protein (●) and free (▲) tryptophan in human milk (from 16 women) and bovine milk (from 20 cows) during first month *post partum*. Values of human versus bovine milk in first few days of lactation are significant (P < 0·001).

Table 1. Levels of total non-protein and free tryptophan in fresh and UHT long life bovine milk and soy 'milk' from a commercial source (mean ± SD)

	Tryptophan (mg/litre)	
	Total	Free
Bovine milk[a]		
Fresh	0·58 ± 0·09	0·34 ± 0·11
UHT long life	0·60 ± 0·05	0·35 ± 0·10
Soy 'milk'[b]		
Soyalac[c]	5·01[f] ± 1·20	3·44[f] ± 0·62
Soya milk[d]	3·24[f] ± 0·33	1·68[f] ± 0·21
Soja drink[e]	3·11[f] ± 0·22	2·82[f] ± 0·19

[a] Twenty samples for each type. These milks supplied 65 kcal/100 ml; protein concentration 3·5 g/100 ml.
[b] Ten samples for each type.
Protein content:
[c] 3·6 g/100 g (kcal = 47/100 g); [d] 3·3 g/100 g (kcal = 36/100 g);
[e] 3·6 g/100 g (kcal = 53/100 g).
[f] Values significantly different from those of bovine milk at level $P < 0{\cdot}001$.

protein, and from 1·28 ± 0·27 mg/litre to 1·12 ± 0·21 mg/litre for free tryptophan. In bovine milk too, a similar decrease is observed.

Table 1 lists mean levels (mg/litre ± Standard Deviation (SD)) of total non-protein and free tryptophan in samples of fresh and UHT long life bovine milk and soy 'milk' from commercial sources. Commercial bovine milk presents the values of both forms of non-protein tryptophan, similar to those in bovine milk on day 30 after delivery as reported in Fig. 1, but they are much lower than those found in soy 'milk', which shows great variations in both concentrations of non-protein tryptophan.

Table 2 shows mean values (mg/litre of solution ± SD) of both forms of non-protein tryptophan in different commercial adapted formulas of bovine milk. It shows contents of total non-protein tryptophan significantly different from those of fresh bovine milk depending on the kind of formula, while the values of the free form are not significant. Instead, the hydrolysate-based formula contains very high levels of both forms of the amino acid.

Table 2. Levels of total non-protein and free tryptophan in bovine milk adapted formulas and in a hydrolysate-based formula

	Tryptophan (mg/litre)	
	Total	Free
PREAPTAMIL[1]	0·69 ± 0·07[a]	0·36 ± 0·06
NAN[2]	0·48 ± 0·08[a]	0·31 ± 0·05
EULAC[3]	0·48 ± 0·04[a]	0·32 ± 0·02
MELLIN-1[4]	0·55 ± 0·07	0·37 ± 0·01
NATIVA'-1[5]	0·52 ± 0·02[b]	0·36 ± 0·02
ALFARE'[6]	450·0 ± 82·31[a]	70·33 ± 17·41[a]

[1–5] Bovine milk formulas; protein content: [1] 1·5 g/100 ml (kcal = 67/100 ml; dilution at 13·0% w/v); [2] 1·5 g/100 ml (kcal = 67/100 ml; dilution at 13·2% w/v); [3] 1·6 g/100 ml (kcal = 67/100 ml; dilution at 13·2% w/v); [4] 1·6 g/100 ml (kcal = 66·8/100 ml; dilution at 13·1% w/v); [5] 1·7 g/100 ml (kcal = 67/100 ml; dilution at 12·9% w/v). [6] Hydrolysate-based formula; protein content: 2·04 g/100 ml (kcal = 65/100 ml; dilution at 13·6% w/v). Tryptophan values are means ± SD of twenty samples for each type of formula. Values significantly different from those of fresh bovine milk (see Table 1) at level [a] $P < 0{\cdot}001$ and [b] $P < 0{\cdot}01$.

Table 3. Levels of total non-protein and free tryptophan in soybean protein formulas for term infants

	Tryptophan (mg/litre)	
	Total	Free
ISOMIL[a]		
Batch a	3·40 ± 0·43	2·76 ± 0·21
Batch b	2·79 ± 0·21	1·76 ± 0·03
HUMANA[1]	3·16 ± 0·34	1·56 ± 0·04
MILUPA SOM[2]	1·86 ± 0·28	1·09 ± 0·03
NEO-SOYAL[3]	3·80 ± 0·41	2·34 ± 0·15

[a] Ten samples for each batch; protein content: 1·8 g/100 ml (kcal = 67/100 ml; dilution at 13% w/v).
[1–3] Twenty samples for each type; protein content: [1] 2·0 g/100 ml (kcal = 68/100 ml; dilution at 13·2% w/v); [2] 2·0 g/100 ml (kcal = 70/100 ml; dilution at 14% w/v); [3] 1·9 g/100 ml (kcal = 69/100 ml; dilution at 13·5% w/v).
Tryptophan values are means ± SD. All values of both forms of non-protein tryptophan versus bovine milk formulas are significant at level $P < 0{\cdot}001$. For Isomil, $P < 0{\cdot}001$ batch a versus batch b. Milupa Som is significant ($P < 0{\cdot}001$) versus the other soy formulas.

Table 3 shows mean contents (mg/litre of solution ± SD) of total non-protein and free tryptophan in soy-based formulas. These levels are much higher ($P < 0{\cdot}001$) in soybean protein than in bovine milk formulas, although the values are significantly lower ($P < 0{\cdot}001$) than those of human milk in the first three days *post partum*. However, there are some differences among the various kinds of formulas, even from batch to batch, depending on different manufacturing processes.

DISCUSSION

Our data indicate that the non-protein tryptophan content of colostrum is much higher than that of mature human and bovine milk and tends to decrease slightly after the initial rapid fall during the first few days of lactation. The high content of free tryptophan in colostrum in the first few days after birth could guarantee an amount available for uptake by the central nervous system and reflect full homeostatic mother-foetus adjustment after sudden removal of the placental supply (Tricklebank *et al.*, 1979; De Antoni *et al.*, 1980; Allegri, 1987; Zanardo *et al.*, 1989).

However, the amount of tryptophan which enters the brain is known to depend, not only on the ratios of free tryptophan/tryptophan bound to serum albumin, but also on the total serum concentrations of the neutral amino acids tyrosine, phenylalanine, valine, leucine and isoleucine (Fernstrom & Wurtman, 1972; Fernstrom *et al.*, 1973), which enter the brain by the same transport system and which compete with tryptophan. In this way, a milk richer in these neutral amino acids and less rich in tryptophan, such as bovine milk (George & Lebenthal, 1981), has the effect of decreasing the availability of tryptophan to the brain, thus influencing the synthesis of the neurotransmitter serotonin (Wurtman, 1980).

Comparing the levels of non-protein tryptophan in fresh soy 'milks' and soy-based formulas, it appears that these are markedly higher than those of fresh bovine milk and bovine-adapted formulas. This content in soy-based formulas may integrate the lower protein concentration of tryptophan in soybean protein.

Janas *et al.* (1987) observed that infants fed formula milk had lower plasma tryptophan concentrations when compared with infants fed human milk at ages 4 and 8 weeks. Our previous studies (Zanardo *et al.*, 1987) indicated that serum levels of total tryptophan in newborn infants fed at the breast or with bovine milk and soybean formulas are approximately equal on days 2 and 6 after birth, in spite of the higher content of this amino acid in human milk. However, concentrations of the serum free form of tryptophan appeared to be significantly higher in breast-feeding infants than those obtained in infants fed with bovine milk formula (Zanardo *et al.*, 1987). This increase in serum free tryptophan makes more tryptophan available for brain serotonin synthesis (Tagliamonte *et al.*, 1973).

Casein hydrolysates are also sometimes used as a source of nutrition in infants with various gastrointestinal disturbances. However, their high levels of free tryptophan cause vitamin B_6-deficiency (Musajo & Benassi, 1964; Wolf, 1974), some enzymes involved in tryptophan metabolism being B_6-dependent.

These results indicate that more rigorous diet control is necessary in the perinatal period, in particular of newborn infants fed casein hydrolysates.

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