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True Ileal Amino Acid Digestibility of Goat and Cow Milk Infant Formulas

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ABSTRACT

Goat milk is used as an alternative to cow milk for the production of infant formulas. However, little is known about the protein quality and, specifically, about the digestible AA pattern of goat milk formulas compared with their cow milk counterparts. In this study, the true ileal AA digestibility of a goat milk infant formula was compared with a premium cow milk infant formula. The 3-wk-old piglet was used as a model for the 3-mo-old infant. Both milk formulas were prepared as described by the manufacturer, with titanium dioxide added as an indigestible marker. The formulas were fed to the piglets over a 2-wk trial period. Digesta from the terminal ileum were collected post euthanasia and analyzed for AA content, along with samples of the formulas. True AA digestibility was determined after correcting for endogenous AA loss at the terminal ileum of pigs fed an enzyme-hydrolyzed casein-based diet, followed by ultrafiltration (5,000 Da) of the digesta. Total urine and feces collection was also undertaken to determine the nitrogen retention from the diets. The true ileal AA digestibility was similar between the goat and cow milk infant formulas for all AA except Gly and Trp. There was no significant difference in the nitrogen retention of piglets fed the two different formulas. The goat milk infant formula and the premium cow milk infant formula were similar in terms of protein quality.
Key words: amino acid, digestibility, goat milk, infant formula

INTRODUCTION

Goat milk is often used as an alternative to cow milk in human nutrition, particularly in the area of infant nutrition. Mothers who are unable to breastfeed their babies often turn to commercial infant formulas as a solution. Cow milk and goat milk are protein sources commonly used for the manufacture of infant formulas. The casein composition in human milk, particularly the level of α_{s1}-casein, is more similar to goat milk than to cow milk, although it should be noted that the α_{s1}-casein level in goat milk does vary considerably across different milks (Brown et al., 1995).

The protein composition of goat milk has been examined by a number of researchers (D'Urso, 2000; Roncada et al., 2002), but for most of these studies, the focus has been on the relationship between the levels of different milk proteins and the possible allergenic response to those proteins (Roncada et al., 2002). Some work has been conducted examining the AA composition of casein in goat milk in relation to the lactation stage (Singh and Singh, 1985) and the composition of whole goat milk in comparison with other mammalian milks (Davis et al., 1994). However, information on the AA digestibility of goat milk or goat milk products is lacking, even though this information is of paramount importance in understanding the nutritional value of goat milk and goat milk infant formulas. In this study, the 3-wk-old piglet was used as a model for the 3-mo-old human infant (Darragh and Moughan, 1995), because the gut physiology of the pig is similar to that of the human (Moughan et al., 1992). The true ileal AA digestibility of a goat milk infant formula and a premium cow milk infant formula was determined and compared. The nitrogen retention was also compared in piglets fed a goat or a cow milk infant formula.

MATERIALS AND METHODS

The goat whole milk infant formula was obtained from the Dairy Goat Co-operative (N.Z.) Ltd. (Hamilton, New Zealand) and the whey-enhanced cow milk infant formula was from Wyeth (Auckland, New Zealand).

Proximate Analysis

The gross energy contents of the goat milk infant formula and the whey-enhanced cow milk infant formula were determined using bomb calorimetry (Miller and Payne, 1959). The nitrogen content was determined on a Leco analyzer (Leco FP-2000, Leco Corp., St. Joseph, MI) using the Dumas method (Agricultural and Food Research Council, 1987), whereas the fat content was determined using the Soxhlet method (Firth et al., 1985).

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Piglet Trial

The two infant formula diets were prepared according to the manufacturers' instructions (goat milk formula: 13.5 g plus 90 mL of water; cow milk formula: 12.7 g plus 90 mL of water). Sixteen entire male piglets (1 wk of age) were housed at the Animal Physiology Unit, Massey University (Palmerston North, New Zealand), in a temperature-controlled room maintained at 28 ± 2°C, with a 16:8 h light:dark cycle, in purpose-built plastic metabolism crates that allowed the separate collection of urine (Darragh and Moughan, 1995). All experimental procedures were approved by the Massey University Animal Ethics Committee. The metabolism crates were equipped with urine-collection assemblies to allow urine collection. The piglets underwent a 14-d acclimation period during which time they received a 1:1 mixture of the 2 infant formulas. At the beginning of the acclimatization period, the piglets were weighed. During the acclimatization period, the piglets received 345.1 g of liquid formula per kilogram of BW per day (Darragh and Moughan, 1995). The piglets were trained to drink using a bottle and teat and were fed every 1.5 h from 0630 to 2130 h. At the end of the acclimation period, the piglets were 3 wk of age and were allocated to 1 of the 2 test diets, with each treatment balanced for littermates. The piglets were also fitted with a stomahesive base plate (Conva Tec Ltd., Deeside, UK; designed to attach to human ostomy bags) for quantitative collection of feces. The plates were glued in place after the anal and tail region of the piglets had been shaved. A preweighed ostomy bag was then attached to allow fecal collection. The piglets were reweighed and the food intakes adjusted for the increase in piglet body weights. The main trial lasted 14 d. During the first 12 d, the daily allowance was given as 7 portions every 2.5 h throughout the day from 0630 to 2130 h. For each meal, the piglets were fed using a bottle and teat. Any formula refused was collected, dried, and weighed.

Total urine and feces collection was undertaken during the trial to determine nitrogen retention. Urine was collected into a bottle containing 25 mL of 1.8 *M* H_2SO_4 per liter of urine, and this and the feces were collected separately each day. The respective urine and feces samples were then pooled over matching collection periods for each piglet.

Each urine sample for each pig and for each collection period was analyzed for creatinine (which acted as a marker) and nitrogen content. The remaining urine from each sample and the fecal samples were then freeze-dried. The freeze-dried feces and urine were analyzed for nitrogen content, and urinary nitrogen output for each piglet was calculated.

On d 13 and 14 of the study, the piglets were fed meals hourly starting at 0530 h and finishing at 2030 h. On the last day, the feeding of the piglets was staggered such that each piglet was fed 30 min apart. The formulas used were the same as for the previous days except that 0.3% of titanium dioxide (wt/wt dry formula) was added to the formula before diet preparation. Seven hours after the start of the hourly feeding regimen, each piglet was anesthetized with halothane and then killed using an intercardial injection of sodium pentobarbitone. The abdominal cavity was opened and 20 cm of ileum immediately anterior to the ileo-cecal junction was then dissected out. The dissected ileum was washed with distilled, deionized water to remove any blood and hair, and was carefully dried on an absorbent paper towel. The digesta were then gently flushed from the ileal section with distilled, deionized water from a syringe. The digesta were freeze-dried and stored at –20°C until AA and titanium dioxide contents were determined.

True ileal AA digestibility was calculated as shown in Equation 1:

$$\text{true ileal AA digestibility (\%)} = \frac{\text{dietary AA intake} - (\text{ileal AA flow} - \text{endogenous AA flow}) \times 100}{\text{dietary AA intake}} \quad [1]$$

where the units are milligrams per kilogram DMI. AA flow was calculated as shown in Equation 2:

$$\text{endogenous AA flow (mg/kg of DMI)} = \frac{\text{AA content}_{(\text{digesta})} \times \text{titanium}_{(\text{diet})}}{\text{titanium}_{(\text{digesta})}} \quad [2]$$

where the units are milligrams per kilogram of DMI. Endogenous AA losses were those reported for the young pig (Rutherfurd and Moughan, 1997).

AA

Amino acids were determined in 5-mg samples of dry formula, diet, and digesta in duplicate using a Waters ion-exchange HPLC system (Waters Corp., Milford, MA), utilizing postcolumn ninhydrin derivatization and detection with absorbance at 570 nm (440 nm for Pro), following hydrolysis in 6 *M* glass-distilled HCl containing 0.1% phenol for 24 h at 110 ± 2°C in evacuated, sealed tubes. Cysteine and Met were determined in the same way except that they underwent a preoxidation step in performic acid at 0°C for 16 h to convert the acid-labile Cys and Met to the more acid-stable derivatives, cysteic acid and methionine sulfone. Tryptophan was determined by HPLC using base hydrolysis prior to quantitation. No correction was made for loss of AA during acid hydroly-

Table 1. Proximate analysis of the goat milk formula and cow milk formula

	Per 100 g of powder		Per 100 mL of formula[1]		Reported on can[2]	
Item	Goat	Cow	Goat	Cow	Goat	Cow
Gross energy, kJ	2220	2280	300	290	290	274
Crude protein, g	11.4	11.8	1.53	1.50	1.5	1.5
Total fat, g	24.1	26.0	3.25	3.30	3.6	3.6

[1]Prepared according to the manufacturer's specifications.
[2]Reported on the food composition label present on the can.

sis. Free AA molecular weights were used to calculate AA weights.

Creatinine

Creatinine was determined using the Jaffe method based on the method of Masson et al. (1981).

Titanium Dioxide

Titanium was determined based on the method of Short et al. (1996). Essentially, samples were ashed before being digested in 60% (vol/vol) sulfuric acid. The mixture was then incubated with 30% H_2O_2 and the absorbance was read at 405 nm.

Data Analysis

Differences between dietary treatments were tested using least significant differences (GLM procedure, SAS Institute, 1999).

RESULTS

Proximate Analysis of the Goat Milk and Cow Milk Infant Formulas

The gross energy, CP (determined as nitrogen and multiplied by 6.38), and total fat contents of the diets are shown in Table 1. The gross energy of the dry powders was similar for the two infant formulas, as was the protein content. The fat content was slightly higher (7%) in the cow milk formula than in the goat milk formula. Based on the formula recipe, the goat milk formula provided slightly more energy (3.6%) and protein (2.3%) but less fat (1.4%) than the cow milk formula when prepared and ready for consumption.

Table 2. Nitrogen composition of the goat and cow infant formulas

Item	Goat	Cow
Total nitrogen, g/100 g of powder	1.78	1.85
AA nitrogen, g/100 g of powder[1]	1.49	1.64
Non-AA nitrogen, % of total nitrogen[2]	16.5	11.1

[1]AA nitrogen is calculated from the AA composition.
[2]Calculated as total nitrogen minus AA nitrogen.

Nitrogen Content and AA Composition of the Goat Milk Formula and the Cow Milk Formula

The total nitrogen contents of the cow milk formula and goat milk formula were similar (3.9% difference; Table 2). The AA nitrogen content was slightly higher in the cow milk formula than in the goat milk formula. For both formulas, the AA nitrogen was lower than the total nitrogen (11 to 16% less), suggesting the presence of non-AA nitrogen. The non-AA nitrogen was 49% higher in the goat milk formula than in the cow milk formula.

The AA composition of the goat milk formula and the cow milk formula is shown in Table 3. For most AA, the levels (g/100 g of powder) were higher in the cow milk formula than in the goat milk formula. For some of the essential AA, such as Cys, Trp, Thr, and Ile, the levels were much higher (23 to 89% higher) in the cow milk

Table 3. Amino acid composition of the goat milk formula and cow milk formula powder

	Composition, g/100 g of powder		Composition, wt %[1]	
AA	Goat	Cow	Goat	Cow
Asp	0.78	1.11	7.48	9.68
Thr	0.50	0.61	4.75	5.32
Ser	0.51	0.57	4.82	4.96
Glu	2.04	2.06	19.48	17.97
Pro	1.09	0.86	10.36	7.46
Gly	0.19	0.22	1.83	1.92
Ala	0.34	0.48	3.21	4.18
Cys	0.10	0.20	1.00	1.72
Val	0.75	0.70	7.17	6.09
Met	0.27	0.32	2.60	2.82
Ile	0.50	0.63	4.80	5.49
Leu	1.01	1.15	9.60	10.00
Tyr	0.40	0.42	3.81	3.62
Phe	0.50	0.44	4.66	3.86
His	0.30	0.28	2.81	2.44
Trp	0.14	0.17	1.33	1.50
Lys	0.80	0.93	7.48	8.08
Arg	0.30	0.33	2.81	2.90

[1]Wt % = Weight of an AA/sum of the weights of all AA × 100.

Table 4. Mean (n = 8) true ileal AA and nitrogen digestibility (%) of the goat milk formula and cow milk formula

	AA digestibility		SE		
	Goat	Cow	Goat	Cow	Significance[1]
AA					
Asp	96.6	99.3	1.79	1.13	NS
Thr	87.9	90.9	2.10	2.61	NS
Ser	95.0	98.1	1.47	1.66	NS
Glu	98.6	99.5	0.73	0.85	NS
Pro	95.9	95.1	1.19	1.87	NS
Gly	55.5	82.3	9.00	5.12	*
Ala	90.9	95.8	3.01	1.98	NS
Cys	92.4	97.3	2.28	1.64	NS
Val	97.5	98.2	1.01	1.36	NS
Met	99.8	100.0	0.57	0.46	NS
Ile	98.2	99.3	0.79	0.85	NS
Leu	98.5	99.3	1.30	0.95	NS
Tyr	97.8	99.5	0.92	0.88	NS
Phe	97.2	98.4	1.27	1.44	NS
His	89.8	90.6	2.06	3.22	NS
Trp[2]	88.7	93.1	1.32	1.42	*
Lys	95.5	95.1	1.54	1.32	NS
Arg	95.2	98.4	2.46	1.82	NS
Mean AA digestibility	92.8	96.0	1.33	1.55	NS
Nitrogen	92.3	92.8	1.00	1.52	NS

[1]NS = Not significant, *$0.05 > P > 0.01$.

[2]Trp digestibility values are apparent ileal digestibility values.

Table 5. Mean (n = 8) true ileal digestible AA and nitrogen content (g/100 g powder) of the goat milk formula and cow milk formula

	Digestible AA content		SE		
AA	Goat	Cow	Goat	Cow	Significance[1]
Asp	0.76	1.10	0.014	0.013	***
Thr	0.44	0.56	0.010	0.016	***
Ser	0.48	0.56	0.007	0.010	***
Glu	2.01	2.05	0.015	0.018	NS
Pro	1.00	0.78	0.013	0.016	***
Gly	0.11	0.18	0.017	0.011	***
Ala	0.31	0.46	0.010	0.010	***
Cys	0.10	0.19	0.002	0.003	***
Val	0.73	0.69	0.008	0.010	***
Met	0.27	0.32	0.002	0.002	***
Ile	0.50	0.63	0.004	0.005	***
Leu	0.99	1.14	0.013	0.011	***
Tyr	0.39	0.41	0.004	0.004	***
Phe	0.48	0.44	0.006	0.006	***
His	0.27	0.25	0.006	0.009	NS
Trp	0.12	0.16	0.002	0.003	***
Lys	0.75	0.88	0.012	0.012	***
Arg	0.28	0.33	0.007	0.006	***
Nitrogen	0.16	0.17	0.002	0.003	*

[1]NS = Not significant, *$P < 0.05$, ***$P < 0.001$.

formula than in the goat milk formula when calculated per 100 g of powder. When the AA content was calculated as weight percentage (weight of an AA divided by the sum of the weights of all the AA), the AA profiles for the 2 infant formulas were very similar, with all the AA levels (except Cys) being within 15% when the 2 formulas were compared. Cysteine concentrations differed greatly (73%) between the two infant formulas.

True Ileal AA Digestibility

The piglets appeared healthy throughout the acclimatization period, although most developed nutritional scours for up to 2 d during this period, after which time they recovered and remained healthy for the main trial period. The mean initial live weight of the piglets after allocation to each diet at the start of the experimental period was 3.4 kg for the piglets on the goat milk formula and 3.2 kg for the piglets on the cow milk formula. These initial live weights were not significantly different between treatments.

The true ileal AA digestibility of the goat milk formula and the cow milk formula is shown in Table 4. The average digestibility of all the AA was 93% for the goat milk formula and 96% for the cow milk formula. Nitrogen digestibility was correspondingly high: 92 and 93% for the goat and cow milk formulas, respectively. The overall digestibility of both formulas was very high and generally consistent with what we would expect for milk protein-based products. With the exception of Gly and Trp, there were no significant differences in the true ileal AA digestibility between the piglets fed the 2 infant formulas for any of the AA tested. For Gly, the digestibility of the goat milk formula was considerably lower than for the cow milk formula (27 percentage units lower). For Trp, the digestibility was 4 percentage units higher in the cow milk formula compared with the goat milk formula. For Cys, there was a large numerical difference in AA digestibility between the 2 formulas, although this difference was not statistically significant.

The true ileal digestible AA content of the goat milk formula and cow milk formula is shown in Table 5. There was no significant difference in the digestible Glu and His levels between the 2 formulas in their powdered form. In contrast, the digestible Pro, Val, and Phe contents in the powered formula were significantly higher in the goat milk formula than in the cow milk formula, whereas for the remainder of AA, the digestible AA content was higher in the cow milk formula. The greatest difference was observed for Cys, with the cow milk formula containing twice the amount present in the goat milk formula.

Urinary Nitrogen Excretion and Nitrogen Retention

The mean daily urinary excretion of nitrogen (g/d) for the 8 piglets fed the goat milk formula diet was not significantly different compared with the piglets fed the cow milk formula diet (Table 6). A similar result was found when nitrogen content was expressed per weight

Table 6. Mean urinary nitrogen excretion

	Mean		SE		
	Goat	Cow	Goat	Cow	*P*
Urinary nitrogen excretion					
g/d	0.31	0.27	0.021	0.014	0.166
g/μmol of creatinine	595	498	83.3	61.4	0.365
Nitrogen retention					
g/d	2.76	2.56	0.17	0.19	0.438

of creatinine excreted. The nitrogen retention is also shown in Table 6. For the piglets fed the goat milk infant formula, nitrogen retention was 2.76 g/d and was not significantly different from that of piglets fed the cow milk infant formula, for which the nitrogen retention was 2.56 g/d.

DISCUSSION

Overall, the gross energy, protein, and fat contents were similar for the goat milk and cow milk infant formulas. There was a high amount of non-AA nitrogen in the goat milk formula compared with the cow milk formula. Amino acid nitrogen will be slightly underestimated, given that not all the AA are recovered completely during AA analysis; despite this fact, a considerable amount of nitrogen still remains unidentified. Part of this non-AA nitrogen is in the form of choline, taurine, and carnitine, some of which are supplemented to the formula during manufacture. Furthermore, human milk is known to contain large amounts of non-AA nitrogen, and a significant proportion of this is in urea (Donovan and Lonnerdal, 1989). Whether urea also contributes significantly to the non-AA nitrogen in goat milk is not known. Further analysis of the non-AA nitrogen component of goat milk might be warranted.

True Ileal AA Digestibility

The true ileal AA digestibility for both formulas was high, with mean digestibilities of 93 and 96% for the goat milk formula and the cow milk formula, respectively. This is consistent with high-quality milk protein products (Rutherfurd and Moughan, 1997). Trp and Gly were the 2 AA for which digestibility differed between formulas. For Trp the difference was relatively small (4 percentage units), whereas for Gly the difference was large (27 percentage units). The reason for the latter is unknown; however, the difference in Gly digestibility between the 2 formulas may not be of nutritional significance, given that Gly is a nonessential AA.

The digestible AA contents of the essential AA relative to Lys were also calculated for each formula and were compared with human milk (LSRO, 1998) and the recommended pattern of AA based on an ideal protein (Food and Agriculture Organization/World Health Organization/United Nations University, 1985). This comparison is shown in Table 7. The pattern of digestible essential AA for the goat milk infant formula matched or exceeded that of human milk for Lys, Met, Cys, Phe, Tyr, and Val and was within 90% for His, Ile, Leu, and Thr. The pattern for the cow milk infant formula matched or exceeded that of human milk for Lys, Ile, Met, Cys, and Thr and was within 90% for Leu, Phe, and Tyr. The recommended AA pattern for the human infant (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) is also shown in Table 7 and is based on the ideal protein, which in turn is based on the AA requirements of the human infant. The digestible AA contents of the infant formulas were compared with the recommended AA pattern for the human infant. Relative to Lys, the digestible His, Leu, Phe, Tyr, and Val contents were higher in the goat milk formula than the cow milk formula. For many of these AA, the levels were close to or exceeded the recommended levels. For the cow milk formula, Ile, Met, Cys, and Thr were present in greater levels than in the goat milk formula and were close to the recommended levels. It should be noted, however, that the Cys and Trp levels

Table 7. True ileal digestible essential AA content of the goat milk formula and the cow milk formula relative to Lys

AA	Goat	Cow	Human milk[1]	Recommended pattern[2]
Lys	100[3]	100	100	100
His	35	29	37[2]	39
Ile	66	71	71	70
Leu	132	129	138	141
Met + Cys	49	59	47	64
Phe + Tyr	115	96	107	109
Thr	58	63	62	65
Val	98	78	73	83
Trp	17	18	21	26

[1]LSRO, 1998.

[2]Food and Agriculture Organization/World Health Organization/United Nations University, 1985.

[3]The digestible Lys content has been normalized to 100, and the digestible contents of the other AA have been calculated relative to Lys.

observed in the goat milk formula batch used in this study were approximately 40 and 20% lower, respectively, than has been found routinely in other batches of this particular goat milk infant formula (D. Lowry, unpublished data).

It must also be noted that these comparisons were based on how the AA pattern within the protein component of the formula matched the pattern of human milk or an ideal protein. They did not show how well the formula met the actual requirements of an infant, because food intake data were required to make this comparison.

Nitrogen Retention Study

In this study, there was no significant difference in retained nitrogen in the piglets fed the 2 infant formulas. This finding is consistent with the urinary nitrogen excretion data, in which no significant differences were observed between formulas, and with the fact that nitrogen absorption (95 and 97% for the goat milk formula and the cow milk formula, respectively) and nitrogen contents in the 2 formulas were similar.

CONCLUSIONS

Overall, the goat milk infant formula was similar to the cow milk infant formula with respect to the AA contents and protein availability. Perhaps the most significant difference in terms of nutritional value was the lower levels of digestible Trp in the goat milk formula. However, other than for Trp, the goat milk formula appears to be a suitable substitute for a whey-enhanced cow milk infant formula for infants.

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