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Gastric protein digestion of cow, goat, and sheep milk is not reflected in the amino acid appearance in the blood of suckling piglets

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ABSTRACT

Structural changes in milk during gastric digestion are a key driving factor for the rate of digestion of nutrients in the gastrointestinal tract. Thus, the influence of gastric coagulation behavior on the kinetics of protein digestion of raw cow, goat, and sheep whole milk in the stomach was investigated using the 3-wk-old suckled male piglet as an animal model for human infants. Piglets received a single meal of fresh raw milk normalized for protein content, and were slaughtered at 0, 30, 90, 150, or 210 min postprandially. Gastric chyme and cardiac blood samples were collected. Gastric pepsin activity, rate of protein hydrolysis, and gastric emptying of AA were determined along with how these changes influence the appearance of AA in the plasma. The disappearance rates of individual proteins (especially β-LG and α_S-CN), total digested proteins entering the small intestine, as well as the gastric emptying of some AA (proline, leucine) were (or tended to be) greater for goat and sheep milk than for cow milk. Differences in plasma concentrations for some AA (e.g., leucine) were observed across milk types, but they did not directly reflect changes in gastric protein digestion and the gastric emptying of AA. In conclusion, a combination of protein (and AA) composition, susceptibility of specific proteins to hydrolysis, and the nature of the curd structure formed influenced the digestion behavior of milk proteins in the stomach and their subsequent release into the small intestine.

Key words: milk of different species, gastric protein digestion, gastric emptying of amino acids, appearance of blood amino acids, piglet model

INTRODUCTION

Cow milk accounts for ~81% of the world's milk production, followed by milk from noncow species such as buffalo (~15%), goat (~2%), sheep (~1%), and camel (~0.4%; FAOSTAT, 2024). The demand for noncow milk such as goat and sheep milk is increasing, likely due to consumer perceptions about ease of digestion and health benefits (Jandal, 1996; Haenlein, 2004; Haenlein and Wendorff, 2006; Park et al., 2007; Park, 2006a,b; Crowley et al., 2017). However, little scientific information is available about the in vivo digestion of noncow milk. A deeper scientific understanding of noncow milk digestion behavior is needed because these milk types are potential options for the development of different milk products.

Milk proteins and lipids from all the mammalian species undergo significant changes in their physicochemical and structural properties during digestion in the stomach, a first key step in the gastrointestinal digestion of milk. In our previous study (Roy et al., 2022), the comparative processes of gastric coagulation of raw cow, goat, and sheep whole milk in bottle-fed (suckled) piglets were investigated for the first time to understand the curd formation and disintegration over time and the impact of these changes on nutrient (protein and lipid) delivery to the small intestine. The bottle-fed piglet was chosen as a model for the human infant, as previous studies have shown that piglets have a similar gastrointestinal tract anatomy and physiology to human infants (Moughan et al., 1990, 1991, 1992; Darragh and Moughan, 1995, 1998; Rutherfurd et al., 2006; Guilloteau et al., 2010; Mudd and Dilger, 2017).

Our previous study observed faster gastric emptying of proteins and lipids for goat and sheep milk than cow milk (Roy et al., 2022). This was attributed to the relatively softer curd consistency (measured using a rheometer) and the less fused protein networks (observed using transmission electron microscopy) of goat and sheep milk curds compared with cow milk curds in the

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stomach (Roy et al., 2022). In the present work, it is hypothesized that the softer curd structures observed for goat and sheep milk compared with cow milk during gastric digestion (Roy et al., 2022) facilitate a greater flow of gastric pepsin into the curd matrix (structure), resulting in faster protein digestion and associated outcomes, such as disappearance of the protein fractions and flow of digested protein and AA into the small intestine. These differences in the kinetics of gastric protein digestion may further affect the concentrations of AA in the bloodstream during milk digestion.

MATERIALS AND METHODS

Animals, Dietary Treatments, and Experimental Design

All procedures involving animals were approved by the Massey University Animal Ethics Committee (MUAEC protocol 18/97). The researchers and technicians involved in the study were aware of all the stages of the experiment starting from group allocation to data analysis. Any data related to the study are available upon request from the researchers of the study.

The animals, housing, dietary treatments, and experimental design of the piglet study have been reported in detail in our previous study (Roy et al., 2022). Briefly, 60 locally sourced Large White × (landrace × Large White) entire male piglets (7–8 d of age; mean BW on arrival 3 kg) were housed in individual metabolism crates at the Animal Physiology Unit of Massey University (Palmerston North, New Zealand).

The experimental dietary treatments were raw cow, goat, and sheep whole milk. Milk composition has been detailed in our previous paper (Roy et al., 2022) and provided in Supplemental Table S1 (see Notes). The piglets were allocated at random to the 3 dietary treatments on arrival. The piglets were fed using a bottle with a rubber teat for 15 d exclusively with cow, goat, or sheep whole milk. The study duration of 15 d was selected because a 3-wk-old piglet has been reported as an appropriate animal model for studying aspects of digestion and absorption due to their similar developmental patterns to a 3-mo-old human infant (Moughan et al., 1992; Guilloteau et al., 2010). Throughout the animal study, the piglets were monitored for their health, changes in BW, body temperature (~38–40°C), diet intake, meal refusals, any adverse signs such as dehydration and scouring, as well as defecation frequency and fecal consistency. The piglets were provided with clean toys and time to play with one another daily under supervision, and they interacted with the research (or technical) staff involved to provide social contact. Any piglet that did not acclimatize by d 6 of starting the study was withdrawn from the study and rehomed as described previously (Roy et al., 2022).

On the last experimental day (i.e., d 15), fasted (18 h) piglets of 21 to 22 d old were slaughtered at 0 min (before feeding meal) and at 30, 90, 150, and 210 min after feeding fresh raw cow, goat, and sheep whole milk based on an amount of equal protein (i.e., 2 g/kg of BW; Supplemental Table S2, see Notes) such that there were 20 piglets per milk type (i.e., milk species) and 4 piglets per milk type × time point combination. The piglets were first anesthetized using Zoletil 100 (50 mg/mL each of zolazepam and tiletamine [Zoetis Inc., Parsippany-Troy Hills, NJ] reconstituted with 2.5 mL each of ketamine and xylazine [both 100 mg/mL]). Cardiac blood samples were drawn in tubes containing EDTA (BD Vacutainer; Franklin Lakes, NJ). Immediately after the blood collection, the blood sample was centrifuged at 2,000 × *g* for 10 min at 4°C, and the plasma removed was stored at −80°C until required for further analysis. The piglets were then slaughtered with an intracardiac injection of sodium pentobarbitone.

Immediately after slaughter, the abdomen was opened, and the stomach was clamped and removed with minimal movement. The exterior surface was gently washed and dried, and the stomach was weighed full before being opened by an incision along the lesser curvature for removal of the contents. The curd and liquid fractions of the gastric chyme were separated using a 1-mm sieve. Gastric curd and liquid samples were collected for various analyses, and subsamples of each fraction were either immediately frozen at –80°C or further freeze-dried, weighed, and ground. Freeze-dried ground samples were stored at –20°C until analysis. The chemical composition (DM, fat, protein [total nitrogen × 6.38], and ash) of each milk and gastric chyme sample was determined following AOAC International (2005) protocols as described previously (Roy et al., 2022).

Potential Pepsin Activity of Gastric Chyme

Potential pepsin activity was measured in the gastric curd and liquid fractions (thawed frozen samples) following the protocol of Meisel and Hagemeister (1984) and Mulet Cabero (2018) with some modifications. Briefly, 0.2 g of chyme (homogenized inner and outer curd or liquid fraction samples) was mixed with 2 mL 0.01 *M* HCl (pH 2). Then, 2.5 mL of hemoglobin solution (2.5%, wt/vol, bovine blood hemoglobin [Sigma-Aldrich, ref: H2500], pH 2) was added, and the samples were incubated at 37°C for 10 min. After incubation, the samples were treated with 3.5 mL 15% tricarboxylic acid (**TCA**) and centrifuged at 3,800 × *g* for 30 min at room temperature (20°C). The absorbance of clear supernatant was measured at 280 nm using a spectrophotometer. The blank for each sample received the same treatment as the sample, except that the addition of the sample was performed after the mixing of TCA and hemoglobin, and then the absorbance of the clear supernatant of the blank was measured at 280 nm. The potential pepsin activity was calculated per gram protein of the chyme fraction at each digestion time point as described in Supplemental Material section S2.1 (see Notes). Considering that the gastric chyme of the milk from different species varied in chemical composition and also that the chyme composition changed as the gastric digestion progressed, the potential pepsin activity was expressed per gram protein of the chyme fraction at each digestion time point.

Total Gastric Protein Digestion

Apparent degree of protein hydrolysis, the release of digested proteins into the small intestine, and the apparent disappearance of individual milk proteins (using tricine SDS-PAGE) were determined in representative freeze-dried samples. In this study, we did not determine the free amino groups (NH_2) and band intensity in SDS-PAGE gels of endogenous proteins (Montoya et al., 2014). Thus, the apparent degree of hydrolysis and apparent disappearance of individual milk proteins in the stomach are presented.

Apparent Degree of Protein Hydrolysis in Gastric Chyme

The apparent degree of protein hydrolysis was determined by measuring the free NH_2 groups in each milk type, total gastric chyme, gastric curd and liquid fractions, as well as the total NH_2 in each milk type. The free NH_2 of each sample was quantified using the *o*-phthaldialdehyde method (Church et al., 1983; Montoya et al., 2014). To quantify the total NH2 in each milk type, samples were hydrolyzed for 24 h in 6 *M* HCl at 110°C. The resulting hydrolyzed samples were dried using a vacuum evaporator, followed by 2 water washes to remove traces of HCl. The final dried samples were then analyzed for NH_2 as described.

The apparent degree of protein hydrolysis may be calculated as the number of free NH_2 in the chyme relative to the total NH_2 in the milk. However, the chemical composition of the chyme changes over time during digestion as the components are released into the small intestine (Ahlborn et al., 2024). Thus, in the calculations, the total and free NH_2 were expressed per gram protein of the chyme sample retained at each digestion time point (Ahlborn et al., 2024). Based on this, the apparent degree of protein hydrolysis at each time point was calculated as described in Supplemental Material section S2.2 (see Notes).

Digested (or Hydrolyzed) Protein Entering the Small Intestine

The amount of protein emptied from the stomach contained both digested (or hydrolyzed) and intact proteins. Thus, the gastric emptying of the digested proteins from the stomach was determined based on the amount of total protein emptied from the stomach and the apparent degree of protein hydrolysis. Thus, the amount of digested protein entering the small intestine (or emptying from the stomach) was calculated as described in Supplemental Material section S2.3 (see Notes).

Apparent Disappearance of Individual Milk Proteins in Gastric Chyme Using Tricine SDS-PAGE

Both the apparent degree of hydrolysis and the amount of digested protein entering the small intestine are representative of overall gastric protein digestion. The quantitative estimation of the apparent disappearance of the individual milk proteins (αs_2-CN, αs_1-CN, β-CN, κ-CN, β-LG, and α-LA) from different species was further determined by using a semiquantitative SDS-PAGE approach (Supplemental Figure S1, see Notes). As mentioned in the "Total Gastric Protein Digestion" section, the term apparent is used as endogenous proteins (e.g., pepsin) could migrate with milk proteins and affect the disappearance values.

Reducing tricine-SDS-PAGE gels were performed by loading equal protein quantities (20 μg) in each well of the gel for freeze-dried milk and gastric chyme (curd and liquid fractions separately) samples from each piglet as described and shown previously (Roy et al., 2022; Supplemental Figure S1). De-stained gels were imaged using a GelDoc XR (Bio-Rad Laboratories Pty Ltd., Auckland, New Zealand), and ImageLab (software version 6.1, Bio-Rad Laboratories Pty Ltd.) was used for SDS-PAGE quantification by measuring the band intensities of protein bands horizontally in the milk and chyme fractions. Band intensities were considered as arbitrary density units (**ADU**). The apparent disappearance of individual proteins in total gastric chyme (curd + liquid) only are reported. However, it should be noted that the curd phase was mainly associated with the retention of caseins (due to casein coagulation), whereas the liquid phase comprised soluble whey proteins.

To calculate the disappearance of intact individual proteins from the whole chyme, the estimated total band intensity (ADU) of each individual protein present in the milk and the whole (or total) chyme (curd plus liquid fractions) was expressed per gram protein present in each sample (milk or whole chyme) and calculated as described in Supplemental Material section S2.4 (see Notes). The semiquantitative analysis of SDS-PAGE for total gastric chyme was only reported for up to 150 min of gastric digestion, as insufficient replicates were available to complete statistical analysis for 210 min digestion due to the small amount of gastric liquid fractions found in some piglets. Considering the limitations of the methodology (Montoya et al., 2014), the results of apparent gastric disappearance of individual proteins should be interpreted with some caution.

AA Analysis and Gastric Emptying

Freeze-dried and ground chyme (solid and liquid fractions) and milk samples were defatted using a diethylether/petroleum ether extraction method (Ahlborn et al., 2023b). Then, the defatted chyme and milk samples were analyzed for AA content following 24 h of HCl hydrolysis, and with *o*-phthaldialdehyde pre-column derivatization, followed by reverse-phase chromatography (Rutherfurd et al., 2012). The physiologically relevant AA groups (branched-chain AA [**BCAA**]; EAA; and NEAA) were calculated for each chyme and milk sample. The retention of each AA (and AA group) in the total chyme in the stomach (solid plus liquid fractions), relative to the total amount of protein consumed, was calculated as described in Supplemental Material section S2.5 (see Notes). Endogenous proteins could affect the AA analysis of the chyme. However, in this study we did not have a protein free diet to determine and correct for endogenous AA.

Blood Plasma AA Analysis

The concentration of AA in plasma was quantified (AgResearch Analytical Laboratory, Palmerston North, New Zealand) using the Pico-Tag method (White et al., 1986). A volume of 500 μL of each plasma sample was used for sample preparation, followed by HPLC analysis using the Pico-Tag C18 column (60 Å, 4 μm, 3.9 mm × 300 mm; Waters Corporation, MA).

Statistical Analysis

Statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc., Cary, NC). Based on the power analysis for structural parameters, 3 to 4 replicates (i.e., piglets) at each time point per milk diet were needed to reach a power higher than 0.8, as described in detail in our previous paper (Roy et al., 2022). A linear model including milk type, time, and their interaction as fixed effects was used to analyze the potential pepsin activity, apparent degree of protein hydrolysis, blood plasma AA, and the apparent release of digested protein into the small intestine. Models using time as a categorical or numerical variable were tested and the model better describing the response was selected using the log-likelihood ratio test. Nonsignificant factors ($P > 0.05$) were removed from the model, except for each main factor.

For individual proteins (α_{S1}-CN, α_{S2}-CN, β-CN, κ-CN, β-LG, and α-LA), the gastric band disappearance half-time (i.e., time taken for the intensity of each individual protein band in the milk to halve) was calculated using the Michaelis–Menten nonlinear model as described by Montoya et al. (2014), with α_{S2}-CN as an example:

$$\text{Gastric disappearance}_{\alpha s2\text{-CN}}\ (\%) = \alpha \times \text{time}/(\beta + \text{time}),$$

where α is the maximum possible disappearance (i.e., 100%), and β is the half-time of the fitted curve.

The power exponential model was used to analyze the retention of individual and grouped AA (mg AA/g protein consumed) in the stomach, as detailed by Montoya et al. (2014), with EAA as an example:

$$\text{Gastric retention}_{\text{EAA}}\ (\text{mg}) = \alpha_0 \exp - (\kappa \times \text{time})^{\beta},$$

where the parameter α_0 is the amount of AA (mg) in the milk as consumed, κ is the logarithmic slope of the curve (mg/min), β is a dimensionless index for the shape of the curve, and time is in minutes.

The parameters κ and β were then used to determine the half gastric emptying time (**$T_{1/2}$**):

$$\text{Gastric } T_{1/2\text{EAA}}\ (\text{min}) = (1/\kappa) \times [\log(1/0.5)]^{(1/\beta)}.$$

For all nonlinear models, the full (i.e., individual model for each milk type) and the reduced model (i.e., a single model for all milk types) were fitted, and an *F*-test was used to identify the best-fitting model. The normal distribution and the homogeneity of variance were evaluated for each statistical analysis. For the linear model where time was a categorical factor, means were compared using an adjusted Tukey test, whereas for the nonlinear models, the parameters (κ, β, and $T_{1/2}$ for gastric AA retention) were compared using a *t*-test, and the difference was declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Potential Pepsin Activity of the Gastric Chyme

Because pepsin is a major protease in the stomach (Heda et al., 2019), its activity in the curd (Figure 1A), liquid fraction (data not shown), and total gastric chyme (Figure 1B) at its optimum pH (i.e., pH 2) was measured. Pepsin activity for the total gastric chyme and curd fraction had a similar trend, both influenced ($P \leq 0.05$) by the interaction between milk type and time (milk type × time). In contrast, the pepsin activity of the liquid

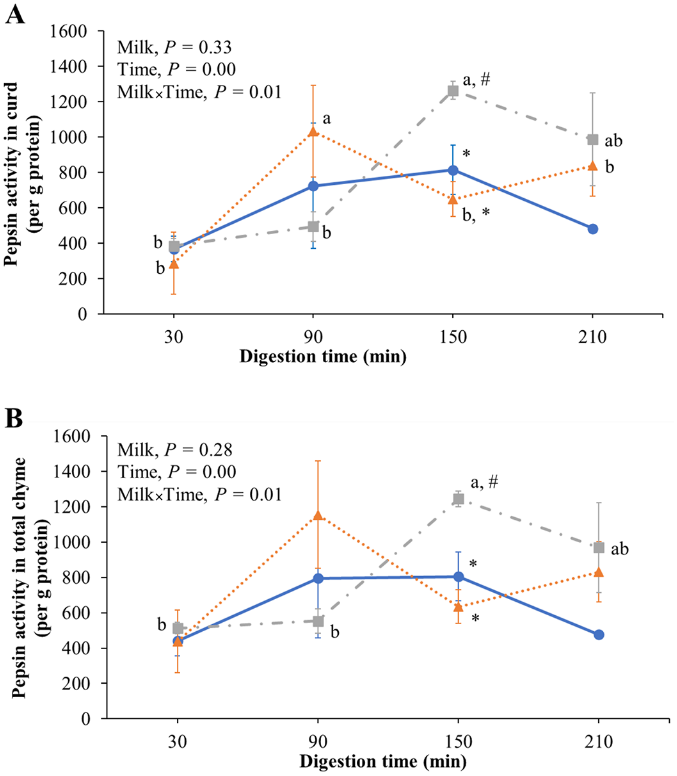

Figure 1. Potential pepsin activity of the (A) gastric curd and (B) total gastric chyme of piglets fed cow (●), goat (▲), or sheep milk (■). Values are reported as mean ± SEM, n = 3 to 4 piglets per milk species and time combination. The best model describing the pepsin activity had the parameter time as categorical. Different symbols (#, *) represent significant differences among milk types at a given digestion time point ($P \leq 0.05$). Different letters (a, b) represent significant differences over time within a particular milk type ($P \leq 0.05$).

fraction was not influenced ($P > 0.05$) by any of the parameters of the model, with a mean activity of 1,676 ± 164 U for cow, 1,504 ± 284 U for goat, and 1,496 ± 177 U for sheep milk. The curd and total chyme pepsin activity across milk from different species was similar at all postfeeding times except for 150 min postfeeding, at which time the pepsin activity was higher ($P \leq 0.05$) for the piglets fed sheep milk compared with those fed cow and goat milk (Figure 1). In general, the curd pepsin activity was lower than the liquid pepsin activity, and this could be related to the limited retention as well as penetration of gastric juices (containing pepsin) in the curd network during digestion (Ye et al., 2016; Mulet Cabero, 2018; Roy et al., 2022).

The structure of the curd network changes (strengthens) during digestion of milk due to changes in pH, pepsin concentration, and gastric motility. This structural change leads to the removal of the liquid entrapped within the curd network, limiting penetration of gastric juices in the curd, as well as continuous curd breakdown (or protein hydrolysis) over time. Previous in vitro studies have reported that the pepsin activity or protein hydrolysis is higher at the external surface of the curd with little activity in the core inside of the curd (Ye et al., 2016; Mulet Cabero, 2018). Such differences in enzyme activity in different locations of the curd might have influenced the overall enzyme activity of the curd in this study.

Little in vivo information is available on pepsin activity in piglets fed milk or milk products, and the methods used to determine pepsin activity also vary, making comparisons difficult. Previous studies in piglets fed infant formulas with intact or hydrolyzed cow milk and isolated soybean protein reported low (198.52 ± 39.36 U/g of DM at 30 min postfeeding) and highly variable pepsin activity in gastric chyme, which was unaffected by the dietary treatment (Moughan et al., 1990, 1991). The total pepsin activity at 30 min postfeeding per gram of DM in this study was similar (154–419 U) to those reported by Moughan et al. (1990, 1991).

In the present study, pepsin activity was measured at its optimum pH (i.e., pH 2) for all digestion time points by adjusting the pH of the gastric chyme to 2. However, the pH of the gastric chyme ranged from 3.0 to 5.9 (Roy et al., 2022). The chief cells of the stomach are known to release pepsinogens that are converted to their active form (i.e., pepsin) at pH <5 and optimally at pH 2 (Herriott 1938). Thus, a limitation of the pepsin activity method used in this study is that the pepsinogens present in chyme at higher pH may have been activated when adjusting to pH 2. This change in pH could have led to overestimation of the pepsin activity in high-pH chyme samples. It is important to note that inherent milk proteases (such as cathepsin D) could also autoactivate during gastric digestion (at pH 3.5–5.0; Demers-Mathieu et al., 2018; Leite et al., 2023) and hydrolyze the substrate (proteins) used to determine pepsin activity, leading to an overestimation of pepsin activity.

Gastric Protein Digestion

Apparent Degree of Protein Hydrolysis. The apparent degree of gastric protein hydrolysis for the whole chyme, curd, and liquid fraction was ($P < 0.05$) or tended ($P < 0.01$) to be influenced by the main factors (milk type and time; Figure 2), but we found no statistically significant interaction (milk type × time). The degree of hydrolysis at time 0 represented the free NH_2 present in the milk before it was given to the piglets. For the whole chyme (Figure 2A) and the curd (Figure 2B), an increase in the apparent degree of protein hydrolysis at 30 min digestion time was observed for all milk types; however, the apparent degree of protein hydrolysis remained at similar levels during the rest of the digestion, indicating that the major effect of protein hydrolysis occurred within the first 30 min. This initial increase in the apparent degree

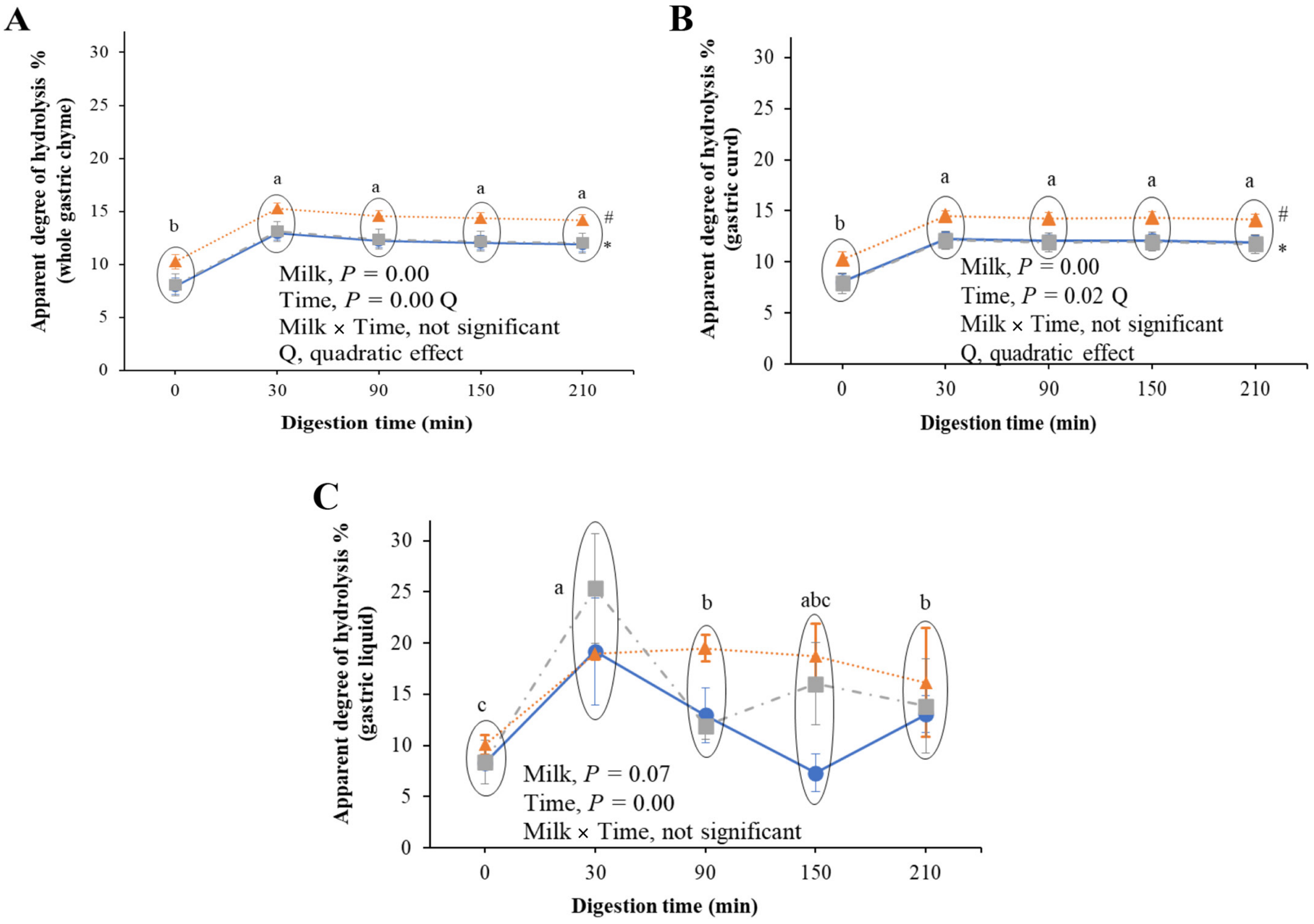

Figure 2. Apparent degree of protein hydrolysis of (A) whole gastric chyme, (B) gastric curd, and (C) gastric liquid of cow (●), goat (▲), or sheep milk (■) during digestion in piglets. Values are reported as mean ± SEM, n = 3 to 4 piglets per milk species and time combination. For the whole and curd degree of protein hydrolysis, the best statistical model had time as a numerical factor, whereas for the liquid fraction, the best model had time as a categorical factor. Different symbols (#, *) represent a significant milk type effect ($P \leq 0.05$) in the absence of interaction. Different letters (a–c) for each of the ovals represent statistically significant differences over time regardless of milk type. Q = quadratic effect.

of protein hydrolysis within the first 30 min was also observed for the liquid fraction (Figure 2C). As in the present study, others have reported an increase in milk protein hydrolysis in the gastric chyme of premature human infants (Demers-Mathieu et al., 2018).

In the whole chyme and the curd (Figure 2A, 2B), the degree of protein hydrolysis was higher for the piglets fed goat milk compared with cow and sheep milk, and this appears to be related to the higher free NH_2 present in the milk before digestion. In the liquid fraction, the apparent degree of hydrolysis also tended ($P = 0.07$) to be higher for goat milk compared with cow milk (Figure 2C). In general, during digestion, the apparent degree of hydrolysis for the curds was 12% to 15%, but for the liquid fractions, it was higher (7%–19% for cow, 16%–19% for goat, and 12%–25% for sheep milk). This could be due to the better accessibility of the enzymes to the proteins (substrate) in the liquid phase of the chyme compared with the dense network of the curd. In addition, the pH of the liquid fractions during digestion was lower than the pH of the curds, as reported in our previous study (Roy et al., 2022), which might be a reason for the observed higher pepsin activity and hydrolysis in the liquid fractions compared with their respective curd fractions at different digestion times.

Apparent Amount of Total Digested Protein Entering the Small Intestine. The apparent amount of digested protein (in the form of peptides) entering the small intestine (or emptying from the stomach) was dependent on both the apparent degree of protein hydrolysis and gastric emptying of protein, and was subject to a milk type × time interaction ($P \leq 0.05$; Figure 3). The initial increase in the

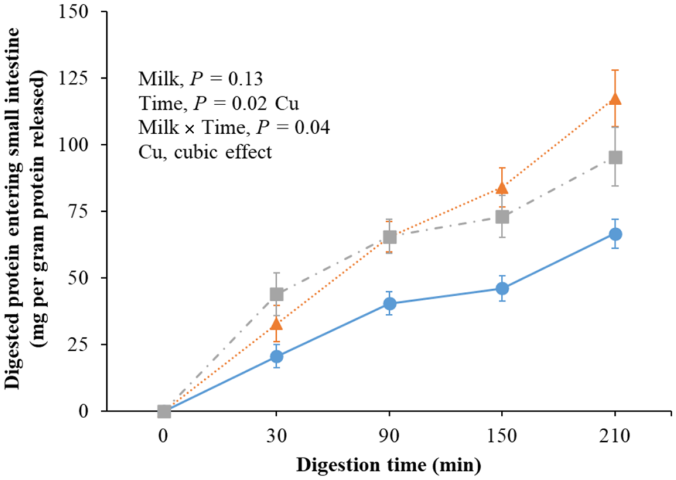

Figure 3. Apparent amount of digested protein entering the small intestine at different postfeeding times for piglets fed cow (●), goat (▲), or sheep milk (■). Values are reported as mean ± SEM, n = 2 to 4 piglets per milk species and time combination. Cu = cubic effect.

amount of digested proteins entering the small intestine could be related to the formation of curd and liquid phase that results in faster emptying of the proteins in the liquid phase (Roy et al., 2022; Ahlborn et al., 2024).

The amount of digested protein entering the small intestine for goat and sheep milk was higher than for cow milk throughout the gastric digestion (Figure 3). Because the apparent degree of protein hydrolysis for the whole chyme was found to be similar in milk from different species, the higher amount of digested protein for goat and sheep milk is likely mainly explained by the faster gastric emptying of goat and sheep milk proteins (Roy et al., 2022) compared with cow milk proteins. The difference in gastric emptying of proteins was related to the relatively softer curds formed by goat and sheep milk compared with cow milk curd (Roy et al., 2022). This indicates the relevance of gastric chyme structure and gastric protein emptying on the amount of total digested protein entering the small intestine. Although the amount of digested proteins entering the small intestine was higher for goat and sheep milk compared with cow milk, peptides (types and amounts) generated during gastric digestion of milk from different species could be similar (or different). Thus, further work to identify and quantify these peptides is warranted.

Gastric Disappearance of Individual Milk Proteins. The apparent disappearance of whey proteins and caseins from milk of different species during digestion is shown in Figure 4. Overall, higher proportions of whey proteins (Figure 4A and 4B) apparently disappeared from the gastric chyme during digestion than casein proteins (Figure 4C and 4D). As the SDS-PAGE at each digestion time was performed on equal protein concentrations, the apparent disappearance of the caseins and whey protein bands is expected to be mainly due to gradual hydrolysis as the digestion progressed; however, the structural changes in milk (curd and liquid phase), gastric contraction forces, and continuous gastric emptying would also have played a role in the apparent disappearance of proteins from the chyme fractions. For example, the faster disappearance of whey proteins could be explained by their solubility in the gastric environment and the fast emptying of the liquid fraction (compared with the caseins in the curd fraction; Roy et al., 2021a,b).

Among the whey proteins, α-LA (Figure 4A) had a faster apparent disappearance than β-LG (Figure 4B). For example, the time to reach $T_{1/2}$ was 8.2 min for α-LA versus 47 to 89 min for β-LG. Although the apparent disappearance of α-LA was the same for all milk types, β-LG disappeared fastest for goat milk (e.g., $T_{1/2}$ 47 min for goat milk versus >60 min for sheep and cow milk). In vitro gastric digestion studies have shown that β-LG from goat milk (Almaas et al., 2006) and sheep milk (El-Zahar et al., 2005) are more susceptible to hydrolysis than that of cow milk; however, the reasons for their greater susceptibility remain unclear.

Among caseins, κ-CN from all the milk species disappeared completely within 30 min of gastric digestion, whereas β-CN did not disappear within 210 min (i.e., it was not hydrolyzed) for all milk species (based on the SDS-PAGE approach; data not shown). α_{S1}-Casein (Figure 4C) and α_{S2}-CN (Figure 4D) slowly disappeared (i.e., were hydrolyzed) over time ($P \leq 0.05$) and disappearances appeared to differ across milk types (e.g., for α_{S1}-CN, the rate of apparent disappearance was slower for piglets fed cow milk than those fed goat or sheep milk). However, these differences were not reflected in statistical differences for $T_{1/2}$ due to the high variability in cow milk results. Differences in the apparent disappearance of α_S-CN could be related to the relatively softer curd networks formed by goat and sheep milk compared with cow milk, as reported previously (Roy et al., 2022). In vitro gastric digestion studies have reported greater hydrolysis of goat milk caseins than cow milk caseins based on SDS-PAGE analysis (Hodgkinson et al., 2018; Ye et al., 2019). The authors also attributed the higher hydrolysis susceptibility of caseins from goat milk to its potential to form softer curds and smaller casein aggregates compared with cow milk.

Gastric Emptying of AA

Differences in the gastric emptying of grouped and individual AA during digestion are shown in Figure 5 and Figure 6, respectively. Cow milk had a slower emptying rate (κ, kappa) of grouped EAA (Figure 5A), NEAA

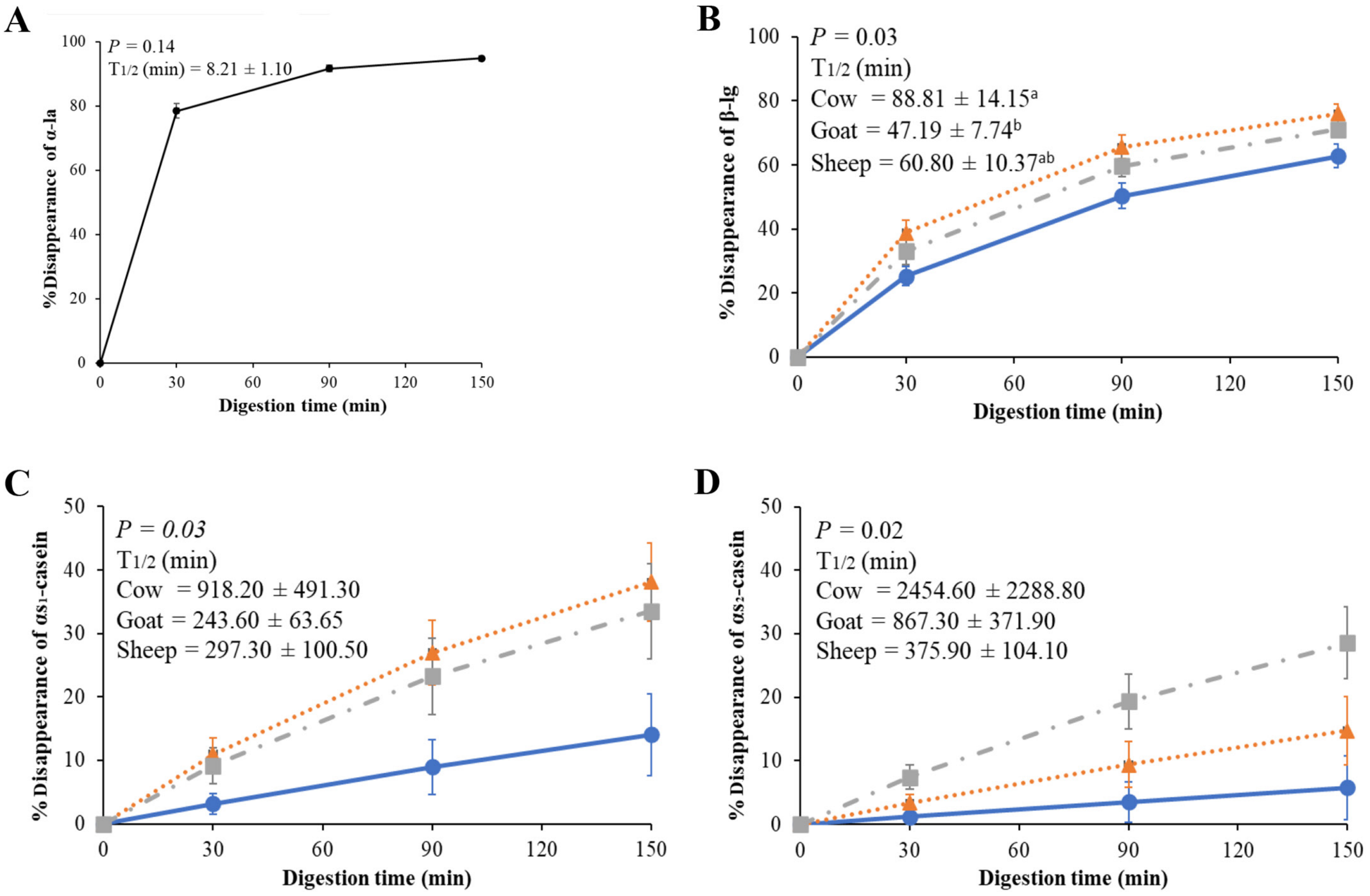

Figure 4. Changes over time in apparent gastric disappearance of (A) α-LA in total gastric chyme in piglets fed all types of milk (i.e., there was no difference across milk types), and (B) β-LG, (C) αs_1-CN, and (D) αs_2-CN in total gastric chyme in piglets fed cow (blue dots), goat (orange triangles), or sheep milk (gray squares). There was no difference ($P > 0.05$) in the disappearance of α-LA for piglets fed different milk types. Values are reported as the mean ± SEM, n = 2 to 4 piglets per milk species and time combination. $T_{1/2}$ is half gastric emptying time (min), $T_{1/2}$ values with different superscript letters (a, b) differ ($P \leq 0.05$).

(Figure 5B), and BCAA (Figure 5C) compared with goat and sheep milk ($P \leq 0.05$), both of which had similar emptying rates. These differences are related to the longer gastric retention of these grouped AA for cow milk (as observed from longer gastric half-emptying time, $T_{1/2}$) followed by goat and sheep milk ($P \leq 0.05$). Similar trends were observed for the gastric emptying of individual AA across milk species (Figure 6; Supplemental Figures S2 and S3, see Notes). Differences in $T_{1/2}$ (Figure 6A) and kappa (Figure 6B) were observed across AA. For example, the $T_{1/2}$ ranged between 153 min (alanine) and 225 min (methionine) for cow milk, whereas it ranged between 72 min (alanine) and 138 min (tyrosine) for goat milk. Sheep milk AA had the lowest $T_{1/2}$ (53–104 min).

To depict the effect of different curd structures on gastric emptying kinetics of AA from milk of different species, proline (an AA more dominant in casein; Rasmussen, 2008; Tang et al., 2009; Burd et al., 2012; van Eijnatten et al., 2024) was considered as an example. The emptying rate of proline AA from cow milk was significantly lower ($P \leq 0.05$) than that of goat and sheep milk (Figure 6; Supplemental Figure S3F). The delayed emptying of cow milk proline could be related to the stronger curd network formed by the cow milk caseins (and lower hydrolysis tendency) compared with goat and sheep milk caseins (Roy et al., 2022). This emphasizes that the nature of the curd structure formed, as well as the susceptibility of specific proteins to enzyme hydrolysis, are important in determining the emptying rates of AA from the stomach. In addition, leucine, an AA that plays a crucial role in protein synthesis (Kimball and Jefferson, 2006; Rasmussen, 2008; Tang et al., 2009), was found to have a faster rate of emptying from goat and sheep milk compared with that of cow milk (Figure 6; Supplemental Figure S2C), which may result in a faster delivery of leucine in the growing infant. In a previous in vivo study with the same animal model and milk types, it was found that at 210 min after feeding, piglets fed cow

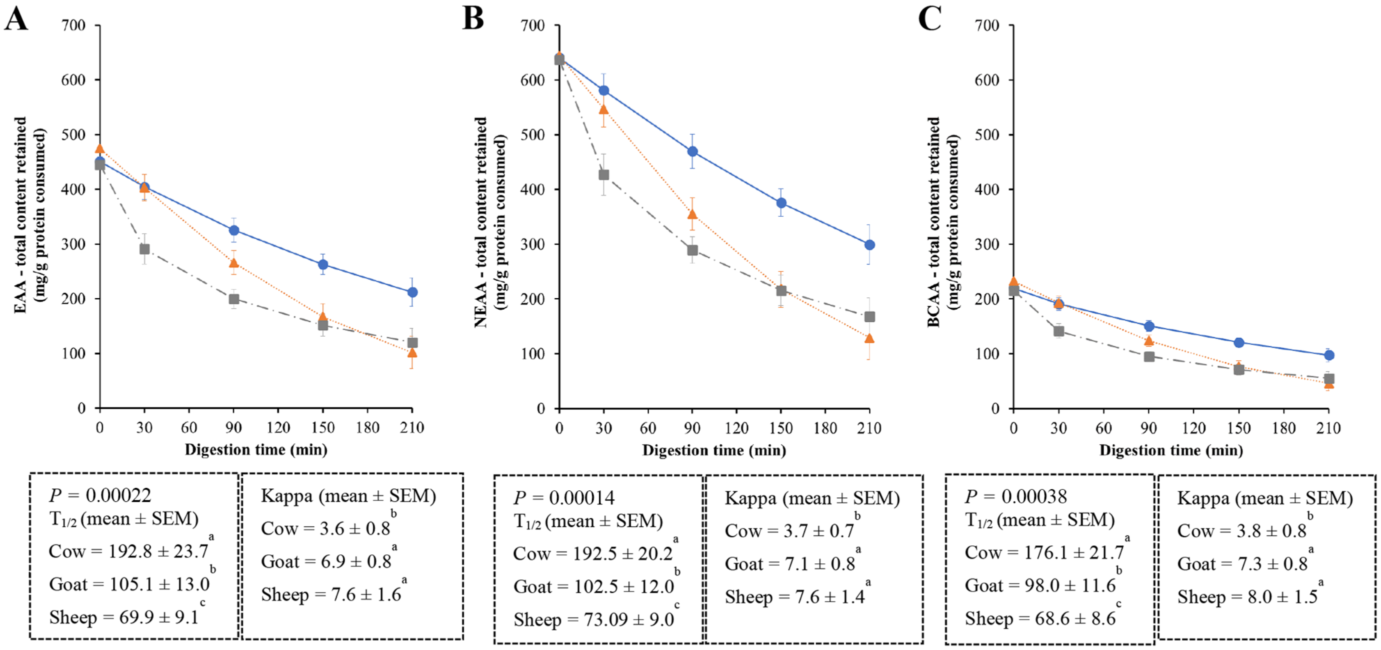

Figure 5. Gastric emptying of (A) total EAA, (B) total NEAA, and (C) total branched-chain amino acids (BCAA) in total gastric chyme for piglets fed cow (●), goat (▲), or sheep milk (■). Values are reported as the mean ± SEM, n = 3 to 4 piglets per species and time combination. Kappa is the slope of the curve (%/min × 10^{-3}), and $T_{1/2}$ is the time to reach half gastric emptying rate (min). Mean values of kappa and $T_{1/2}$ with different superscript letters (a–c) differ ($P \leq 0.05$).

milk had lower apparent small intestinal AA digestibility than those fed goat and sheep milk, and these differences were related to the differences in gastric retention of AA (Ahlborn et al., 2023a). In addition, previous studies using other types of foods have reported that the rates of digested protein entering the small intestine influence the rates of AA absorption in the first half of the small intestine (Montoya et al., 2018). Thus, it could also be expected that the piglets fed goat and sheep milk would have a faster rate of small intestinal AA absorption.

Appearance of Blood Plasma AA

The concentrations of AA in blood plasma over time were determined (Figure 7) as a proxy to determine the impact of gastric emptying on small intestinal absorption (Loveday, 2023; Milan et al., 2023; Mayar et al., 2024; van Eijnatten et al., 2024). We observed a significant ($P \leq 0.05$) interaction (time × milk) for the appearance of blood plasma total EAA (Figure 7A). At times greater than 60 min of digestion, the plasma EAA were lower for cow milk (Figure 7A), but not for NEAA (Figure 7B) and BCAA (Figure 7C), though for the BCAA, there was a significant ($P \leq 0.05$) effect of milk type. For some individual AA (Supplemental Figures S4 and S5, see Notes), the concentrations in plasma were higher for sheep and (or) goat (e.g., proline) across all postfeeding times ($P \leq 0.05$ milk effect), whereas for other AA, the concentrations were higher for a specific milk at specific postfeeding times (e.g., histidine at 30 min was higher for cow milk compared with other milk types). This indicates that the AA in blood plasma did not directly reflect (or follow) the differences observed in their gastric emptying rates. For example, blood plasma leucine concentration showed a significant interaction between milk type and time ($P \leq 0.05$; Supplemental Figure S4C), with a similar increase in leucine concentration for all milk types within the first 30 min after feeding, but only the piglets fed sheep milk retained higher plasma leucine concentrations. However, for all postfeeding times, the amount of leucine entering the small intestine (i.e., the gastric emptying) was higher for piglets fed sheep and goat milk than those fed cow milk (Supplemental Figure S2C). Scant information has been published on the appearance of blood plasma AA concentrations in infant models. However, a study in adult humans found no differences in blood plasma leucine concentrations after consumption of cow and goat protein–based milk drinks (Milan et al., 2018). In contrast, the same group also found that leucine concentrations were higher in blood plasma after consumption of sheep milk compared with cow milk (reconstituted from the spray dried powders) in adults (Milan et al., 2020).

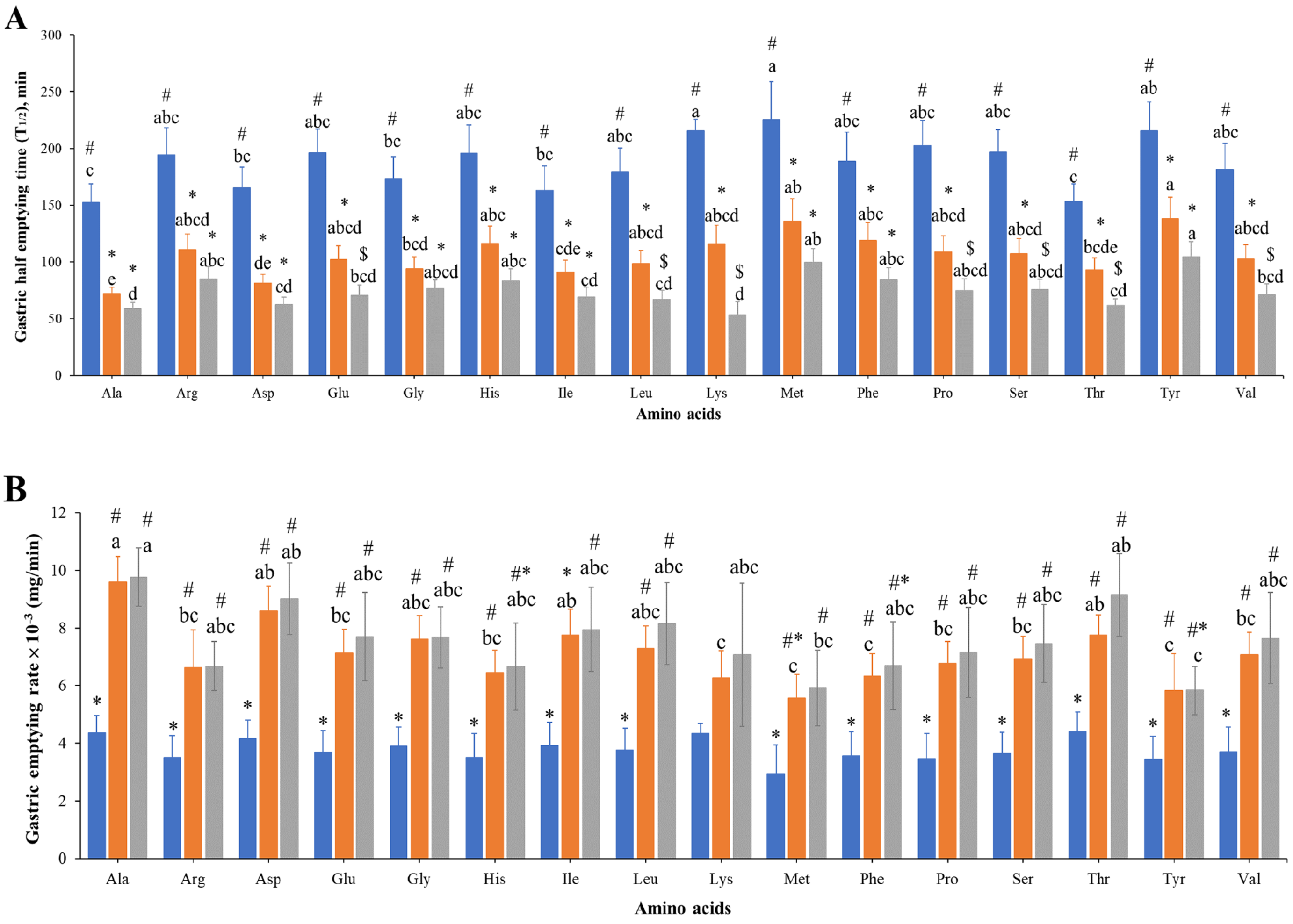

Figure 6. Gastric emptying of amino acids in total chyme in piglets fed cow (blue bars), goat (orange bars), or sheep (gray bars) milk: (A) gastric emptying half-time ($T_{1/2}$) of AA; (B) gastric emptying rates (mg/min × 10^{-3}) of AA. For an AA, different symbols (#, *, $) above bars represent significant differences across milk types ($P \leq 0.05$). Different letters (a–e) above the mean values represent significant differences across AA within a particular milk type ($P \leq 0.05$). Values are means ± SEM, n = 3–4.

The postprandial appearance of AA in the blood is a dynamic process, with levels of AA in blood fluctuating depending on a combination of factors such as the composition (and structure) of the milk-based meals, the rates of protein digestion and absorption, and the metabolic demands of the body. Although studies have indicated that the appearance of AA in blood relates to AA gastric emptying (Boirie et al., 1997), our study shows that interpretations based on blood plasma AA concentrations need to be made with caution.

Considering the results presented in the current study are from raw milk and infants do not consume raw milk, the results provide a fundamental mechanistic information on the effect of curd structure on gastric protein digestion. Thus, further work to understand the effect of different milk processing treatments on infant gastric digestion is warranted.

CONCLUSIONS

Overall, physiological levels of gastric pepsin activity and apparent degree of protein hydrolysis in whole chyme were similar for piglets fed cow, goat, and sheep milk. However, the rate of disappearance of individual protein fractions (e.g., β-LG and α_S-CN) were (or tended to be) greater for goat and sheep milk, indicating greater susceptibility to enzyme hydrolysis. In addition, AA gastric emptying was greater for goat and sheep milk compared with cow milk, emphasizing that a combination of protein composition, susceptibility of specific proteins to enzyme hydrolysis, and the nature of the curd structure formed may be a key mechanism influencing the emptying rates of AA from the stomach. However, the appearance of AA in blood plasma did not always reflect the differences observed in their gastric empty-

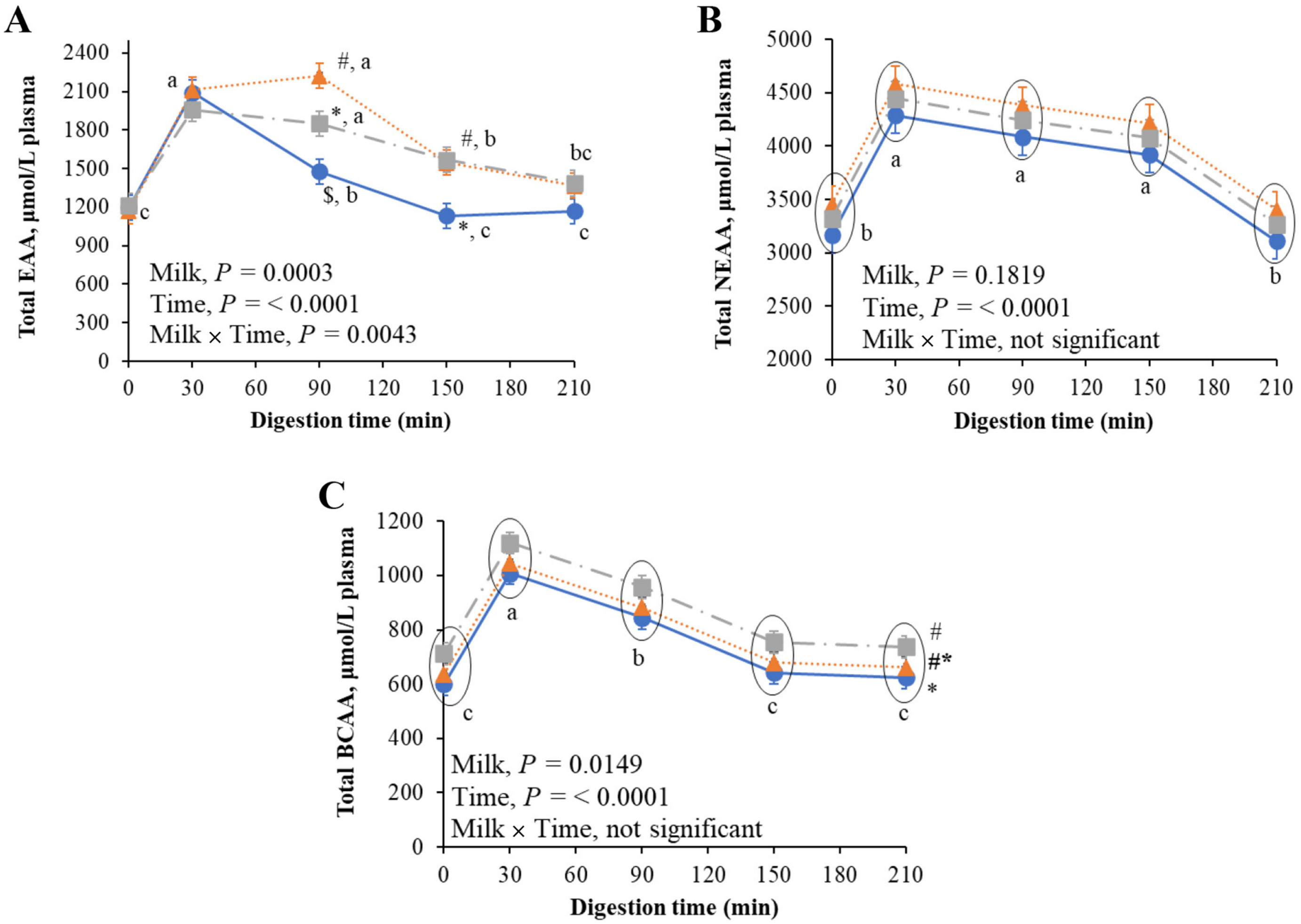

Figure 7. Blood plasma appearance of (A) total EAA, (B) total NEAA, and (C) total branched-chain amino acids (BCAA) over time in blood plasma of piglets fed cow (●), goat (▲), or sheep milk (■). Values are reported as the mean ± SEM, n = 4 piglets per milk type and time combination. Different symbols (#, *, $) represent significant differences ($P \leq 0.05$) among milk types at a given digestion time point when the interaction was significant or represents a significant milk type effect ($P \leq 0.05$) in the absence of interaction. Different letters (a–c) above the mean values represent significant differences ($P \leq 0.05$) over time within a particular milk type when the interaction was significant or represents a significant time effect ($P \leq 0.05$) in the absence of interaction by including all milk types within the same oval.

ing rates, indicating that the blood appearance of AA is a complex phenomenon and may not directly reflect the gastric emptying of AA.

NOTES

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Nonstandard abbreviations used: ADU = arbitrary density units; BCAA = branched-chain AA; $T_{1/2}$ = half gastric emptying time; TCA = tricarboxylic acid.

REFERENCES

Ahlborn, N. G., C. A. Montoya, D. Roy, N. C. Roy, N. Stroebinger, A. Ye, L. M. Samuelsson, P. J. Moughan, and W. C. McNabb. 2023a. Differences in small intestinal apparent amino acid digestibility of raw bovine, caprine, and ovine milk are explained by gastric amino acid retention in piglets as an infant model. Front. Nutr. 10:1226638. https://doi.org/10.3389/fnut.2023.1226638.

Ahlborn, N. G., C. A. Montoya, S. M. Hodgkinson, A. Dave, A. Ye, L. M. Samuelsson, N. C. Roy, and W. C. McNabb. 2023b. Heat treatment and homogenization of bovine milk loosened gastric curd structure and increased gastric emptying in growing pigs. Food Hydrocoll. 137:108380. https://doi.org/10.1016/j.foodhyd.2022.108380.

Ahlborn, N. G., C. A. Montoya, N. C. Roy, A. Ye, L. M. Samuelsson, R. J. Wieliczko, and W. C. McNabb. 2024. Heat treatment and homogenization influence the gastric digestion of bovine milk protein in growing pigs as an adult human model. J. Nutr. 154:2097–2107. https://doi.org/10.1016/j.tjnut.2024.04.035.

Almaas, H., A.-L. Cases, T. G. Devold, H. Holm, T. Langsrud, L. Aabakken, T. Aadnoey, and G. E. Vegarud. 2006. In vitro digestion of bovine and caprine milk by human gastric and duodenal enzymes. Int. Dairy J. 16:961–968. https://doi.org/10.1016/j.idairyj.2005.10.029.

AOAC International. 2005. Official Methods of Analysis. Accessed Mar. 1, 2025. http://www.eoma.aoac.org/Methods.

Boirie, Y., M. Dangin, P. Gachon, M. P. Vasson, J. L. Maubois, and B. Beaufrère. 1997. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc. Natl. Acad. Sci. USA 94:14930–14935. https://doi.org/10.1073/pnas.94.26.14930.

Burd, N. A., Y. Yang, D. R. Moore, J. E. Tang, M. A. Tarnopolsky, and S. M. Phillips. 2012. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. Br. J. Nutr. 108:958–962. https://doi.org/10.1017/S0007114511006271.

Church, F. C., H. E. Swaisgood, D. H. Porter, and G. L. Catignani. 1983. Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. J. Dairy Sci. 66:1219–1227. https://doi.org/10.3168/jds.S0022-0302(83)81926-2.

Crowley, S. V., A. L. Kelly, J. A. Lucey, and J. A. O'Mahony. 2017. Potential applications of non-bovine mammalian milk in infant nutrition. Pages 625–654 in Handbook of Milk of Non-Bovine Mammals. 2nd ed. Y. W. Park, G. F. W. Haenlein, and W. L. Wendorff, ed. John Wiley & Sons Ltd., Chichester, UK. https://doi.org/10.1002/9781119110316.ch13.

Darragh, A. J., and P. J. Moughan. 1995. The three-week-old piglet as a model animal for studying protein digestion in human infants. J. Pediatr. Gastroenterol. Nutr. 21:387–393. https://doi.org/10.1002/j.1536-4801.1995.tb11956.x.

Darragh, A. J., and P. J. Moughan. 1998. The amino acid composition of human milk corrected for amino acid digestibility. Br. J. Nutr. 80:25–34. https://doi.org/10.1017/S0007114598001731.

Demers-Mathieu, V., Y. Qu, M. A. Underwood, R. Borghese, and D. C. Dallas. 2018. Premature infants have lower gastric digestion capacity for human milk proteins than term infants. J. Pediatr. Gastroenterol. Nutr. 66:816–821. https://doi.org/10.1097/MPG.0000000000001835.

El-Zahar, K., M. Sitohy, Y. Choiset, F. Métro, T. Haertlé, and J.-M. Chobert. 2005. Peptic hydrolysis of ovine β-lactoglobulin and α-lactalbumin: Exceptional susceptibility of native ovine β-lactoglobulin to pepsinolysis. Int. Dairy J. 15:17–27. https://doi.org/10.1016/j.idairyj.2004.06.002.

FAOSTAT. 2024. Compare Data. Accessed Jul. 11, 2024. https://www.fao.org/faostat/en/#compare.

Guilloteau, P., R. Zabielski, H. M. Hammon, and C. C. Metges. 2010. Nutritional programming of gastrointestinal tract development. Is the pig a good model for man? Nutr. Res. Rev. 23:4–22. https://doi.org/10.1017/S0954422410000077.

Haenlein, G. F. W. 2004. Goat milk in human nutrition. Small Rumin. Res. 51:155–163. https://doi.org/10.1016/j.smallrumres.2003.08.010.

Haenlein, G. F. W., and W. L. Wendorff. 2006. Sheep milk. Pages 137–194 in Handbook of Milk of Non-Bovine Mammals. Y. W. Park and G. F. W. Haenlein, ed. Blackwell Publishing Professional, Ames, IA. https://doi.org/10.1002/9780470999738.ch7.

Heda, R., F. Toro, and C. R. Tombazzi. 2019. Physiology, pepsin. StatPearls Publishing, Treasure Island, FL.

Herriott, R. M. 1938. Kinetics of the formation of pepsin from swine pepsinogen and identification of an intermediate compound. J. Gen. Physiol. 22:65–78. https://doi.org/10.1085/jgp.22.1.65.

Hodgkinson, A. J., O. A. M. Wallace, I. Boggs, M. Broadhurst, and C. G. Prosser. 2018. Gastric digestion of cow and goat milk: Impact of infant and young child in vitro digestion conditions. Food Chem. 245:275–281. https://doi.org/10.1016/j.foodchem.2017.10.028.

Jandal, J. 1996. Comparative aspects of goat and sheep milk. Small Rumin. Res. 22:177–185. https://doi.org/10.1016/S0921-4488(96)00880-2.

Kimball, S. R., and L. S. Jefferson. 2006. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J. Nutr. 136:227S–231S. https://doi.org/10.1093/jn/136.1.227S.

Leite, J. A., C. A. Montoya, S. M. Loveday, J. A. Mullaney, T. S. Loo, W. C. McNabb, and N. C. Roy. 2023. The impact of heating and drying on protease activities of ruminant milk before and after in vitro infant digestion. Food Chem. 429:136979. https://doi.org/10.1016/j.foodchem.2023.136979.

Loveday, S. M. 2023. Protein digestion and absorption: The influence of food processing. Nutr. Res. Rev. 36:544–559. https://doi.org/10.1017/S0954422422000245.

Mayar, M., M. de Vries, P. Smeets, J. van Duynhoven, and C. Terenzi. 2024. MRI assessment of pH and coagulation during semi-dynamic in vitro gastric digestion of milk proteins. Food Hydrocoll. 152:109866. https://doi.org/10.1016/j.foodhyd.2024.109866.

Meisel, H., and H. Hagemeister. 1984. Influences of different technological treatments of milk on the digestion in the stomach. II. Gastric passage of different milk constituents. Milchwissenschaft 39:262–266.

Milan, A. M., M. P. G. Barnett, W. C. McNabb, N. C. Roy, S. Coutinho, C. L. Hoad, L. Marciani, S. Nivins, H. Sharif, T. R. Angeli-Gordon, P. Du, A. A. Gharibans, G. O'Grady, P. Sharma, A. Shrestha, and R. F. Mithen. 2023. Heat treatment of bovine milk impacts gastric emptying and nutrient appearance. Medical Sciences Forum 18:8. https://doi.org/10.3390/msf2023018008.

Milan, A. M., A. J. Hodgkinson, S. M. Mitchell, U. K. Prodhan, C. G. Prosser, E. A. Carpenter, K. Fraser, and D. Cameron-Smith. 2018. Digestive responses to fortified cow or goat dairy drinks: A randomised controlled trial. Nutrients 10:1492. https://doi.org/10.3390/nu10101492.

Milan, A. M., L. M. Samuelsson, A. Shrestha, P. Sharma, L. Day, and D. Cameron-Smith. 2020. Circulating branched chain amino acid concentrations are higher in dairy-avoiding females following an equal volume of sheep milk relative to cow milk: A randomized controlled trial. Front. Nutr. 7:553674. https://doi.org/10.3389/fnut.2020.553674.

Montoya, C. A., D. L. Cabrera, M. Zou, M. J. Boland, and P. J. Moughan. 2018. The rate at which digested protein enters the small intestine modulates the rate of amino acid digestibility throughout the small intestine of growing pigs. J. Nutr. 148:1743–1750. https://doi.org/10.1093/jn/nxy193.

Montoya, C. A., S. M. Rutherfurd, T. D. Olson, A. S. Purba, L. N. Drummond, M. J. Boland, and P. J. Moughan. 2014. Actinidin from kiwifruit (*Actinidia deliciosa* cv. Hayward) increases the digestion and rate of gastric emptying of meat proteins in the growing pig. Br. J. Nutr. 111:957–967. https://doi.org/10.1017/S0007114513003401.

Moughan, P. J., M. Birtles, P. D. Cranwell, W. Smith, and M. Pedraza. 1992. The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. World Rev. Nutr. Diet. 67:40–113. https://doi.org/10.1159/000419461.

Moughan, P. J., P. D. Cranwell, and W. C. Smith. 1991. An evaluation with piglets of bovine milk, hydrolyzed bovine milk, and isolated soybean proteins included in infant milk forumlas. II. Stomach-emptying rate and the postprandial change in gastric pH and milk-clotting enzyme activity. J. Pediatr. Gastroenterol. Nutr. 12:253–259. https://doi.org/10.1002/j.1536-4801.1991.tb10227.x.

Moughan, P. J., M. Pedraza, W. C. Smith, M. Williams, and M. N. Wilson. 1990. An evaluation with piglets of bovine milk, hydrolyzed bovine milk, and isolated soybean proteins included in infant milk formulas. I. Effect on organ development, digestive enzyme activities, and amino acid digestibility. J. Pediatr. Gastroenterol. Nutr. 10:385–394. https://doi.org/10.1002/j.1536-4801.1990.tb10015.x.

Mudd, A. T., and R. N. Dilger. 2017. Early-life nutrition and neurodevelopment: use of the piglet as a translational model. Adv. Nutr. 8:92–104. https://doi.org/10.3945/an.116.013243.

Mulet Cabero, A. 2018. Effect of dairy structures on gastric behaviour and nutrient digestion kinetics using a semi-dynamic model. PhD thesis. Quadram Institute Bioscience and Teagasc Food Research Centre. Faculty of Science, School of Biological Sciences, University of East Anglia, Norwich, Norfolk, UK. https://ueaeprints.uea.ac.uk/id/eprint/70235/.

Park, Y. W. 2006a. Minor species milk. Pages 393–406 in Handbook of Milk of Non-Bovine Mammals. Y. W. Park and G. F. W. Haenlein, ed. Blackwell Publishing Professional, Ames, IA. https://doi.org/10.1002/9780470999738.ch17.

Park, Y. W. 2006b. Sow milk. Pages 371–381 in Handbook of Milk of Non-Bovine Mammals. Y. W. Park and G. F. W. Haenlein, ed. Blackwell Publishing Professional, Ames, IA. https://doi.org/10.1002/9780470999738.ch15.

Park, Y. W., M. Juarez, M. Ramos, and G. F. W. Haenlein. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88–113. https://doi.org/10.1016/j.smallrumres.2006.09.013.

Rasmussen, C. J. 2008. Nutritional supplements for endurance athletes. Pages 369–407 in Nutritional Supplements in Sports and Exercise. M. Greenwood, D. S. Kalman, and J. Antonio, ed. Humana Press, Totowa, NJ.

Roy, D., P. J. Moughan, A. Ye, S. M. Hodgkinson, N. Stroebinger, S. Li, A. C. Dave, C. A. Montoya, and H. Singh. 2022. Structural changes in milk from different species during gastric digestion in piglets. J. Dairy Sci. 105:3810–3831. https://doi.org/10.3168/jds.2021-21388.

Roy, D., A. Ye, P. J. Moughan, and H. Singh. 2021a. Impact of gastric coagulation on the kinetics of release of fat globules from milk of different species. Food Funct. 12:1783–1802. https://doi.org/10.1039/D0FO02870C.

Roy, D., A. Ye, P. J. Moughan, and H. Singh. 2021b. Structural changes in cow, goat and sheep skim milk during dynamic in vitro gastric digestion. J. Dairy Sci. 104:1394–1411. https://doi.org/10.3168/jds.2020-18779.

Rutherfurd, S. M., K. Bains, and P. J. Moughan. 2012. Available lysine and digestible amino acid contents of proteinaceous foods of India. Br. J. Nutr. 108(Suppl. 2):S59–S68. https://doi.org/10.1017/S0007114512002280.

Rutherfurd, S. M., A. J. Darragh, W. Hendriks, C. Prosser, and D. Lowry. 2006. Mineral retention in three-week-old piglets fed goat and cow milk infant formulas. J. Dairy Sci. 89:4520–4526. https://doi.org/10.3168/jds.S0022-0302(06)72500-0.

Tang, J. E., D. R. Moore, G. W. Kujbida, M. A. Tarnopolsky, and S. M. Phillips. 2009. Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J. Appl. Physiol. 107:987–992. https://doi.org/10.1152/japplphysiol.00076.2009.

van Eijnatten, E. J., J. J. Roelofs, G. Camps, T. Huppertz, T. T. Lambers, and P. A. Smeets. 2024. Gastric coagulation and postprandial amino acid absorption of milk is affected by mineral composition: A randomized crossover trial. Food Funct. 15:3098–3107. https://doi.org/10.1039/D3FO04063A.

White, J. A., R. J. Hart, and J. C. Fry. 1986. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. J. Anal. Methods Chem. 8:170–177. https://doi.org/10.1155/S1463924686000330.

Ye, A., J. Cui, E. Carpenter, C. Prosser, and H. Singh. 2019. Dynamic in vitro gastric digestion of infant formulae made with goat milk and cow milk: Influence of protein composition. Int. Dairy J. 97:76–85. https://doi.org/10.1016/j.idairyj.2019.06.002.

Ye, A., J. Cui, D. Dalgleish, and H. Singh. 2016. Formation of a structured clot during the gastric digestion of milk: Impact on the rate of protein hydrolysis. Food Hydrocoll. 52:478–486. https://doi.org/10.1016/j.foodhyd.2015.07.023.

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